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Epiphytic bryophytes as bio-indicators of atmospheric nitrogen deposition in a subtropical montane cloud forest: Response patterns, mechanism, and critical load☆

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ABSTRACT

Increasing trends of atmospheric nitrogen (N) deposition due to pollution and land-use changes are dramatically altering global biogeochemical cycles. Bryophytes, which are extremely vulnerable to N deposition, often play essential roles in these cycles by contributing to large nutrient pools in boreal and montane forest ecosystems. To interpret the sensitivity of epiphytic bryophytes for N deposition and to determine their critical load (CL) in a subtropical montane cloud forest, community-level, physiological and chemical responses of epiphytic bryophytes were tested in a 2-year field experiment of N additions. The results showed a significant decrease in the cover of the bryophyte communities at an N addition level of 7.4 kg ha^{-1} yr^{-1}, which is consistent with declines in the biomass production, vitality, and net photosynthetic rate responses of two dominant bryophyte species. Given the background N deposition rate of 10.5 kg $ha^{-1}yr^{-1}$ for the study site, a CL of N deposition is therefore estimated as *ca.* 18 kg N ha^{-1} yr^{-1}. A disordered cellular carbon (C) metabolism, including photosynthesis inhibition and ensuing chlorophyll degradation, due to the leakage of magnesium and potassium and corresponding downstream effects, along with direct toxic effects of excessive N additions is suggested as the main mechanism driving the decline of epiphytic bryophytes. Our results confirmed the process of C metabolism and the chemical stability of epiphytic bryophytes are strongly influenced by N addition levels; when coupled to the strong correlations found with the loss of bryophytes, this study provides important and timely evidence on the response mechanisms of bryophytes in an increasingly N-polluted world. In addition, this study underlines a general decline in community heterogeneity and biomass production of epiphytic bryophytes induced by increasing N deposition.

1. Introduction

Nitrogen (N) is an essential macronutrient critical for plant growth and a key element controlling the dynamics of community composition and the functioning of many terrestrial and aquatic ecosystems (Vitousek et al., 1997). However, increased atmospheric N deposition resulting from intensive anthropogenic activities has altered the global N cycle substantially, and been recognized as an important driver that threatens the functioning of ecosystems and their floristic diversity on a global scale (Galloway et al., 2003, 2004; Liu et al., 2013; Pardo et al., 2011; Solga and Frahm, 2006). To protect the ecosystems and their services from air pollution, the concept of critical load (CL) has been widely implemented across Europe and North America (Bobbink et al., 2003; Geiser et al., 2014; Giordani et al., 2014; Mitchell et al., 2005; Pearce et al., 2003), which is defined as the level of pollution below which there are no significant harmful effects on the environment, according to current best knowledge (Nilsson, 1988). In China, a long-term increase in both urban development and the human population is expected during this century, changes that will certainly lead to increased

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anthropogenic emissions of reactive N and thus elevate atmospheric N deposition (Liu et al., 2013). Nevertheless, the lack of empirical studies in the determination of CL implies that the sensitivity and responses of major ecosystem components to N deposition remains unknown (Liu et al., 2011). Although the sources of atmospheric N deposition in recent decades over China have been quantified (Liu et al., 2013), an enhanced level of N deposition and its possible impacts on terrestrial plants and ecosystems, both require careful monitoring.

Increasing concern on the surveillance of elevated N pollution in natural ecosystems calls for timely feedback. Because of the absence of cuticle barrier, bryophytes are very sensitive to environmental change, pollution, nutrient change and ecosystem health, and have been identified reliable for monitoring atmospheric N deposition ranging from highly localized to regional or even global (Bates, 2002; Bobbink et al., 2003; Liu et al., 2008; Zotz and Bader, 2009). Previous works on N deposition as it affects bryophytes have focused on dynamics in peatland, dry grassland, and heathland communities, finding major declines in bryophyte abundance and negative effects on the growth and coverage of particular bryophyte species (Nordin et al., 2005; Pearce et al., 2003; Song et al., 2012; Street et al., 2015). Accordingly, the CLs of N pollution in ecosystems dominated by bryophytes have been determined based on the responses of these plant groups (Bobbink et al., 2003; Geiser et al., 2014; Giordani et al., 2014; Nordin et al., 2005). Physiological responses of bryophytes to elevated N deposition involve alterations in plant N metabolism, such as an increased concentration of N in tissues and an increased allocation of N to photosynthesis, as well as direct N toxicity, which together may induce and hasten membrane leakage, reduce the activity of nitrate reductase, and ultimately shoot death (Arróniz-Crespo et al., 2008; Granath et al., 2009; Limpens et al., 2011; Pearce et al., 2003). In tandem, the decreased productivity associated with impaired photosynthesis—from the intensified biotic interactions and an altered nutrient stoichiometry—point to a strong inhibitory effect of excessive N addition on carbon (C) storage in bryophytes (Bobbink et al., 2010; Du et al., 2014; Limpens et al., 2011). Although previous studies have provided several important insights into the likely causes of bryophyte decline under conditions of increased N deposition, we still lack a detailed mechanistic analysis of such responses at multiple scales (i.e., community, physiological, and chemical).

Previous studies have clearly improved our understanding of N deposition and its ecological impacts on bryophytes; however, many of these were conducted in regions having a high ambient N deposition or used too few elevated N treatments over relatively short time periods (Arróniz-Crespo et al., 2008; Mitchell et al., 2004; Pearce et al., 2003; Salemaa et al., 2008). Recent studies have highlighted that the results derived from the rapid responses of bryophytes to high concentrations of applied N may underestimate its effects occurring at lower concentrations in the real world (Armitage et al., 2012). For example, a previous study in a subtropical montane cloud forest (hereinafter referred to as montane forest) reported a significant decrease in epiphytic bryophyte abundance and growth at locations receiving N additions at 15 kg ha^{-1} yr^{-1} (Song et al., 2012). Accordingly, several sensitive bryophyte species were selected as potential bio-indicators of atmospheric N deposition (Song et al., 2012). However, the data we collected at that time were insufficient to either accurately predict the threshold of N deposition, or to enable a comprehensive evaluation that would provide a plausible explanation for the observed detrimental effects on the epiphytic bryophytes.

Considering these limitations, we carried out a field manipulation experiment in the montane forest of the Ailao Mountain National Nature Reserve (NNR) in southwest (SW) China. Multiple parameters—from the scale of plant tissue up to that of community—were repeatedly measured during the applied N treatments over the course of 2 years. The objectives of this study were to address the physiological and chemical mechanisms driving the decline in bryophytes and to determine the CL for epiphytic bryophytes in a natural forest ecosystem. The specific questions we addressed were: 1) How do community-level, physiological, and chemical variables of epiphytic bryophytes respond to increasing N additions, and what are the relationships among these variables? 2) What are the mechanisms that drive the decline of bryophytes under increased N loads? 3) What is the CL of N deposition for the epiphytic bryophytes in the subtropical montane forest?

Based on the previous studies mentioned above, we formulated three hypotheses: 1) Increasing N doses should have detrimental effects on the community-level, physiological, and chemical variables of the epiphytic bryophytes (Arróniz-Crespo et al., 2008; Pearce et al., 2003; Song et al., 2012), and some of these variables ought to be closely correlated with the bryophyte loss. 2) Increased N loads should cause alternations in plant N metabolism, resulting in an imbalance of C metabolism, and thus driving a decline in the bryophytes (Du et al., 2014). 3) Considering a severe decline in bryophyte cover (and possibly even species extinction) at the additional N deposition rate of 15 kg N ha^{-1} yr^{-1} (Song et al., 2012) set against the background N deposition rate of this region of *ca.* 10.5 kg N $ha^{-1}yr^{-1}$ (Liu et al., 2002), together suggests that the CL of N deposition for epiphytic bryophytes in the montane forest should fall within the range of 10–25 kg N $ha^{-1}yr^{-1}$.

2. Materials and methods

2.1. Study site and experimental design

The field experiment was performed in montane forest in the Xujiaba region of Yunnan Province (24°32′ N, 101°01′ E), situated at the northern crest of the Ailao Mountain NNR, at an altitude range of 2000–2600 m (You, 1983) (Fig. 1). Here, the mean annual temperature is 11.6 °C and the mean annual relative humidity is 84%; the mean annual precipitation is 1859 mm, with 86% of this amount falling in the rainy season from May to October followed by a pronounced dry season (November to April) (Song et al., 2015). The tree species *Castanopsis rufescens* (Hook. f. et Th.) Huang et Y.T. Chang, *Lithocarpus hancei* (Benth.) Rehder, and *Lithocarpus xylocarpus* (Kurz) Markgr. among others, are the main co-dominant trees hosting a diverse community of epiphytes (Ma et al., 2009; Xu and Liu, 2004; You, 1983). The ambient wet N deposition rate at the study site is estimated at 10.5 kg ha^{-1} yr^{-1} (Liu et al., 2002).

In January 2013, five sites (each 2 ha), in the cloud forest in Xujiaba were chosen to conduct the experiment. These sites were located in the same region, at similar altitudes, and they also had similar slopes (Fig. 1). At each site, 10 plots (each 10 m × 10 m) dominated by *Lithocarpus xylocarpus*, *Lithocarpus chintungensis*, *C. rufescens*, and *Schima noronhae*, for which *Plagiochila assamica* Steph. and *Homaliodendron montagneanum* (C. Muell.) Fleisch. were the dominant bryophyte species present on the tree trunks, were randomly assigned to receive N additions at a range of levels. Five trees in each plot (stem diameter at breast height, DBH, > 20 cm) were selected as the target hosts for sampling the bryophyte community.

Based on our previous study (Song et al., 2012), we applied N solutions following a natural exponential increment at 10 doses as follows: 0 (distilled water only), 1.0, 1.7, 2.7, 4.5, 7.4, 12.2, 20.1, 33.1, and 54.6 kg $ha^{-1}yr^{-1}$, so as to observe more nuanced responses and to identify the critical N load for epiphytic bryophytes in the

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