

## A single phosphorus treatment doubles growth of cyanobacterial lichen transplants

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**Abstract.** Lichens are reputedly slow growing and become unhealthy or die in response to supplements of the usual limiting resources, such as water and nitrogen. We found, however, that the tripartite cyanobacterial lichen *Lobaria pulmonaria* doubled in annual biomass growth after a single 20-minute immersion in a phosphorus solution ( $K_2HPO_4$ ), as compared to controls receiving no supplemental phosphorus. This stimulation of cyanolichens by phosphorus has direct relevance to community and population ecology of lichens, including improving models of lichen performance in relation to air quality, improving forest management practices affecting old-growth associated cyanolichens, and understanding the distribution and abundance of cyanolichens on the landscape. Phosphorus may be as important a stimulant to cyanobacterial-rich lichen communities as it is to cyanobacteria in aquatic ecosystems.

**Key words:** cyanobacteria; cyanolichen; growth rate; lichen; *Lobaria pulmonaria*; nitrogen fixation; Oregon, USA; Pacific Northwest, North America; phosphorus; transplant experiment.

### INTRODUCTION

Lichens are reputedly slow growing, difficult to grow indoors or in growth chambers, and apt to become unhealthy or die in response to supplements of the usual limiting resources, such as water or nitrogen (Galun et al. 1972, Bublick 1988, Bando and Sugino 1995). “Overirrigation or excess nutrients usually leads to death or dissolution of the symbiosis” (Bublick 1988:127). It would be notable, therefore, if some factor stimulated lichen growth rates beyond those observed in the field.

While most lichens are bipartite symbioses between algae and fungi, some lichens contain cyanobacteria. “Cyanolichens” contain cyanobacteria, either in a bipartite association between a fungus and a cyanobacterium or a tripartite association among a fungus, a primary green algal photobiont, and a secondary cyanobacterial photobiont that is restricted to discrete external or internal packets called “cephalodia.” Tripartite epiphytic lichens in the genus *Lobaria* can achieve biomass > 1 kg/ha dry mass in the Pacific Northwest of North America (McCune 1993, Berryman and McCune 2006). The air-pollution sensitivity of

cyanolichens makes them important components of models of lichen communities in relation to air quality (e.g., Geiser and Neitlich 2007). Cyanolichens are sensitive to many pollutants, most importantly  $SO_2$ ,  $NH_3$ ,  $NO_x$ , and acidic forms of S and N.

These and other cyanolichens fix atmospheric nitrogen that is subsequently released by leaching, decomposition, and herbivory (Antoine 2004, Holub and Lajtha 2004), providing a significant nutrient input to a wide range of boreal, temperate, and tropical ecosystems (Crittenden and Kershaw 1978, Gunther 1989, Nash 1996). Nitrogen fixation is an energy-intensive process, requiring phosphorus (P) in the form of approximately 15 ATP (adenosine triphosphate) to reduce each molecule of  $N_2$  to  $NH_3$ . Crittenden et al. (1994) suggested that P availability would limit N fixation in lichens; Kurina and Vitousek (1999) demonstrated this. Phosphorus control over N fixation has been suggested at ecosystem scales (Vitousek and Howarth 1991, Vitousek and Field 1999), over long periods of pedogenesis, or during vegetation succession (Gorham et al. 1979).

Benner and Vitousek (2007) and Benner et al. (2007) demonstrated long-term and short-term stimulation of epiphytic cyanolichens in Hawaii, in response to P fertilization of the soil. Presumably the P was taken up by the trees, then leached onto the trunk and branch

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surfaces, where it became available for uptake by lichens. The increases in cyanolichens were dramatic, with potentially profound consequences for our understanding of epiphytic lichen biology, including population dynamics, community structure, and contribution of fixed nitrogen. Because the Hawaiian field site has P-deficient soils, a strong response to P by organisms that have high demand for P is not entirely surprising.

We sought a rapid evaluation of whether P limitation of cyanolichens might be important even in temperate-forest ecosystems that are not traditionally considered P limited. The Pacific Northwest of North America is ideal for such an evaluation, because cyanolichens are naturally abundant and the young volcanic soils are relatively P rich. To evaluate P limitation, we applied a single dose of P in solution to transplants of the tripartite cyanolichen *Lobaria pulmonaria*, returned the transplants to the field, then measured growth over one year.

#### METHODS

We studied the effects of a single immersion in potassium-phosphate solutions on growth rates of transplants of lobes of the lichenized fungus *Lobaria pulmonaria*, using a logarithmic P-concentration series. Transplants were prepared by attaching a nylon monofilament loop to lobes with clear silicone sealer (McCune et al. 1996). Lobes about 4 × 4 cm were cut from much larger individuals. Source material was from healthy thalli on branches in the same general area to which the transplants were returned (see below).

Transplants were air-dried on a laboratory bench for 24 hr, then weighed. Biomass was corrected to an oven-dry basis by calculating the percentage of moisture in air-dried vs. oven-dried sacrificial samples similar to the transplants (McCune et al. 1996). Transplants were then activated physiologically by spraying with distilled water, kept moist for 1 h by occasional misting, then immersed in open beakers in one of four different P solutions for 20 min, air dried for 24 h on cardboard egg cartons on a laboratory bench, then hung in the field on the next day, 23 October 2006. Transplants were retrieved in November 2007, air dried, weighed, and corrected to oven-dry mass as before. Biomass growth was expressed as the difference in mass divided by the initial mass, after subtracting the mass of the nylon and silicone.

Phosphorus solutions were 0, 6, 60, and 600 mg/L of P made with anhydrous  $K_2HPO_4$  dissolved in distilled water. The treatment of 60 mg/L equals the P concentration in Bold's basal medium (Stein 1973) and is approximately 10 000 times rainwater concentration and 1000 times throughfall concentration in nearby conifer forests in western Oregon (Sollins et al. 1980). The high concentrations were used to compensate for our use of a single application, rather than chronic exposure to elevated P. Fifteen transplants received each

of the two lower concentration treatments and 25 transplants received each of the two higher treatments.

Transplants that appeared healthy were re-used from a previous transplant experiment that applied dilute solutions of various forms of nitrogen and calcium carbonate to the thalli. These treatments had weak effects (to be reported elsewhere). P treatments were randomly assigned to individual transplants with the constraint that each P treatment received equal numbers of transplants from the preceding treatments. Carryover effects of previous treatments, including interactions with the P treatments, were sought statistically but not found, regardless of whether or not the previous year's growth was used as a covariate.

Transplants were hung at a rural site that was selected to have the following characteristics: away from the Willamette Valley to minimize exposure to urban and agricultural pollutants, away from the immediate vicinity of regular fertilizer use and large concentrations of domestic animals, presence of healthy lichen communities with pollution-sensitive species, and availability of numerous branches of *Fraxinus latifolia* at a convenient height. *Fraxinus latifolia* was chosen because it is conspicuously favorable for cyanolichens. A site on the floodplain of Marys River, near Wren, west of Corvallis met these criteria: 44°35'39" N, 123°24'37" W; elevation 131 m; Benton County, Oregon, USA.

Transplants were hung on branches 2–3 m high arranged in a wiggly transect around a clump of trees on the edge of a grassy, little-used driveway. Treatments were randomly assigned to locations. Transplants and labels were attached loosely with plastic zip-ties that were color coded by treatment. Plastic transplant labels were numbered with both an engraving tool and a black alcohol marker. Several branches broke off during the year, destroying 14 transplants, leaving 66 for the final analysis.

Because growth rates were not skewed within treatment groups, we did not transform the data. We used a Type III sum of squares to accommodate the unbalanced design. We compared growth rates among treatments with analysis of variance (ANOVA). In addition to one-way ANOVA, we used growth rate in the preceding year as a covariate. Post hoc Dunnett's tests were used to contrast individual treatments with controls, applying the test to a one-factor design based on residuals from the covariate.

#### RESULTS AND DISCUSSION

*Lobaria* transplants grew more rapidly with higher doses of inorganic P (Fig. 1, Table 1). These differences were strengthened somewhat by factoring out the previous year's growth, before application of P, as a covariate (Table 2). Biomass growth averaged 14.8% in controls receiving no supplemental P, more than doubling to 32.7% in the transplants receiving a single

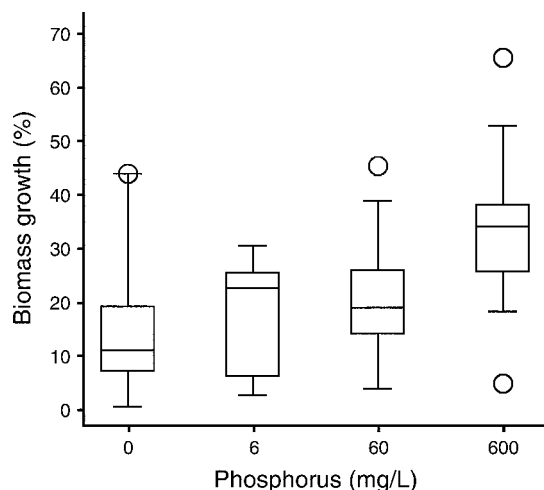


FIG. 1. Annual biomass growth by phosphorus treatment level. Boxplots show the median, interquartile range, 5th and 95th percentiles, and outliers exceeding 1.5 times the interquartile range.

dose of 600 mg/L of P (Table 1). Dunnett's test showed the highest dose to differ from the controls; others were not significantly different, but because of the small sample size, the power is quite low.

The strong relationship between current year's growth and the previous year's growth, apart from their response to P, (Table 2) suggests a carryover in vigor between years. These differences among transplants could be partly genetic and partly environmental. Healthy, vigorous transplants in one year are more likely to be vigorous in the next year. Conversely, transplants with critically low energy reserves or inherently slower growth are likely to express those traits repeatedly.

The fact that a single immersion in elevated-P solution resulted in faster growth effect suggests that the supplemental P was probably retained and recycled within the lichen thallus. Farrar (1976) found that P taken up by *Hypogymnia physodes* was rapidly immobilized, presumably as insoluble polyphosphates for long-term storage. Polyphosphates are polymers of inorganic P with energy-rich phospho-anhydride bonds, similar to ATP (Kulaev 1975). High concentrations of polyphosphate granules occur in the developing lobes of *L. pulmonaria* (Giordani and Brunialti 2002). If the supple-

TABLE 1. Annual biomass growth by phosphorus treatment level.

Phosphorus (mg/L)	No. transplants	Growth (%)	
		Mean	SD
0	11	14.8	13.0
6	9	17.9	10.5
60	21	20.4	11.2
600	25	32.7	12.5

TABLE 2. Results of ANCOVA comparing mean annual biomass growth (%) of *Lobaria pulmonaria* among four levels of phosphorus treatment.

Source	SS	df	MS	F	P
Previous growth	2656	1	2656	26.1	<0.001
Phosphorus	2658	3	886	8.7	<0.001
Error	6218	61	102		
Total	12304	65			

Note: The previous year's growth was used as a covariate.

mental P had been rapidly leached by fall rains, we would not have expected a pronounced growth response.

The surprisingly strong growth response to P fertilization leads to several specific predictions. (1) Cyanolichens should be more abundant in areas with higher P contents in soils or bedrock, all other factors being equal. (2) Fertilizing forests with P should indirectly increase N-fixation rates by stimulating the N-fixing cyanolichens. (3) Re-establishment of old-growth-associated cyanolichens in young forests may be accelerated by application of P, since the old-growth structure per se is not necessary for these cyanolichens to thrive (Sillett et al. 2000a, b). (4) Models of lichen communities in relation to pollutants (Jovan and McCune 2006, Geiser and Neitlich 2007) or topography (Berryman and McCune 2006) can be strengthened by factoring in local P availability, perhaps using P content of standard common species (e.g., see Geiser and Neitlich 2007) as a covariate.

Our results also suggest further research into cyanolichen physiology by exploring Hyvärinen and Crittenden's (2000) suggestion of long-term within-thallus recycling of P. For example, how long can a pulse of P stimulate growth? How do N-fixation rates of cyanolichens respond to supplemental P?

Phosphorus may be as important a stimulant to cyanobacterial-rich lichen communities as it is to cyanobacteria in aquatic ecosystems (e.g., Chorus and Bartram 1999). Lichen communities rich in cyanolichens occur in biotic soil crusts, on mesic rock outcrops and talus, on boreal-forest floors, and in oceanic temperate forests, tropical cloud forests, and tundra. Phosphorus may play a critical role in regulating biomass, diversity, and species composition of these communities.

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