Oecologia
DOI 10.1007/s00442-012-2341-9

GLOBAL CHANGE ECOLOGY - ORIGINAL RESEARCH

Response of epiphytic bryophytes to simulated N deposition in a subtropical montane cloud forest in southwestern China

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Received: 1 September 2011 / Accepted: 16 April 2012

Abstract A field manipulation experiment was conducted in a subtropical montane cloud forest in southwestern China to determine the possible responses of epiphytic bryophytes to increasing nitrogen (N) deposition from community to physiology level, and to find sensitive epiphytic bryophytes that may be used as indicators for assessing the degree of N pollution. N addition had significantly negative effects on species richness and cover of the epiphytic bryophyte community. Harmful effects of high N loads were recorded for chlorophyll, growth, and vitality of the species tested. The decline of some epiphytic bryophytes may result from detrimental effects on degradation to photosynthetic pigments. *Bazzania himalayana* (Mitt.) Schiffn., *Bazzania ovistipula* (Steph.) Mizut., and *Homaliodendron flabellatum* (Sm.) Fleisch. are candidates in atmospheric nitrogen monitoring. Epiphytic bryophytes in the montane cloud forest are very sensitive to increasing N deposition and often difficult to recover once they have been destroyed, providing early detection of enhanced N pollution for trees or even the whole forest ecosystem. The inference that increasing N pollution may lead to loss of biodiversity is a concern to the developing economy in western China, and should alert the government to the adverse impacts caused by increased industrial pollution during the process of China's West Development.

Keywords Biodiversity · Cover · Epiphyte · Pollution · Recovery

Communicated by Meelis Pärtel.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-012-2341-9) contains supplementary material, which is available to authorized users.

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Introduction

Rapid economic development has increased usage and emissions of nitrogen (N) in Asia, especially in China, and is predicted to increase in the future (Zheng et al. 2002; Richter et al. 2005), although N deposition appears to be decreasing slightly in Europe in recent years (EEA Management Plan 2010). This human-driven process may have serious impacts on ecosystems at a global scale (Vitousek et al. 1997). In fact, artificial N enrichment, as a result of chronically accumulating deposition, has already caused a wide range of adverse influences in terrestrial ecosystems (Lü et al. 2007). Excessive N input to forest ecosystems should influence the growth, structure, function, and dynamics of the receiving forests (Li et al. 2003).

Published online: 12 May 2012

There has been increasing concern in recent years about the effects of atmospheric N deposition on lower plants such as bryophytes and lichens, which represent the most sensitive types of vegetation to N pollution (Mitchell et al. 2005; Cape et al. 2009). Although bryophytes exhibit a larger range of sensitivities to pollutants compared with lichens, attention has focused less on the impacts of N pollution on bryophytes (Bates 1994a). In bryophytes, water, nutrients, and even toxic substances are absorbed directly from air and precipitation through the entire plant surface (Zechmeister et al. 2007). Given their typically high leaf area indices and high surface area to volume ratios, the fact that they do not have a rooting system, and lack a cuticular barrier, naturally growing bryophytes are very sensitive to environmental changes and in recent decades have been proposed to be good bio-indicators for assessing the potential effects of a number of contaminants (Farmer et al. 1992; Solga et al. 2005; Liu et al. 2007; Zotz and Bader 2009). Furthermore, bryophytes appear to be particularly sensitive to increased atmospheric N deposition (Pitcairn et al. 1995, 2003; Mitchell et al. 2004).

Bryophytes respond to increased N deposition mainly through changes in community composition, changes in growth, and changes in physiological function (Mitchell et al. 2004). Detailed studies have indicated that enhanced N input alter species abundance and growth of certain bryophyte species (Bergamini and Pauli 2001; Mitchell et al. 2004; Curtis et al. 2005), which may be attributed to physiological and chemical responses, such as change of tissue N (Solga et al. 2005; Wilson et al. 2009), chlorophyll concentrations (Baxter et al. 1992; Bignal et al. 2008), and enzyme activity of plant tissues (Soares and Pearson 1997). Some species may ultimately decrease in coverage or even die back under high N deposition (Pitcairn et al. 2003; Mitchell et al. 2005). However, previous studies have mainly focused on just one or two aspects of the above influences with few integrating the possible mechanisms for those changes. Furthermore, a core issue concerning the effects of N deposition is the re-establishment rate of the original flora following decreases in N input (Strengbom et al. 2001), but few studies have focused on the recovery process of bryophyte communities after the cessation of N addition.

Mitchell et al. (2004) carried out an interesting study to assess the potential effects of increased or decreased N deposition on growth and tissue N of epiphytic bryophytes by reciprocal transplantation in Atlantic oak woods; however, the result may be confounded by the transplantation effect and habitat difference (e.g., climate, terrain, host) between sites. A field manipulation experiment was conducted to study the effects of N deposition on epiphytic bryophytes in a subtropical montane cloud forest (MCF) in Ailao Mountain National Nature Reserve in southwestern China. There are several important reasons that impel us to undertake this study. Firstly, atmospheric N deposition is expected to accelerate with rapid economic development in China (Richter et al. 2005). Industrial pollution, particularly in western China, is becoming more severe due to both low efficiency of the traditional industry and pollution industry transmission from developed areas during China's Western Development (Gu 2010). The detrimental ecological outcomes that this policy is likely to have in western China have not yet been reported. Secondly, few studies have looked at the impacts of enhanced N deposition on epiphytic bryophytes, despite their being much more sensitive to atmospheric N pollution compared with ground-rooted plants (Benzing 1998; Hietz 1998). Thirdly, epiphytic bryophytes play an important role in the biodiversity, biomass, and nutrient cycling of the whole forest ecosystem in the Ailao Mountains (Ma et al. 2009; Chen et al. 2010; Han et al. 2010).

The aims of this study were to: (1) assess the potential response of epiphytic bryophytes to simulated increases in N deposition, from the community level down to the impact on physiology (including species richness and cover, growth and vitality, concentrations of tissue C, N, and P, and chlorophyll content) in the MCF; (2) reveal the possible mechanisms underlying those responses; (3) find sensitive epiphytic bryophytes which may be used as indicators for assessing the degree of N pollution in the future; and (4) assess the recovery rate of bryophyte communities after cessation of N addition.

Materials and methods

Study site

We conducted our research in the MCF at Xujiaba region (24°32′N, 101°01′E), a core area of the Ailao Mountain National Nature Reserve (Fig. S1). The MCF usually occur as "islands" of evergreen forests on mountain tops with altitudes higher than 2,600 m (Shi and Zhu 2009). The forest is mainly influenced by the southwest monsoon and exposed to frequent and intense wind and mist events throughout the year. Branch and trunk surfaces of nearly all mature trees support abundant epiphytes (mainly bryophytes) in interwoven root–humus mats up to 10 cm thick, and thus the MCF is commonly called "top mountain dwarf mossy forest" (Shi and Zhu 2009). In total, 89 epiphytic bryophytes have been recorded from 26 families and 47 genera, of which *Plagiochila arbuscula* (Brid. ex Lehm. et Lindenb.) Lindenb., *Sinskea phaea* (Mitt.) Buck., and *Homaliodendron flabellatum* (Sm.) Fleisch. appear to be dominant species (Ma 2009). Details of the meteorology and structure of the forest can be found in Chen et al. (2010).

Experimental treatment

We measured total N input at 10–15 kg N ha^{-1} $year^{-1}$ in the study region in a former study (Liu et al. 2002), with expectations of increased N input with time. In addition, rates of wet N deposition have reached 30–73 kg N ha^{-1} $year^{-1}$ in some forests in southern China due to anthropological activities (Lu et al. 2010). Based on these figures, we implemented the following treatment levels in our simulations of N input with three replicates of each: control (no N addition), low N (15 kg N ha^{-1} $year^{-1}$), medium N (30 kg N ha^{-1} $year^{-1}$), and high N (60 kg N ha^{-1} $year^{-1}$). In total, we established and marked 12 representative plots of 100 m^2 (10 × 10 m), each surrounded by a 10-m-wide buffer strip to avoid mutual interference. These plots were located in the same region at similar altitudes and slopes. We laid out the field treatments randomly. To provide more reasonable realistic response of epiphytic bryophytes to N, we sprayed NH_4NO_3 solution (NH_4NO_3 dissolved in 5 L distilled water) monthly, from April 2009 to March 2010 (maximum 5 kg N ha^{-1} $month^{-1}$), rather than large, pulsed doses used in some experiments in the past (Fog 1988). We carried out spraying on foot, using a backpack sprayer held at roughly 1.5 m above the forest floor of each 100 m^2 plot (Mo et al. 2007). We sprayed each of the control plots with 5 L of water only. The concentrations of the NH_4NO_3 solutions used for the control, low, medium, and high N groups were 0, 0.09, 0.18, and 0.36 M in each application, respectively. We also investigated recovery of bryophyte communities in each plots in the year after N treatment (April 2011).

Sampling and measurement

Species richness and cover

We applied a nested design (plot nested within N treatment) to examine the response of species richness and cover of the epiphytic community to enhanced N deposition. Selection of hosts was critical because the diversity of epiphytic species appeared to vary with variables such as tree size, tree species, location, and direction of the quadrat sampled (González-Mancebo et al. 2003; Song et al. 2011). In total, we selected 36 *Rhododendron irroratum* (three hosts from each 100 m^2 plot) with diameter at breast height ranging from 6 to 8 cm, the most common size in this region (Chen et al. 2010). We investigated epiphytic bryophytes growing on trunks of the selected trees at 1–1.5 m southwest using 10 × 40 cm metal-frame quadrats with 256 standard square-shaped grid cells (1.25 × 1.25 cm each) equal in size (Song et al. 2011). We marked the position of each quadrat at the four corners of each rectangular frame using small red plastic sheets, which we fixed with thumbtacks, before applying N solution. In each quadrat, we recorded both total cover of all epiphytic bryophytes and a detailed cover of each species (calculated as percent of grids occupied by the species in the 256 grid cells of the quadrat) in April 2009 (before N treatment), April 2010 (at the end of N treatment), and April 2011 (1 year after N treatment ceased). Nomenclature follows Gao and Cao (2000) for liverworts and Li (2002, 2005) for mosses.

Growth measurement and vitality rating

We also applied a nested design to examine the growth and vitality of the trunk-dwelling epiphytic liverwort, *Plagiochila arbuscula*, which is the most common epiphytic species in the study region (Ma 2009), in response to each N treatment. In total, we tagged 96 healthy shoots (eight shoots per 100 m^2 plot) of *P. arbuscula* at around 10 mm from the apex of each shoot with a single thread of embroidery cotton before N addition in April 2009 (Mitchell et al. 2004). In April 2010 (at the end of N treatment), we measured the shoots again according to the tags placed the previous year using calipers. We rated the vitality of all shoots on the proportion of brown coloration, as indication of the health of the shoot: 0, mostly brown, dead looking (more than 90 % brown); 1, partially brown or die back (10–90 % brown); 2, healthy and green (less than 10 % brown).

Tissue C, N, and P measurement

We selected two common epiphytic bryophytes, *Plagiochila assamica* Steph. and *Dicranum japonicum* Mitt., to study the response of tissue carbon (C), N, and phosphorous (P) concentrations to enriched N deposition as they were abundant on trunks and easy to sample in the field. We collected samples of these two species in April 2009 before the initial addition of N, and again in April 2010, at the end of N treatment. We sorted the collected samples to first obtain a pure healthy sample of the desired species, and then washed with deionized water to remove any dry N deposition on the surface of the samples (Leith et al. 2008). We took three samples (one mixed sample per plot) of each treatment for each epiphytic species selected from respective mixed samples collected from ten samples within each plot. We then oven-dried the samples at 70 °C for 48 h and hammer-milled to less than 0.8 mm (Mitchell et al. 2004). We analyzed total C and N concentration using an auto-analyzer (Vario MAX CN; Elementar Analysensststeme, Germany). We determined total P concentration by digestion in HNO_3–$HClO_4$, dissolved in HCl, and analyzed using an ICP-AES (Thermo Jarrel Ash, USA).

Chlorophyll extraction and measurement

We extracted and measured chlorophyll following the method of Barnes et al. (1992) (see also Granath et al. 2009 for the use of dimethyl sulfoxide in bryophyte studies; see Appendix S1 for more details). We sampled one healthy shoot of each target species (*P. assamica* and *D. japonicum*) in each plot for chlorophyll measurement both before and after N treatment. We used Barnes's equations (Barnes et al. 1992) to calculate chlorophyll contents. In addition, we calculated the phaeophytinization quotient (PQ) as absorbance values of the extracts at 435 nm divided by that at 415 nm, which was used to quantify degradation of chlorophyll as it decreased under stress (Bignal et al. 2008).

Statistical analysis

We subjected all data to normality and homoscedasticity tests before statistical analysis. We analyzed health data by nonparametric statistical methods (Kruskal–Wallis *H*) because they were categorical. We transformed cover (arcsine transformation) to meet the assumption of variance homogeneity before further statistical testing. We conducted repeated measure ANOVAs on all the repeated observations. We conducted comparisons of species richness, cover, growth and vitality of *Plagiochila arbuscula*, and chlorophyll content of *Plagiochila assamica* and *Dicranum japonicum* between different N levels within observation periods using GLM or one-way ANOVA with LSD's or Game-Howell's post hoc tests. We conducted all analyses using SPSS 13.0 (SPSS, Chicago, IL, USA).

Results

Species richness, cover, and species composition

We found 46 epiphytic bryophytes (22 mosses and 24 liverworts) belonging to 24 genera and 16 families on all 36 sampled trunks. The most common moss families sampled in our study site were Meteoriaceae (8 species) and Sematophyllaceae (4 species), and Plagiochilaceae (8 species) and Lepidoziaceae (5 species) were the most common liverwort families.

Repeated measure ANOVAs indicated that there were significant effects of N treatment and time, and also a significant interaction between N treatment and time, on species richness and cover of epiphytic bryophytes growing on host trunks of *Rhododendron irroratum* (Table 1). Before treatment (April 2009), we found no significant difference in species richness ($F_{3,24} = 2.480$, $P = 0.085$) and total cover ($F_{3,24} = 2.341$, $P = 0.099$) among different N treatment groups. After 12 months of N addition, an

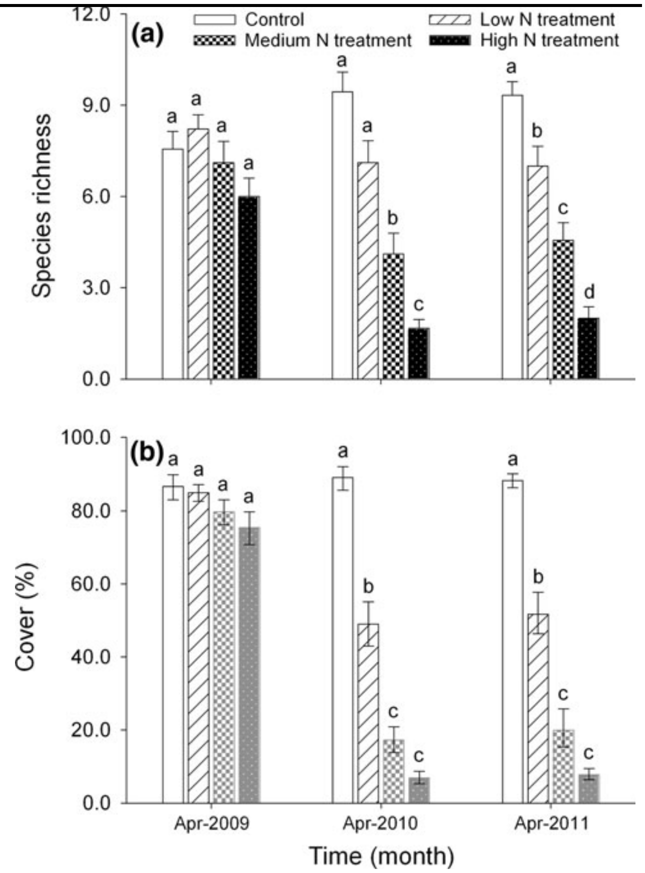

Fig. 1 Treatment effects of nitrogen solutions on **a** species richness and **b** cover of epiphytic bryophytes (mean ± SE, $n = 9$ per treatment). *Letters* above *columns* indicate significant differences for $\alpha = 0.05$

average of 1.9 new species were counted and bryophyte cover increased by 2.2 % in the quadrats of the control group. However, even low N treatment (i.e., 15 kg N ha^{-1} $year^{-1}$) resulted in a remarkable decline in both species richness and cover. More bryophyte species disappeared along with increasing concentrations of N addition. High N deposition killed almost all epiphytic bryophytes with a very low cover (mean 6.9 %) recorded from the few remaining species (Fig. 1).

Epiphytic bryophytes in the control plots grew well after regular spraying of water for 12 months; most species kept their cover, and some new species such as *Syrrhopodon gardneri* (Hook.) Schwaegr. and *Herbertus fragilis* (Steph.) Herz. appeared in the plots. *Bazzania himalayana* (Mitt.) Schiffn., *H. fragilis*, *Homaliodendron flabellatum*, *Bazzania ovistipula* (Steph.) Mizut., *Porella perrottetiana* (Mont.) Trev., and *Sinskea phaea* disappeared after low N addition for one year. Cover of *Plagiochila arbuscula* and *Homaliodendron scalpellifolium* (Mitt.) Fleisch. decreased remarkably under low N addition, and disappeared completely under medium N addition. Though average cover of *Dicranum japonicum*, *Wijkia deflexifolia* (Ren. et Card.)

Table 1 Results of repeated measure ANOVAs for species richness (*A*) and cover (*B*) of epiphytic bryophyte communities and growth of *Plagiochila arbuscula* (*C*) under different N treatment levels from April 2009 to April 2010

Source	*df*	MS	*F*	*P*
(A) Species richness				
N treatment	3	84.61	16.69	<0.001
Error: N treatment × replication	24	5.07		
Time	1	43.56	53.15	<0.001
N treatment × time	3	30.19	36.84	<0.001
Error: N treatment × time × replication	24	0.82		
(B) Cover				
N treatment	3	0.73	53.52	<0.001
Error: N treatment × replication	24	0.01		
Time	1	2.90	302.46	<0.001
N treatment × time	3	0.44	45.96	<0.001
Error: N treatment × time × replication	24	0.01		
(C) Shoot length of *Plagiochila arbuscula*				
N treatment	3	141.95	16.54	<0.001
Error: N treatment × replication	84	8.58		
Time	1	3,504.05	592.35	<0.001
N treatment × time	3	123.49	20.88	<0.001
Error: N treatment × time × replication	84	5.92		

df Degrees of freedom, *MS* mean square

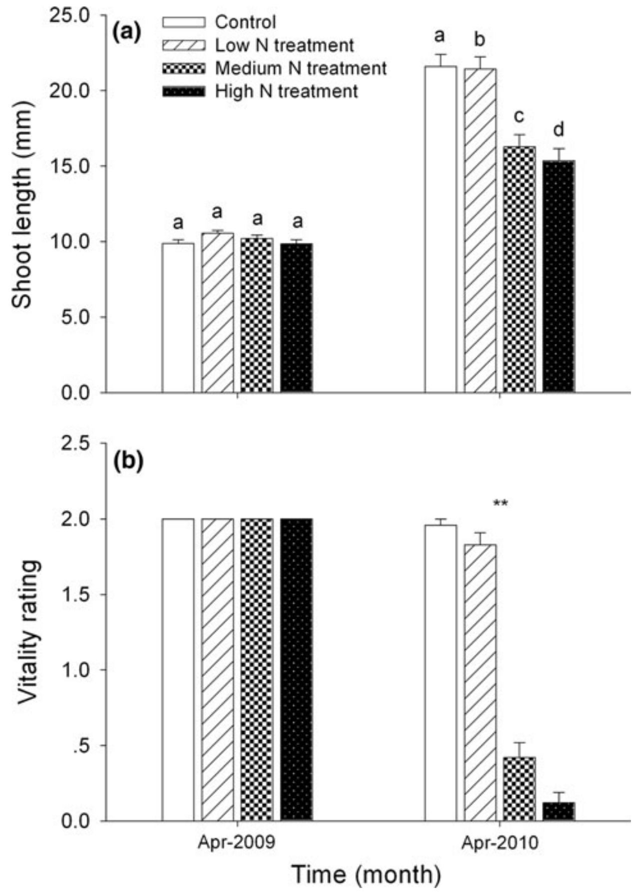

Fig. 2 Treatment effects of nitrogen solutions on **a** growth and **b** vitality rating of *Plagiochila arbuscula* (mean ± SE, $n = 24$ per treatment). *Letters* above *columns* indicate significant differences for $\alpha = 0.05$. **Significant difference of nonparametric test (Kruskal–Wallis *H*) for $\alpha = 0.01$

Crum, *Plagiochila assamica*, *Plagiochila fruticosa* Mitt. had declined to a certain degree, they survived under medium N addition. Nevertheless, no bryophytes with an average cover higher than 2 % were found under high N addition (Table S1).

Comparison of species survey data between April 2010 and April 2011 indicated that epiphytic bryophytes had not recovered 1 year after N application ceased. We found no significant difference in species richness (Repeated measure ANOVA $F_{3,24} = 1.633$, $P = 0.213$) and cover (repeated measures ANOVA $F_{3,24} = 1.512$, $P = 0.231$) between April 2010 and April 2011. Most of the epiphytic bryophytes retained their cover 1 year after N spraying ceased, except *Wikia deflexifolia*, which disappeared from plots treated with low N addition, and a new species, *S. phaea*, recovered in the quadrats of low, medium, and high N groups (Table S1). *S. gardneri* disappeared from the control plots, while *Lejeunea subacuta* Mitt. appeared as a new species (Table S1).

Growth and vitality

Repeated measures ANOVAs indicated significant effects of N treatment and time, as well as a significant interaction between N treatment and time, on growth (shoot length) of *P. arbuscula* (Table 1). Over the year of N addition, *P. arbuscula* grew an average of 12 mm in the control group, while medium and high N treatments resulted in significantly slower growth of *P. arbuscula* compared to the control group (Fig. 2).

While all sampled *P. arbuscula* were healthy and green before treatment, the addition of N had a significant negative effect on the vitality of *P. arbuscula* (Kruskal–Wallis $\chi^2 = 80.033$, $P < 0.001$). We found samples of *P. arbuscula* in the plots of control and low N treatment were still in good condition (vitality >1.8), but samples under the medium and high N treatments were often in poor condition (vitality <0.5) at the end of the 12-month period of N addition (Fig. 2).

Tissue C, N, and P

Repeated measures ANOVAs showed that the effect of N treatment on concentrations of tissue C, N, P, and N/P ratio of *P. assamica* and *D. japonicum* were not significant (Table S2). Before treatment, there was no significant difference in tissue C ($F_{3,8} = 0.180$, $P = 0.907$; $F_{3,8} = 0.931$, $P = 0.469$), N ($F_{3,8} = 0.342$, $P = 0.796$; $F_{3,8} = 0.197$, $P = 0.896$), P ($F_{3,8} = 1.134$, $P = 0.392$; $F_{3,8} = 1.813$, $P = 0.223$), and N/P ratio ($F_{3,8} = 0.099$, $P = 0.958$; $F_{3,8} = 1.294$, $P = 0.341$) among plots of different N treatments for both *P. assamica* and *D. japonicum*. Over the year of N addition, concentrations of tissue C, N, P, or N/P ratio among the N treatments were not significantly different in either species (Table S3).

Chlorophyll

Repeated measure ANOVAs showed a significant effect of N treatment on PQ value of *P. assamica* (Table S4). Prior to treatment (April 2009), there was no significant difference in chlorophyll *a* ($F_{3,8} = 1.615$, $P = 0.261$; $F_{3,8} = 0.581$, $P = 0.644$), *b* ($F_{3,8} = 0.084$, $P = 0.967$; $F_{3,8} = 0.905$, $P = 0.480$), $a + b$ ($F_{3,8} = 0.694$, $P = 0.581$; $F_{3,8} = 0.633$, $P = 0.614$) content or the PQ value ($F_{3,8} = 3.056$, $P = 0.092$; $F_{3,8} = 1.360$, $P = 0.323$) in the tissues of *P. assamica* and *D. japonicum* among plots of different N treatments. After 1 year of N addition the average chlorophyll *a* content of *P. assamica* under high N addition was significantly lower than that in the control group (Fig. 3a). Values of PQ in the high N groups were significantly lower than that in the control group for both species (Fig. 3d).

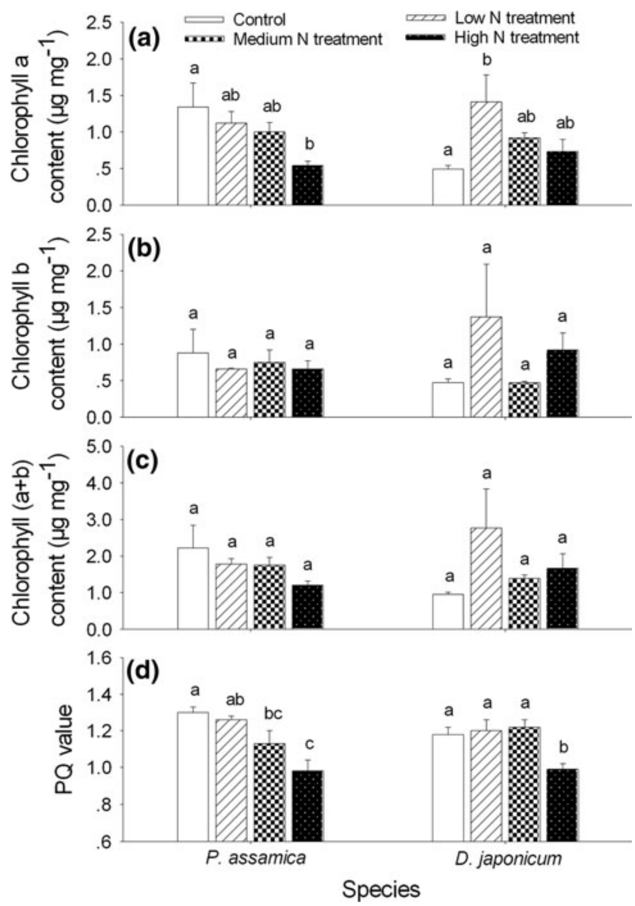

Fig. 3 Multiple comparisons of contents of **a** chlorophyll a, **b** chlorophyll *b*, **c** chlorophyll ($a + b$), and **d** PQ values of *Plagiochila assamica* and *Dicranum japonicum* (mean ± SE, $n = 3$ per treatment) after 1 year of N treatment (in April 2010). Chlorophyll contents are expressed as µg chlorophyll per mg air-dry weight of shoot. *Letters* above *columns* indicate significant differences for $\alpha = 0.05$

Discussion

Effects of N enrichment

In this study, species richness and cover of epiphytic bryophytes were negatively related to the concentration of simulated N addition; even low N addition (15 kg N ha^{-1} year^{-1}) resulted in a remarkable decline in cover and species richness in the MCF (Table 1; Fig. 1). However, in a tropical forest located at a similar latitude in southern China, even medium levels of N addition (100 kg N ha^{-1} year^{-1}) did not alter the overall diversity of terrestrial plants over 5 years (Lu et al. 2010). Another study indicated that seedling growth of two tropical tree species exhibited positive effects under low N addition (50 kg N ha^{-1} year^{-1}) (Mo et al. 2008). The sharp contrast above demonstrates that epiphytic bryophytes are much more sensitive to N pollution than terrestrial trees. Although some bryophytes may benefit from N supply at certain rates (Gordon et al. 2001), many bryophytes, epiphytic species as well as grassland and wetland species, respond to excessive N input in a similar way as the epiphytic bryophytes studied here. For example, in a shrub heath in subarctic Sweden, the addition of 100 kg ha^{-1} year^{-1} of N and P reduced total bryophyte cover by 50 % (Potter et al. 1995). Within a montane heath in the UK, N additions had a highly detrimental effect on *Racomitrium* cover after 5 years of treatment (Pearce and van der Wal 2002). The decline of bryophyte diversity with N addition was also observed in an arctic dwarf shrub heath (Press et al. 1998) and within a pine woodland (Strengbom et al. 2001). All the above studies demonstrate the detrimental effects of N deposition on the abundance and richness of bryophyte communities.

Usually, enhanced N deposition is deleterious to the growth and vitality of many bryophyte species. Results in this study indicated that relatively high N additions (30 kg N ha^{-1} year^{-1}) had a significant negative impact on the growth and vitality of *P. arbuscula* (Table 1; Fig. 2). Similarly, the growth rates of *Hylocomium* *splendens* and *Pleurozium schreberi* were strongly reduced by the addition of similar concentrations of N (Dirkse and Martakis 1992), as was that of species such as *Isothecium myosuroides*, *Dicranum scoparium*, and *Frullania tamarisci* following an increase in atmospheric N deposition (Mitchell et al. 2004). Similar results have been reported in other bryophyte species (Pearce and van der Wal 2002; Pearce et al. 2003). Although the growth of *Pseudoscleropodium purum* was significantly stimulated by a nutrient pulse, this species turned out to be an opportunist and depended on unpredictable nutrient input (Bates 1994b).

Studies using bryophytes to monitor N deposition have generally shown that the tissue N concentration of the plants accurately reflects actual or predicted rates of atmospheric N deposition (Pitcairn et al. 2003; Solga and Frahm 2006; Solga et al. 2006; Wilson et al. 2009). However, no significant effect of N treatments on concentrations of tissue C, N, P, and N/P ratio were detected in either *P. assamica* or *D. japonicum* (Table S2), and many other studies reported similar results (Arróniz-Crespo et al. 2008). No relationship between N deposition and tissue N concentration was found for the moss species *Rhytidiadelphus squarrosus* (Arróniz-Crespo et al. 2008). Mitchell et al. (2004) also found that increasing N input had no effect on the tissue N in an epiphytic bryophyte *Ulota crispa*. Tissue N, and the N:P ratio of bryophytes are not necessarily higher in the heavier N addition groups because bryophytes may already be N saturated under low rates of N deposition (Arróniz-Crespo et al. 2008), and they could not absorb any more extra N. Relying on absolute concentrations of tissue N in epiphytic bryophytes may therefore not be useful for determining N deposition or ecosystem N status in the subtropical MCF studied here.

Possible mechanisms

Added N may have an indirect negative effect on bryophytes because increased shrub and graminoid growth in response to N fertilization resulted in most bryophytes being shaded out (Press et al. 1998; Pearce and van der Wal 2002). However, epiphytic bryophytes need not compete with ground-rooted shrubs and graminoids for resources because they live on tree trunks or branches (Mitchell et al. 2004). Other arboreal plants such as vascular epiphytes or epiphytic algae may benefit from increased N deposition and hence put competitive pressure on bryophytes (Limpens et al. 2003), but these issues are not relevant to this study because very little algae or vascular epiphytes have been observed on trunks throughout the experimental period. Decline of epiphytic bryophytes may result from direct toxicity of excess N input. Some bryophyte species were severe damaged by toxic effects of superfluous ammonium ion (Solga and Frahm 2006), and excessive N addition can induce biochemical distortions (Nordin et al. 2005). In the bryophyte species *Racomitrium lanuginosum*, increased N caused potassium leakage from cells where loss of membrane integrity resulted from N toxicity (Pearce et al. 2003).

This study shows that relatively high N input may degrade chlorophyll and hence hinder photosynthesis for some sensitive epiphytic bryophytes. Normally, when N input increases slightly, N may be taken up, and then used to enhance the chlorophyll content of the cell, thus enlarging photosynthetic capacity (Baxter et al. 1992; Limpens and Berendse 2003). However, excessive N loads are detrimental to chlorophyll, inducing an adverse effect on the photosynthetic system, and thus net photosynthesis is reduced (Van Der Heijden et al. 2000). Here, the PQ of *P. assamica* was significantly negatively affected by N treatment (Fig 3; Table S4), which is an indication of chlorophyll degradation (Bignal et al. 2008). The actual concentrations of NH_4NO_3 solutions used for the high N plots were 0.36 M, which may be the primary stress on the bryophytes here. Direct deleterious effects of excess N on the physiology of some epiphytic bryophytes may be one of the mechanisms that explain negative impacts of increased atmospheric N deposition on them. Nevertheless, the role that other potential mechanisms play in detrimental effects of high N loads calls for further work.

Recovery of epiphytic bryophyte

Previous researchers have documented that epiphytic bryophytes would be very difficult to recover once they have been destroyed or extirpated from a site (Acebey et al. 2003; Zotz and Bader 2009; Song et al. 2011). Strengbom et al. (2001) found that N induced decreases in the abundance of specific bryophytes may persist even after N input has been terminated. After 12 months of recovering from excess N input, minimal changes in species richness, cover, and species composition of trunk-dwelling epiphytic bryophytes were detected. This indicates that the epiphytic bryophytes studied here are vulnerable to high N input over a short period of time, but that recovery over the same period is difficult. Considering the N that accumulated on the tree trunks during N treatments would have been washed off by rainfalls within a few weeks after the treatments ceased, excessive N in the microhabitat is unlikely to be the cause of the absence of recovery. The lack of bryophytes propagules and dispersal limitation are also unlikely to be the main causes because abundant epiphytic bryophytes in the neighborhood without N treatments provide plentiful propagules. A possible explanation might be an irreversible change in conditions for establishment due to the development of the microbial community on barks (During and van Tooren 1987).

Differences in sensitivity of epiphytes in response to N addition

Our results showed that species with high abundance, such as *Bazzania himalayana*, *B. ovistipula*, and *Homaliodendron flabellatum*, disappeared completely from quadrats of low N treatment, suggesting that these species were so sensitive to N loads that slight increases in atmospheric N pollution may kill them. The disappearance of *Porella perrottetiana* from quadrats of low N treatment may be attributed either to its sensitivity to N deposition or from an unknown cause, as they were recorded from a single treatment and had relatively low cover. Interestingly, the reappearance of *Sinskea phaea* in the low, medium, and high N quadrats 1 year after N treatment ceased, might imply a strong dispersal ability. The fact that *Plagiochila arbuscula* and *Homaliodendron scalpellifolium* died back dramatically under low N addition and disappeared under medium N addition indicates they cannot withstand excessive N input. In contrast, the fact that part of *Dicranum japonicum*, *Wijkia deflexifolia*, *P. assamica*, and *P. fruticosa* could survive under medium N addition indicates that they may be tolerant to increased N input to a certain degree. The variation in tolerances amongst epiphytic bryophyte species to N addition in the MCF of southwestern China demonstrates that they should not be treated as a single group when considering susceptibility to changing conditions in N deposition. Nevertheless, only a few bryophyte species survived under high N treatment, which demonstrates that the epiphytic bryophyte community is vulnerable to high N deposition.

Implication for management

Epiphytic bryophytes are highly sensitive to N addition and often difficult to recover once they have been destroyed from a locality, and they appear in many cases to be more vulnerable than co-occurring plants such as the forest trees that support them. In this preliminary exploration looking at how a epiphytic bryophyte community responds to enhanced N input, we found several bryophyte species, *B. himalayana*, *B. ovistipula*, and *H. flabellatum*, to be very sensitive to increasing N deposition with potential as candidates in atmospheric nitrogen monitoring. However, more quantitative data over a larger scale is needed if we want to fully understand the potentiality of these epiphytic bryophyte species as bioindicators.

In this study, we determine that excess N deposition significantly reduces cover and species richness of epiphytic bryophytes in a subtropical MCF in southwestern China. As epiphytic bryophytes play an important role in the MCF (Chen et al. 2010; Ma 2009; Han et al. 2010), increasing N deposition may result in serious ecological consequences to the whole forest ecosystem. Considering excess N input is projected to be a serious threat to biodiversity (Lu et al. 2010), the risk of irreversible damage to this biodiversity hotspot seems inevitable if industrial pollution is not properly managed during China's Western Development phase. We need to appreciate the uniqueness of the biodiversity of western China, and, that it represents a priceless treasure worthy of conservation and preservation for future generations.

Acknowledgments This study was supported by the National Natural Science Foundation of China (No. U1133605, 30870437), the QCAS Biotechnology Fund (No. GJHZ1130), and Knowledge Innovation Program of Chinese Academy of Science (No. KSCX2-YW-Z-1019). The Management Authority of Ailao Mts. National Nature Reserve and the Ecological Station of Ailao Mts. are thanked for granting permission and facilitating this research. Ms Fu Yun et al. are particularly thanked for their assistance with chemical analysis of bryophyte samples. The authors declare that they have no conflict of interest and that the experiment complies with the current laws of China. We wish to thank Mr. Jia-Fu Wu for preparing Fig. S1. We are also grateful to two anonymous reviewers, as well as Dr. Katherine Gross, Dr. Meelis Pärtel, Dr. Yong-Jiang Zhang, Mr. James Shevock, Dr. Zheng-Hong Tan, Dr. Pelin Kayaalp, and Mrs. Rene Wilson for their constructive comments and corrections to the manuscript.

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