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Dry-hot stress significantly reduced the nitrogenase activity of epiphytic cyanolichen

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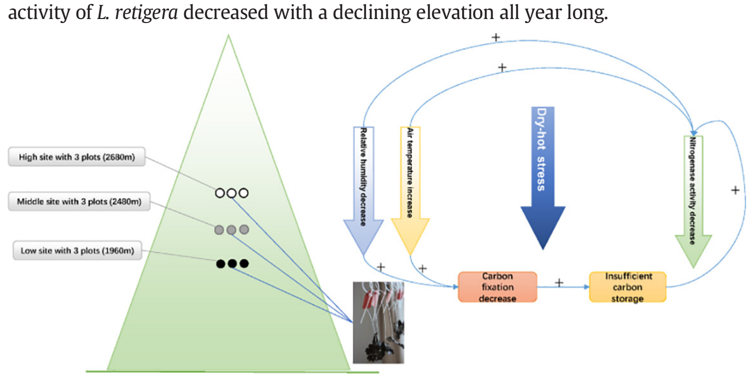
HIGHLIGHTS

- Nitrogen fixed by cyanolichens is an important resource in some forest ecosystems.
- Dry-hot stress reduced the nitrogenase activity of cyanolichens.
- Drought and warming is responsible for reduced nitrogenase activity in the early stage.
- Imbalances in the C budgets will be the ultimate arbiter in later stages.

GRAPHICAL ABSTRACT

We simulated climate change conditions by transplanting *Lobaria retigera*, a common cyanolichen in the area, to lower elevations, and measured nitrogenase activity in response to warmer and drier conditions. The nitrogenase activity of *L. retigera* decreased with a declining elevation all year long.



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ABSTRACT

Nitrogen (N) fixed by epiphytic cyanolichens (i.e. lichens that contain cyanobacterial symbionts) is thought to be the most important resource of this nutrient in some natural forest ecosystems. Although a great deal of work has been carried out to evaluate the biomass of this group as well as its contribution to ecosystem N budgets, empirical studies are needed to confirm the N input responses by cyanolichens under climate change conditions (dry-hot stress) as well as to determine the factors that control this process. We simulated climate change conditions by transplanting *Lobaria retigera*, a common cyanolichen in the area, to lower elevations, and measured nitrogenase activity in response to warmer and drier conditions. In addition, we conducted a series of laboratory and greenhouse experiments to determine the dominant factors influencing nitrogenase activity in this species. The results of this study show that mean annual nitrogenase activity at the higher site was 1.5 and 2.4 times that at the simulated warmer and drier (middle and lower) sites, respectively. Combining laboratory experimental conclusions, we show that thallus water content is a key factor determining the nitrogenase activity of *L. retigera* in early transplantation while insufficient carbon storage resulting from a combination of warming and desiccation was likely responsible for reducing nitrogenase activity in later months of the transplant experiment. The results of this study imply that the negative impact of climate change (dry-hot stress) on ecosystems not only impacts the distribution and growth of species, but also nutrient circles and budgets.

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1. Introduction

The element N most commonly limits net primary production in terrestrial ecosystems (Johnson and Turner, 2014). The main reactive N source in terrestrial ecosystems is inert N_2 gas, comprising 78% of the atmosphere (Marschner and Rengel, 2007). The conversion of N_2 to reactive N for uptake by plants can be due to biological and industrial processes (as well as lightning). Of these processes, biological N fixation (BNF) is responsible for a substantial input of N in many terrestrial ecosystems and has a major impact on its biogeochemical dynamics and ecosystem productivity (Thomas et al., 2006). Previous estimates of global N budgets have suggested that input via BNF accounts for 25% of total fixation, whereas industrial fixation and the combustion of fossil fuels together account for about 75% (Galloway et al., 1995). However, in the absence of industrial input and low deposition of N, the dominant source of reactive N into natural terrestrial ecosystems comes from BNF. In this context the Azotobacter bacteria—which occur in forest floor soils, in plant foliage, in legume root nodules, and in epiphytic lichens and bryophytes—can function as the primary participants in forest ecosystem N fixation. Available estimates, however, suggest that the contribution of free-living bacteria and cyanobacteria-bryophyte symbionts to BNF are small (Vitousek, 1994; Son, 2001; Matzek and Vitousek, 2003). Nonetheless, montane forests will often sustain relatively small numbers of legumes and actinorhizal fixers (Gentry et al., 1995; Crews, 1999) yet support a greater proportion of epiphytes than typically found in other forested ecosystems; the biomass of epiphytes in these forests can rival that of tree foliage (Coxson and Nadkarni, 1995). The biomass of the epiphytes can reach about 44,000 kg ha^{-1}, including 20.4 kg green tissue per tree (Bryophytes,14.77 kg; Lichens, 1.9 kg; Ferns, 2.8 kg; Vascular plants, 1.51 kg) (Hofstede et al., 1993). Meanwhile, the biomass of cyanolichens (*Lobaria oregana*) can reach about 10–15 kg per tree, or approximately 500 kg ha^{-1} in Douglas fir forests (Denison, 1979). Thus, while N fixation by symbiotic vegetation is probably not a major component in montane forests, the N fixed by cyanolichen may represent the major input in those forest ecosystems where they are most abundant (Denison, 1979; Pike et al., 1972; Benner et al., 2007). For example, in New Zealand forest, the N fixed by the cyanolichen genera *Pseudocyphellaria* and *Sticta* can reach 10 $kg \cdot N \cdot ha^{-1} \cdot yr^{-1}$, approximately 5–10-fold the N input from atmospheric deposition (Green et al., 1980). Data show that the N fixed by *Lobaria oregana* in old-growth Douglas fir forests in northeastern Oregon is between 3 $kg \cdot ha^{-1} \cdot yr^{-1}$ and 4 $kg \cdot ha^{-1} \cdot yr^{-1}$, and between 2 $kg \cdot ha^{-1} \cdot yr^{-1}$ and 17 $kg \cdot ha^{-1} \cdot yr^{-1}$ in the Pacific Northwest (Denison, 1979; Antoine, 2004). In addition, the decomposition of epiphytic lichens is faster than that of biological soil crusts and arboreal litter fall (Campbell et al., 2010). As such, these lichens can quickly release nutrients into the surrounding environment and contribute significantly to the N input into forest ecosystems (Pike, 1978; Knops et al., 1996). It follows that determining the rates and controls of cyanolichen BNF under changing ambient conditions is crucial to better understanding the role of these vital cyanolichens in forest ecosystem functioning and in future climate change impacts (Elbert et al., 2012).

While epiphytic cyanolichens may tolerate climatic changes, they are nevertheless sensitive to environmental fluctuations (Nadkarni and Solano, 2002). The nitrogenase activity of these lichens is particularly sensitive to changes in the environment (Belnap, 2001). In the last 100 years, a trend of warming and increasing temperature extremes has been recorded across most regions of Asia that is predicted to continue into the next millennium (Pachauri et al., 2014). In addition, local precipitation varies strongly among different regions and seasons, yet it is reportedly both increasing and decreasing (Pachauri et al., 2014). In Yunnan Province (southwestern China), for example, the mean annual temperature has increased by 1.2 °C over the last 40 years (Fan et al., 2011), whereas the mean annual precipitation is predicted to decrease by 130 mm by the 2050s when compared with current conditions in montane moist evergreen broad-leaved forests (Hijmans et al., 2005; Ramirez and Jarvis, 2008). Therefore, empirical research that monitors the influence of this ensuing 'dry-hot stress' environment on the nitrogenase activity of cyanolichens is necessary.

The available data suggest that water availability or thallus moisture content are the most important abiotic factors influencing N fixation by cyanolichens and cyanobacteria (Nash, 2008), because these organisms are only physiologically active in wet conditions. Ambient temperature is also an important factor that affects the rate of N fixation (Coxson and Kershaw, 1983a), in that BNF is inherently constrained by low temperature, in part due to the optimum of nitrogenase activity, which reaches its maximum efficiency at about 25 °C (Vitousek, 2002; Houlton et al., 2008). Conversely, high temperatures are also known to inhibit the nitrogenase activity of cyanolichens (Hitch and Stewart, 1973). In a study focused on a particular cyanolichen, Antoine (2004) showed that an approximate 10 °C increase in temperature led to a 3–4-fold increase in the *L. oregana* N_2-fixation rate. However, short-term increases in N fixation in response to warming may reflect kinetic responses (Gundale et al., 2012). Other researchers have suggested that long-term warming may result in decreased biomass, abundance and N_2-fixation rate (Lindo et al., 2013). In addition to these direct effects, factors including light, temperature and water supply can each limit nitrogenase activity in an indirect way by influencing both C fixation and consumption processes, as N fixation requires the availability of photosynthetic products (Belnap, 2001). At the same time, evidence suggests that plant productivity and biomass exert stronger controls on N_2-fixation (Lindo et al., 2013). A linear relationship has been demonstrated between biomass production and N fixation in vascular plants (Pimratch et al., 2008), and other studies have shown a similar trend between biomass accumulation and N fixation (Goh and Bruce, 2005). This is mainly because N_2-fixation requires an abundant supply of carbohydrates to meet its high-energy demands. Not surprisingly then, N-fixing organisms are reportedly often abundant in high light environments (Vitousek, 2002; Houlton et al., 2008) where the potential for C accumulation is greatest (Pimratch et al., 2008).

The Ailao Mountains in southwestern China contains one of the largest known tracts of natural evergreen broad-leaved forest, where epiphytic macro- and microlichens are abundant and widely distributed, consisting of 217 species in 76 genera (Li, 2013). Because previous surveys in our field study area have recorded 30 species of epiphytic cyanolichens dominated by *Lobaria retigera* (Li et al., 2011; Li, 2013), this species was selected for further investigation. Based on current research and the distribution of cyanolichens in this region, we hypothesized that (1) The nitrogenase activity of cyanolichens is strongly depressed by a warmer and drier environment, and (2) A decrease in C (energy) to cyanobionts, as driven by reduced lichen growth caused by 1-year warming and drought, should account for any negative impact on the nitrogenase activity of cyanolichens. To test these two hypotheses, we transplanted *L. retigera* at sites at different elevations, as a proxy for estimating the effect of dry-hot stress on N fixation, and conducted laboratory and greenhouse experiments to understand the interacting influences of water supply, temperature, and light on nitrogenase activity.

2. Materials and methods

2.1. Study site

This study was carried out in the Ailao Mountains (23°35′–24°44′N, 100°54′–101°30′E). This forest covers 50,400 ha across elevations that range between approximately 2000 m and 2600 m (Qiu and Xie, 1998; Liu et al., 2002a, 2002b). A mosaic of primary montane forest and secondary forests characterizes this landscape, and a decade of meteorological observations (1996 through 2005) indicated a mean precipitation of 1947 mm, most of which (85%) occurs in rainy season between May and October. The annual mean temperature of this region is 11.3 °C, with average annual evaporation of 1192 mm, and 85% annual