Cryptogamie, Bryologie, 2014, 35 (2): 165-172

Responses of non-structural carbohydrate levels of *Polytrichastrum formosum* and the co-occurring understory fern *Parathelypteris nipponica* to different gap creations by thinning in a dense spruce plantation

Zhe WANG[a, b, c], *Weikai BAO*[a*], *Xiaoli YAN*[a], *Honghui LIN*[b]

[a] *Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization,*
Chengdu Institute of Biology, Chinese Academy of Sciences,
Chengdu 610041, China

[b] *Ministry of Education Key Laboratory for Bio-Resource and Eco-environment,*
College of Life Science, Sichuan University, Chengdu 610064, China

[c] *University of Chinese Academy of Sciences, Beijing 100049, China*

Abstract – Effect of the forest gap on the physiology of forest-floor plants remains poorly understood. The present work explored the responses of a moss, *Polytrichastrum formosum* (PF), and a co-occurring fern, *Parathelypteris nipponica* (PN), to four treatments (control and three gap creations with size at 74 m^2, 109 m^2 and 196 m^2 three replicates each treatment) in a 30-year-old spruce (*Picea asperata*) plantation forest according to the levels and components of non-structural carbohydrates (NSC). Our study revealed that PF significantly presented higher NSC levels at gaps than at the control. But the concentrations of starch and NSC for PN were lower at those gaps than at the control. The result confirmed the shade-tolerant moss PF can benefit from the varied environmental conditions after gap creation. In contrast, the disturbance might inhibit the photosynthesis of PN or increase its NSC translocation rate from source to sink organs. In summary, PF and PN had distinct responses of NSC levels to the gap formation for their different eco-physiological characteristics.

Forest floor plant / Gap formation / Light radiation / Polytrichaceae / Shade-tolerant moss

INTRODUCTION

The growth, development and survival of bryophytes and ferns are closely related to the circumstances (Vanderpoorten & Goffinet, 2009; Walker & Sharpe, 2010). Compared with seed plants, they are highly sensitive to varied environmental factors because of the lack of protective cuticles, low growth rate, poor control of water loss and the water-dependent life cycle (Page, 2002; Glime, 2007). Extreme irradiance, temperature and water stress may lead to cell structure damage, enzyme denaturation, pigment destruction, and negative photosynthetic gain (Glime, 2007). Researches in laboratories have revealed that both moss and

---

* Corresponding author : baowk@cib.ac.cn

doi/10.7872/cryb.v35.iss2.2014.165

fern plants can adjust their metabolic process rapidly to relieve the impact of environmental change (Vanderpoorten & Goffinet, 2009; Walker & Sharpe, 2010). However, previous studies only focused on controlled experiments of single- or two- environmental factors and few investigations examined the physiological responses of these plants to the habitat disturbance under natural conditions.

Gap creation in dense forests is an important method of forest management which can alter the ambient conditions such as light intensity, air temperature, vapor pressure deficit and diurnal patterns (Rambo & North, 2009). Hence, it has great impact on the growth performance of understory plants (Kariuki *et al.*, 2006). Thomas (1998) indicated that forest gap formation allows the understory plants to escape the severe shading environment and promotes their development. Furthermore, Dalling *et al.* (2004) found that the maximum net photosynthetic rates of either shade-tolerant or pioneer tree species were significantly correlated with gap size. Nevertheless, relevant studies on either bryophyte or fern were rare and mostly focused on biodiversity, community abundance and structure (Rambo & Muir, 1998; Fenton & Frego, 2005; Nelson & Halpern, 2005; Shields *et al.*, 2007). The effects of gap creation on the physiological status of these plants are still open questions.

The present study aimed to reveal and compare the responses of moss and fern to gap thinning according to its non-structural carbohydrate (NSC) level. NSC is the greatest fraction of the mobile carbon pool of plants which indicates the carbon 'fuelling' status and reflects the balance between carbon uptake (source) and investment (sink) (Hoch *et al.*, 2003; Körner, 2003; Würth *et al.*, 2004). The authors selected two forest-floor plants as investigated subjects, the moss *Polytrichastrum formosum* (Hedw.) G.L. Smith (PF) and a co-occurring fern *Parathelypteris nipponica* (Franch. *et* Sav.) Ching (PN). We deduced the limiting factor for understory plants in the dense plantation would be irradiance (light intensity and photoperiod). Previous studies reported that the 95% light saturation values of relative electron transfer rate (RETR) are approximate to full sunlight for the Polytrichaceae species (Marschall & Proctor, 2004) but only about 2% to 30% for shade-adapted ferns (Proctor, 2012). Therefore, we presumed that gap creation could improve the net photosynthetic rate of PF and thus increase its NSC level. In addition, the highest NSC level might appear at the small or intermediate gap because the extreme light intensity, air temperature and evaporation rate under a large gap would become stress factors for the moss (Glime, 2007). In contrast, we speculated that the NSC levels of PN would be lower in the gap sites than in the control. Consequently, we hypothesized that the two species might have distinct responses of NSC level to gap creation.

MATERIALS AND METHODS

Focal species

Specimens of studied species were deposited in the herbarium at the Chengdu Institute of Biology, CAS. The nomenclature followed *Flora of China* and *Flora Bryophytorum Sinicorum*. PF is a typical acrocarpous turf moss with 3-8 cm height. It is a widespread species in the northern hemisphere, commonly forming loose tufts in shade and wet soil on forest ground. PN is a fern with long creeping rhizomes, 40-60 cm in height and leaves about 30-40 cm long and 7-10 cm wide. It mainly grows under the forest in hilly areas at elevations from 400 to 2500 m.

Experimental layout and sample collection

The study in the field was conducted in the thinning experiment sites of the 30-year-old *Picea asperata* plantation forest near the Maoxian Mountain Ecosystem Research Station of Chinese Academy of Sciences at Fengyi Township of Maoxian County, Sichuan, China (103°54E, 31°42N, Alt. 1826 m). The region belongs to the typical temperate climate with annual sunshine time of 1373.8 hours, annual mean temperature 9.3°C, annual precipitation 825.2 mm and annual evaporation 968.7 mm (Wu & Liu, 2007). The thinning experiment was designed to create four treatments with size at 0, 74, 109 and 196 m^2, respectively (hereafter called as control, small gap, intermediate gap and large gap) with three replicates on four similar blocks [12 sites (nine gaps and three controls) to collect plant samples]. The operation was implemented in winter 2008 (Jiang *et al.*, 2011).

We synchronously measured fundamental microclimate parameters at those sites before sampling (measurements were conducted at noon of 3, 7, 12 and 17 September 2010, all sunny days). Photosynthetically active radiation (PAR) was measured by Skye Instruments type SKR1800 dual channel radiometers (Skye Instruments, Llandrindod, Powys, UK) at several spots in the center of each site. Temperature and moisture at the soil surface on each site were measured by temperature/humidity data logger (DS1923 iButton, Maxim Integrated Products). The PAR and the temperature of the soil surface in daytime were significant higher in gap sites than that in control and both parameters increased with gap size (Table 1). Additionally, community investigation was done before sample collection (Yan & Bao, unpublished data). The total cover of bryophytes at the intermediate and large gaps was significantly lower than in the controls. However, PF had similar cover among the four treatments. In contrast, PN had higher cover at intermediate gaps than at the control sites.

Table 1. Photosynthetically active radiation (PAR), soil temperature and moisture at soil surface in the four thinning treatments in a spruce plantation forest at Maoxian County, southwestern China. The data were synchronously measured at noon of 3, 7, 12 and 17 September 2010. All data were analyzed by one-way ANOVA using LSD to test the significant differences ($p \leq 0.05$). Values are expressed as mean ± SE

Sites	*PAR (μmol m^{-2} s^{-1})*	*Temperature (°C)*	*Moisture (v %)*
Controls	59.47 ± 6.74^{d}	15.28 ± 0.11^{b}	30.73 ± 1.16^{ab}
Small gaps	234.94 ± 19.16^{c}	16.44 ± 0.32^{a}	30.80 ± 1.58^{ab}
Intermediate gaps	595.62 ± 39.25^{b}	16.67 ± 0.27^{a}	28.78 ± 1.38^{b}
Large gaps	786.06 ± 44.29^{a}	18.20 ± 0.36^{a}	33.07 ± 0.83^{a}

We collected the samples from three sampling points in the central zone of each site (about 2.0 g for each) on 18 September 2010, a sunny day. Only complete leaves at the top layer of PN and the PF population without cover from other plants and litter were collected. The samplings were stored in an ice box (about 4°C) and brought to the station laboratory immediately. Only green tissues for PF and the 10-20th pinna from the leaf apexes for PN were selected as final samples. All samples were swiftly washed with deionized water and then put into oven at 115°C for 10 minutes to stop the physiological activities. After that, the samples were oven-dried at 65°C for 48 hours. Dried samples were ground and stored at –4°C for chemical analysis.

Chemical analysis

All samples were pretreated following the method of Baninasab & Rahemi (2006). HPLC with evaporative light-scattering detector was used to measure the concentrations of simple sugars: glucose, fructose and sucrose (Sun *et al.*, 2004). Starch content was determined by anthrone colorimetric protocol following Li *et al.* (2008). All the results were described on a dry weight basis (mg g^{-1} d.w.).

Statistical analysis

NSC for each sample was counted by summing up starch and soluble sugars (SS). The SS concentration was calculated as the sum of glucose, fructose and sucrose. Data of all components (NSC, starch, SS, glucose, fructose and sucrose) were checked for normality and homoscedasticity prior to statistical analyses. The LSD multiple comparison test was used to compare the differences of all data among the four treatments of each species. Games-Howell post hoc test was used when the data did not meet the ANOVA assumption of homogeneity variance. All statistical analyses were performed using SPSS (version 16.0) and Origin (version 8.6). All values were considered significant when $P \leq 0.05$.

RESULTS

Starch was the most prominent non-structural carbohydrate component in the two species and the concentrations of glucose were higher than those of fructose and sucrose. The two understory species presented various levels of NSC and its components among the four treatments (Fig. 1). For PF, the concentrations of NSC were significant higher in thinning gaps than in the control and reached the peak point at the intermediate gaps. While the NSC levels of PN were lower at small and intermediate gaps than at control (Fig. 1A). Much higher starch levels in PF were observed at the thinning treatments than the control, contrary to those of PN (Fig. 1B). For components of SS, much higher concentration levels of glucose and sucrose and thus SS were observed at the gap treatments than at the control for PF, whereas for PN only the fructose level showed a significant difference between the control and the small gap (Figs 1 C, D, E and F).

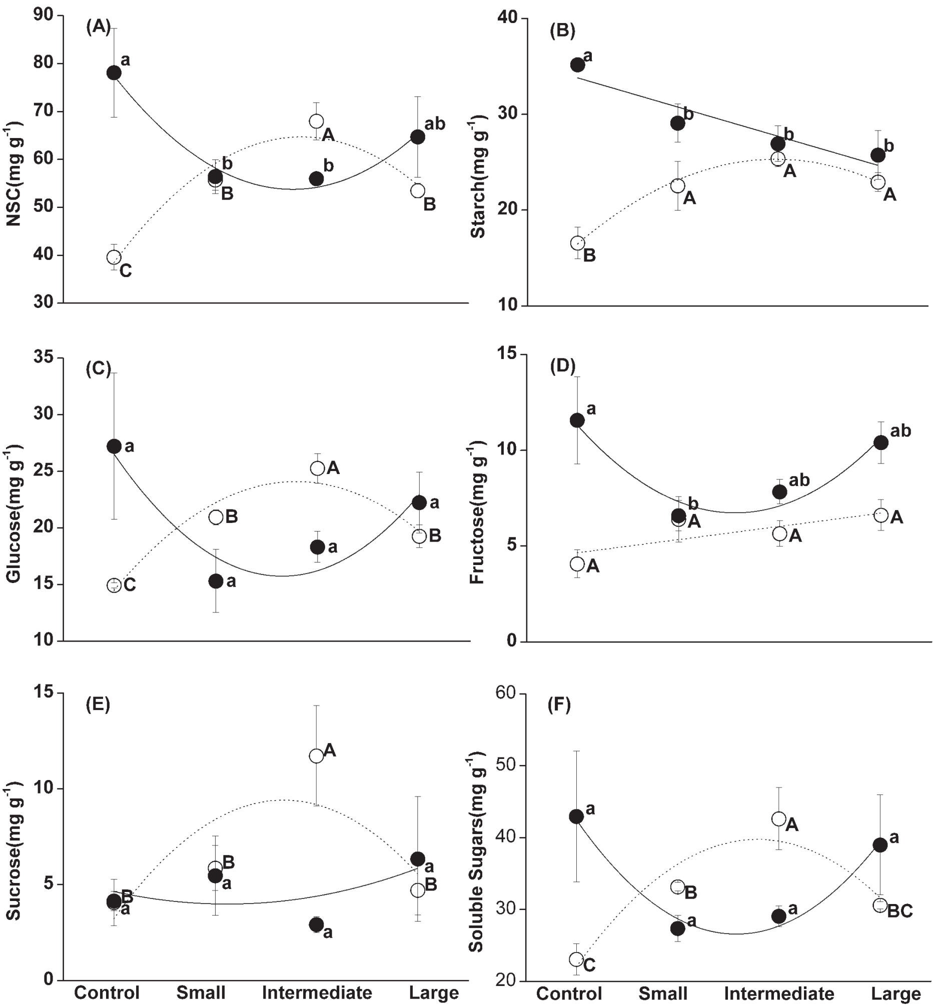

Fig. 1. The effects of gap creation on concentrations of the non-structural carbohydrates (NSC) and its components (mg g^{-1} d.w.) for the two understory plant species under a spruce plantation at Maoxian county, Southwestern China. *Polytrichastrum formosum* —○ with capital letters and *Parathelypteris nipponica* —● with lowercase letters to indicated significant differences among the four treatments respectively ($P \leq 0.05$). The dot and solid lines display the general trends across treatments for these two species, respectively.

DISCUSSION

Previous studies have revealed that starch, glucose, fructose and sucrose were the major fraction of plant carbohydrate components (Hoch *et al.*, 2003). Our study confirmed the starch was the predominant component of NSC for the two species, and their concentrations of glucose were higher than those of fructose and sucrose. However, the results deviated from previous studies of bryophytes which suggested that sucrose was the principal NSC component especially for its important role in carbon transportation (Riitten & Santarius, 1992; Robinson *et al.*, 2000; Forsum *et al.*, 2006; Wasley *et al.*, 2006; Lenné *et al.*, 2010). The relatively lower sucrose levels but higher glucose levels of our species can be attributed to the following reasons: 1). the proportions of various NSC components depend on plant species, e.g. the main photosynthetic product of garlic and onion are glucose and fructose (Pan, 2004); 2). Translocation of hexoses is an important carbohydrate transfer mode by the phloem equivalent to that of sucrose for many plants (van Bel & Hess, 2008); and 3). The proportions of NSC components also vary a lot at different living conditions or growth stages (Baninasab & Rahemi, 2006; Palacio *et al.*, 2008).

The present study also revealed that PF possessed higher NSC levels in gap sites compared with those in controls. Generally, the understory mosses are thought to be shady and humid plants (Waite & Sack, 2010). Nevertheless, studies on boreal feather mosses indicated the limited light condition under the dense forest could restrict their normal growth (Natalia *et al.*, 2008). Shields *et al.* (2007) also suggested that appropriate openings were beneficial to disturbance-adapted moss species. We believe the higher NSC levels of PF in gaps can be ascribed to its specific morphological and physiological features as well as the comparatively greater acclimation capacity to disturbance. Firstly, Polytrichaceae species have relatively high chlorophyll content and light saturation points of RETR (Marschall & Proctor, 2004). And secondly, relatively higher leaf area index and the lamella system of PF provides additional CO_2 uptake area to make better use of higher irradiance (Proctor, 2005). In addition, when suffering environmental stress like extremely high illumination, heat or drought, Polytrichaceae plants can curl against the stem and wrap their lamina rapidly to decrease the exposed area and hide the adaxial surface (Vanderpoorten & Goffinet, 2009). Therefore, we confirmed that although PF was a shade-tolerant moss species, it could benefit from the forest gap creation. Our research also showed that the NSC level of PF at the large gap was lower than at the intermediate gap (Fig. 1). It was possibly because some of the environmental factors such as light intensity, ambient temperature and evaporation rate in the large gaps had exceeded the optimal ranges for PF and had become the limiting factors. In contrast, we found the gap treatments restricted the accumulation of starch of PN and thus led to lower NSC levels. Reduction of NSC levels indicates either the low activity of source and sink or the carbon demand exceeds current supply (Körner, 2003). The disturbance might affect the net photosynthetic rates of PN because the fern was more inclined to the shady and humid environment in the understory rather than gaps for its low growth rate and poor water loss control, as well as the intolerance of varied environmental factors (Page, 2002). And another possibility is the carbon demands of PN in gap sites were higher, so the NSC was translocated to the sink organs (rhizomes) at a faster rate. Relevant studies on gas exchange and carbon isotope should be done to determine which one is the cause for the reduction of NSC levels in gap sites for PN.

CONCLUSION

The present study investigated the effects of gap thinning on levels of NSC and its components of PF and the co-occurring fern PN in the dense spruce plantation. The results supported our assumption that the two species had distinct responses of NSC levels to the gap creation. PF possesses higher NSC levels in gap sites than in the understory. However, the result was reversed for PN. Our study indicated that the shade-tolerant moss PF could benefit from gap formation. On the contrary, gap creation might inhibit the net photosynthesis of PN or accelerate its translocation rate of NSC from leaves to sink organs. More physiological measurements such as gas exchange and carbon isotope are needed to more deeply understand the responses of the forest floor plants to gap creation. Wider researches focus on plants within different growth or life forms are also required to extend our knowledge on the eco-physiological impact of gap formation to the understory plants.

Acknowledgements. The investigation was supported by the National Science & Technology Pillar Program in 12th Five-year Plan of China (No. 2011BAC09B0402) and the Strategic Priority Research Program of the CAS (No. XDA05070306). We gratefully acknowledge Yufei Li for helping HPLC analysis, Dr. Xueyong Pang and Qihua He for providing the background information.

REFERENCE

BANINASAB B. & RAHEMI M., 2006 — Possible role of non-structural carbohydrates in alternate bearing of pistachio. *European journal of horticultural science* 71:277-282.

DALLING J. W., WINTER K. & HUBBELL S. P., 2004 — Variation in growth responses of neotropical pioneers to simulated forest gaps. *Functional ecology* 18:725-736.

FENTON N. & FREGO K., 2005 — Bryophyte (moss and liverwort) conservation under remnant canopy in managed forests. *Biological conservation* 122:417-430.

FORSUM Å., DAHLMAN L., N SHOLM T. & NORDIN A., 2006 — Nitrogen utilization by *Hylocomium splendens* in a boreal forest fertilization experiment. *Functional ecology* 20:421-426.

GLIME J. M. 2007 — Volume 1. Physiological Ecology. Chapter 6 Limiting factors and limits of tolerance. In Glime J.M., *Bryophyte Ecology*. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Accessed on 16 August 2010 at http://www.bryoecol.mtu.edu/.

HOCH G., RICHTER A. & KORNER C., 2003 — Non-structural carbon compounds in temperate forest trees. *Plant, cell and environment* 26:1067-1081.

JIANG Y., PANG X. & BAO W., 2011 — Soil microbial biomass and the influencing factors under *Pinus tabulaeformis* and *Picea asperata* plantations in the upper Minjiang River. *Shengtai Xuebao/Acta ecologica Sinica* 31:801-811.

KÖRNER C., 2003 — Carbon limitation in trees. *Journal of ecology* 91:4-17.

KARIUKI M., KOOYMAN R. M., SMITH R. G. B., WARDELL-JOHNSON G. & VANCLAY J. K., 2006 — Regeneration changes in tree species abundance, diversity and structure in logged and unlogged subtropical rainforest over a 36-year period. *Forest ecology and management* 236:162-176.

LENNÉ T., BRYANT G., HOCART C. H., HUANG C. X. & BALL M. C., 2010 — Freeze avoidance: a dehydrating moss gathers no ice. *Plant, cell and environment* 33:1731-1741.

LI M. H., XIAO W. F., SHI P. L., WANG S. G., ZHONG Y. D., LIU X. L., WANG X. D., CAI X. H. & SHI Z. M., 2008 — Nitrogen and carbon source-sink relationships in trees at the Himalayan treelines compared with lower elevations. *Plant, cell and environment* 31:1377-1387.

MARSCHALL M. & PROCTOR M. C. F., 2004 — Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. *Annals of botany* 94:593-603.

NATALIA S., VICTOR J. & SIMON M., 2008 — Effects of leaf litter on the growth of boreal feather mosses : Implication for forest floor development. *Journal of vegetation science* 19:253-260.

NELSON C. R. & HALPERN C. B., 2005 — Short-term effects of timber harvest and forest edges on ground-layer mosses and liverworts. *Canadian journal of botany* 83:610-620.

PAGE C. N., 2002 — Ecological strategies in fern evolution: a neopteridological overview. *Review of palaeobotany and palynology* 119:1-33.

PALACIO S., MILLA R., ALBUIXECH J., PÉREZ-RONTOMÉ C., CAMARERO J. J., MAESTRO M. & MONTSERRAT-MARTÍ G., 2008 — Seasonal variability of dry matter content and its relationship with shoot growth and nonstructural carbohydrates. *New phytologist* 180:133-142.

PAN R. C. (ed.), 2004 — Chapter 3. The photosynthesis of plant. In *Plant Physiology*. *5th edition*. Beijong, Higher Education Press, p. 82 (in Chinese).

PROCTOR M. C. F., 2005 — Why do Polytrichaceae have lamellae? *Journal of bryology* 27:221-229.

PROCTOR M. C. F., 2012 — Light and desiccation responses of some Hymenophyllaceae (filmy ferns) from Trinidad, Venezuela and New Zealand: poikilohydry in a light-limited but low evaporation ecological niche. *Annals of botany* 109:1019-1026.

RAMBO T. R. & MUIR P. S., 1998 — Forest floor bryophytes of *Pseudotsuga menziesii-Tsuga heterophylla* stands in Oregon: influences of substrate and overstory. *The bryologist* 101:116-130.

RAMBO T. R. & NORTH M. P., 2009 — Canopy microclimate response to pattern and density of thinning in a Sierra Nevada forest. *Forest ecology and management* 257:435-442.

RÜTTEN D. & SANTARIUS K. A., 1992 — Relationship between frost tolerance and sugar concentration of various bryophytes in summer and winter. *Oecologia* 91:260-265.

ROBINSON S., WASLEY J. & POPP M., 2000 — Desiccation tolerance of three moss species from continental Antarctica. *Functional plant biology* 27:379-388.

SHIELDS J. M., WEBSTER C. R. & GLIME J. M., 2007 — Bryophyte community response to silvicultural opening size in a managed northern hardwood forest. *Forest ecology and management* 252:222-229.

SUN Y. A., WANG G. Q., ZHANG Y. J. & YAN K. Y., 2004 — Determination of the water-soluble carbohydrates in tobacco by HPLC-ELSD. *Journal of analytical science* 20:531-533.

THOMAS A. K., 1998 — Relating tree physiology to past and future changes in tropical rainforest tree communities. *Climatic change* 39:363-379.

VAN BEL A. J. E. & HESS P. H., 2008 — Hexoses as phloem transport sugars: the end of a dogma? *Journal of experimental botany* 59:261-272.

VANDERPOORTEN A. & GOFFINET B., 2009 — Physiological Ecology. *in:* A. Vanderpoorten & B. Goffinet (eds). *Introduction to bryophytes*. New York, Cambridge University, pp. 185-213.

WÜRTH M. K. R., PELÁEZ-RIEDL S., WRIGHT S. J. & KÖRNER C., 2004 — Non-structural carbohydrate pools in a tropical forest. *Oecologia* 143:11-24.

WAITE M. & SACK L., 2010 — How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. *New phytologist* 185:156-172.

WALKER L. R. & SHARPE J. M., 2010 — Ferns, disturbance and succession. *in:* K. Mehltreter, L. R. Walker, & J. M. Sharpe (eds), *Fern Ecology*. New York, Cambridge University Press, pp. 177-219.

WASLEY J., ROBINSON S., LOVELOCK C. & POPP M., 2006 — Some like it wet – biological characteristics underpinning tolerance of extreme water stress events in Antarctic. *Functional plant biology* 33:443-455.

WU N. & LIU X.L., 2007 — Chapter 2.The ecological environment and social economy of the upper of Minjiang River. *In* Wu N. (ed.), *Restoration and Rehabilitation of Degraded Mountain Ecosystem on the Upper Minjiang River.* first edtion. Sichuan Scientific Technological Press, Chengdu, pp. 19-28 (in Chinese).