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ANALYSIS ON VARIATION PATTERNS OF *PARMOTREMA TINCTORUM* INDIVIDUALS UNDER DIFFERENT ENVIRONMENTS

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Abstract

Twelve *Parmotrema tinctorum* individuals were collected from the Yaoluoping national nature reserve of Anhui Province, China. The relationships between biological characters of lichen individuals and their environmental factors were analyzed by methods included Redundancy analysis (RDA). Based on the information, variation patterns of different individual's biological characters were described. The analysis results manifested that the biological characters showed a certain extent stability in a individual, but exhibit more variation among all twelve individuals, of which the hyphae diameter and anatomical character, had minimum variation coefficients not only within a individual, but also between all the analyzed lichen individuals. Redundancy analysis showed that environment factor moisture had a significant negative correlation with illumination, had the most-positive correlation and the most-negative correlation with hyphae diameter and biological index medulla width, respectively; While environment factor altitude had the most-negative correlation with lower cortex width and the most-positive correlation with rhizoid density. The atranorine content could response to illumination condition sensitively; whereas the algae layer thickness could reflect comprehensive environment change very well. The nutriment investment strategy within different function parts of a lichen thallus deserve to investigate deeply, which is of great importance in revealing the response model of lichens to environment change.

Key words: *Parmotrema tinctorum*, Characters, Environment factors, Individuals, Redundancy analysis individual.

Introduction

Lichens are a symbiotic phenotype of nutritionally specialized fungi, and are considered ecologically obligate biotrophs (Honegger & Bartnicki-Garcia, 1991). Lichenized fungi exhibit characteristics such as various adaptions to symbiotic life, dual features, low metabolic rates, slow growth, and a long life cycle. Therefore, lichens are more sensitive to the environment, rendering it a good biomonitoring plant (Firdous *et al*., 2017; Sulaiman *et al*., 2018). Sancho *et al.*, (2007) found that lichens show stable, gradual ecological variation along latitude, which is believed to be a response to regional precipitation and temperature differences. Using lichens as indicator plants to monitor long-term climate change can yield very good results. Güvenç & Öztürk (2017) found that the diversity of epiphytic lichens on *Quercuscerris* varied depending on the effects of macroclimatic and microclimatic factors, anthropogenic and agricultural activities. Species richness is associated with climatic factors such as mean annual temperature and precipitation (Giordani & Incerti, 2008). Response patterns to climate differ within and between lichen communities (Ellis & Coppins, 2006). As altitude increases, lichen species richness gradually changes (Ellis & Coppins, 2006; Baniya *et al*., 2010). Hauck (2011) suggested that lichen distribution was determined by the continuity of living conditions. Based on the response pattern of lichens to climatic variables, Ellis *et al.*, (2007) used the prediction model to suggest that intensified summer drought in the 2020*s* and the 2050*s* might result in the overall increase in lichen species abundance and expanded distribution. Forest precipitation and chemical composition of substrates have a significant impact on lichen abundance (Hauck & Spribille, 2005). Lichens also react to substrates, resulting in a complex ecological relationship between the substrate and the lichen (Wedin *et al*., 2004). Therefore, an epiphytic lichen can affect its host tree, thereby affecting the growth and distribution of forest tree species (Hauck, 2011; Li *et al*., 2007). Changes in precipitation and temperature resulting from climate change might affect the plants' usage of nutrients, in turn affecting the distribution of the lichen population or individual lichens. Lichen activities may also affect the structure and function of other vegetation in the forest ecosystem.

Parmotrema tinctorum, a lichenized fungus, belongs to the class *Ascomycetes*, order *Lecanorales*, and family *Parmeliaceae*. It is widely distributed on rocks and trees in the tropics and the temperate region. The diameter of the thallus is generally 10-30 cm, and the lobe width is 10-20 mm. There are rhizines on the lower surface, and bare brown marginal zones. The upper cortex contains secondary metabolites such asatranorin. It is mainly distributed in the middle elevation zone, often in disturbed habitats. It is also a common, large foliose lichen in the Dabie Mountains (Li & Wang, 2013; Wang *et al*., 2014).

The Dabie Mountains are in the northern subtropical and the northern temperate transitional zone, and occupy the watershed between the Huai and Yangtze Rivers. It is generally believed that ecological transition zones are more sensitive to environmental changes, and therefore the impact of climate change on this ecosystem may be more prominent. We selected the Yaoluoping Nature Reserve, in the Dabie Mountains, as the study site and 12 *P. tinctorum* individuals from different habitats as the study subjects. By analyzing the environmental factors, biological indicators, and their relationship, the change patterns of lichen morphology, structure, and chemical substances in different habitats were clarified. Our study aimed to elucidate the trend in lichen responses to environmental changes in the forest ecosystem of the climate transition zone.

Study region overview: The study area was in the Yaoluoping Nature Reserve (E 116°02′20″-116°10′53″, N 30°57′20″-31°06′10″), in the Dabie Mountains at the junction between the Anhui and Hubei provinces. This region is the main watershed between the Huai and Yangtze Rivers. The overall topology was high in the south and low in the north. The highest peak is Duozhijian (1,721 m) in the south (Fig. 1), and the lowest point is Liyuwei (500 m) in the north. The total area of the reserve is 12,300 hm^2. The region has a north subtropical monsoon climate, with humid hair and mild climate. The coldest month is January, with an average temperature of 2°C, and the hottest month is July, with an average temperature of 26.3°C. The annual average temperature is 11.5°C, and the average annual precipitation is 1,400 mm. Yaoluoping Nature Reserve is dominated by the typical northern subtropical alpine forest ecosystem. The vertical distribution of vegetation is richly diverse. The forest coverage rate is above 90% and there are more than 1,400 species of vascular plants, including most plant species in the Dabie Mountains present almost all types of vegetation.

Material and Methods

Sampling sites and sample collection: After thorough consideration of the environmental factors affecting lichen growth, twelve individual *P. tinctorum* thalli were selected as study subjects. The environmental factors in their habitats were measured and recorded, and lichen samples were collected for subsequent analysis. Twelve *P. tinctorum* samples were collected and, based on their altitude, from low to high, the samples were given a number from P_1 to P_{12}. Regarding underside conditions, the diameter of the lichen thallus was between 10 and 15 cm, the lobe tips were round, and apothecia were absent. The location and environmental information for each lichen thallus sample are shown in Fig. 1 and Table 1.

Environmental factor measurement: A total of four environmental factors were selected in this study: altitude, substrate, illumination, and moisture of the lichen growth environment. The altitude was measured and recorded. Three types of substrates were studied: rock surface, moss, and tree bark, denoted with numbers 0, 1, and 2, respectively. The amount of illumination was determined relatively. The lichen growth environment was observed and the degree of shade was estimated. Illumination was determined by subtracting the degree of shade from 1. Six categories were assigned to represent moisture levels (Wang *et al.*, 2012): bare rock (0), low water-holding capacity and poorest moisture condition in the lichen growing environment; moss on the rock (1), better water-holding capacity; tree bark (2), better shading but poor water-holding capacity due to its smooth surface; *Pinus taiwanensis* bark (3) in the forest, a high degree of shade and better water-holding capacity because of its peeled bark; rock in the forest (4), high environmental moisture; and moss in the forest (5), a high degree of shade, moist environment, and strong water-holding capacity and is considered as the highest moisture environment for lichen growth (Fig. 1; Table 1).

Morphological and anatomical indices: Healthy, undamaged thallus tissues were selected and the ventral surface was observed under a stereomicroscope to measure three indices: width of the bare marginal zone (mm), rhizine density (roots/mm^2), and rhizine length (mm).

Cross sectioning was performed approximately 5 mm from the tip of the lobe, and sections were observed under a microscope to measure five anatomical indices: the thicknesses of upper cortex (μm), algal layer (μm), medulla (μm), and lower cortex (μm), and the diameter of hyphae (μm) in the upper cortex.

These indices were measured from 10 randomly selected lobes, and the mean value and the individual coefficient of variation were calculated. The total coefficient of variation for each index was calculated. Furthermore, the total coefficient of variation for each index for each individual was calculated.

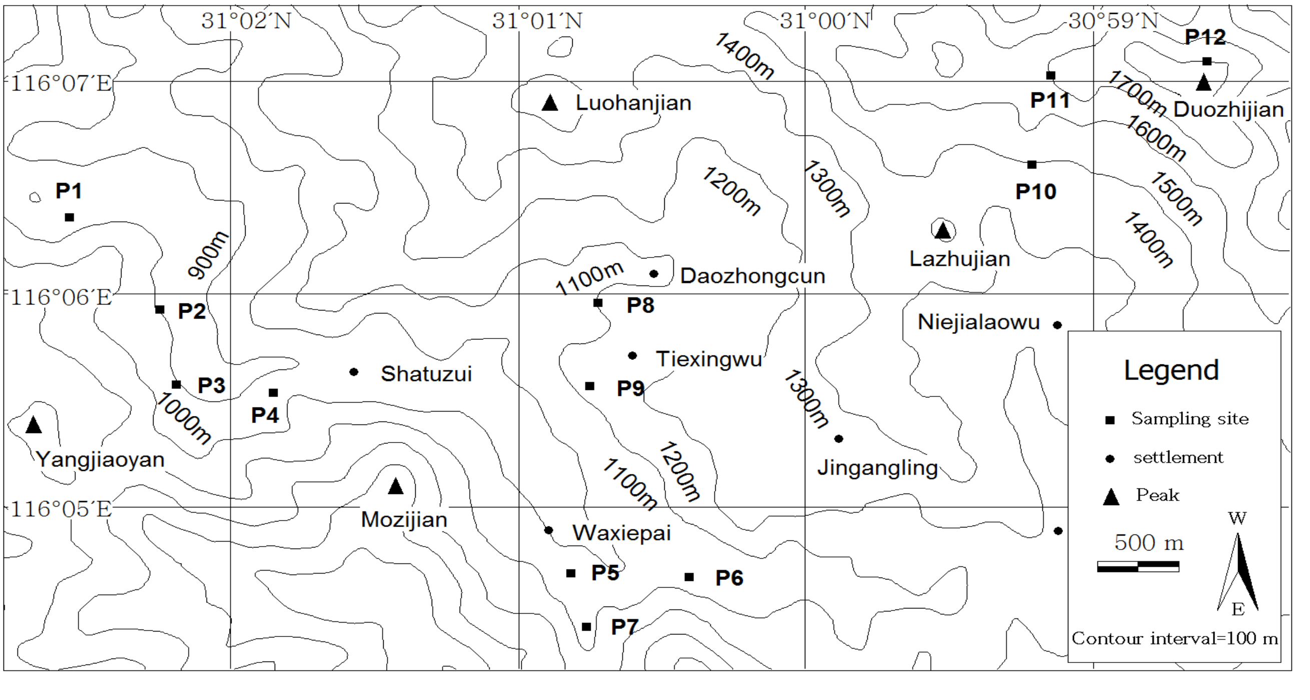

Fig. 1. Survey region.

Table 1. Situation of collection sites.

No.	Altitude	Substrate	Illumination	Moisture %	Sites
P_1	850	1	0.95	21	Fancheling
P_2	900	0	0.40	62	Meilicun
P_3	910	2	0.35	35	Luojiawan
P_4	950	0	0.95	13	Luojiawan
P_5	1 024	2	0.40	54	Waxiepai
P_6	1 024	2	0.70	34	Shiwuchong
P_7	1 080	1	0.30	82	Menkanling
P_8	1 100	0	0.80	23	Houtang
P_9	1 130	0	0.50	64	Tiexingwu
P_{10}	1 400	1	0.80	23	Dongchongwan
P_{11}	1 600	0	0.85	11	Duozhijian
P_{12}	1 700	2	0.90	19	Duozhijian

Note: The means of column No. to see Fig. 1 and text; The means of number in column moisture to see text

Chemical substances: The thallus atranorin content was analyzed using high-performance liquid chromatography (HPLC). The lichen surface was carefully cleaned, and a fixed amount of lichen sample (generally 0.5 g) was weighed and ground, followed by acetone extraction for 24 h. After filtration, the remaining residue was washed, and the volume was adjusted to 10 mL. A Shimadzu LC-2010liquid chromatograph was used for detection (wavelength=254 nm), and the experiment was conducted at a flow rate of 1.0 mL/min at 25°C. The sample injection volume was 1 μL. The reference material for atranorin (purity 98.9%; format 10 mg) was manufactured by ChromaDex, Inc. (USA). The atranorin content in dried lichen samples was calculated based on the HPLC analysis results, and the adopted formula:

$$\omega = ncm^{-1} \tag{1}$$

where ω is the amount of atranorin (mg/g) in the lichen sample; n is the dilution factor; and m is the mass (g) of the collected lichen sample.

Ultrastructure: Ultrastructural analysis was performed on samples to help explain the relationship between environmental factors and biological traits. The impurities and dust on the lichen surface were gently brushed off. A small sample, approximately 5 mm × 5 mm, was dissected 1 cm from the tip of the lobe. The sample's dorsal side was facing up and the sample was attached to double-sided tape on the sample holder, sprayed with gold, and observed under a Hitachi S-4800 scanning electron microscope (SEM) and photographed. The thickness of gold coating was generally 10 nm. The samples were examined mainly for hyphae characteristics and upper surface features.

Statistical analysis: General calculations and analyses were performed using Microsoft Excel. The fitting of the relationship between environmental and biological indices was performed using SPSS 20. The redundancy analysis (RDA) was conducted using Canoco 4.5 software. The species matrix was log-transformed and the data were graphed using Cano Draw 4.5. The ordination result was depicted using a biplot of the relationship between species and environmental factors.

Results and analysis

Variation within and between individuals: The statistical analysis was performed on nine quantitative indices from the 12 lichen thalli, to obtain the mean and the coefficient of variation for each biological index (except for the atranorin content) within each thallus, the total coefficient of variation of indices within a thallus, and the total coefficient of variation for each index of all samples (Table 2). As shown in Table 2, there was a difference in biological index in each individual, and the difference varied. Individual P_2 had the largest difference in biological index coefficient of variation, and the largest coefficient of variation (0.47, width of the bare marginal area) was 9.4 times higher than the smallest (0.05, hyphae diameter). Individual P_7 had the smallest difference in index coefficient of variation, where the largest coefficient of variation (0.23, rhizine density) was 3.8 times higher than the smallest (0.06, hyphae diameter). Of all individuals, without exception, the hyphae diameter was the index with the smallest coefficient of variation. Comparison of variations among individuals suggested the individual with the highest degree of variation was P_9 (0.30), and the one with the lowest degree of variation was P_7 (0.16). Comparison of the changes in the same index among individuals revealed that the index with the largest change in the mean was bare marginal zone width, with the largest value (P_9) being 34.5 times higher than the lowest value (P_2). The atranorin level had the second largest change, with the highest mean value (P_8) being 8.4 times higher than the lowest (P_2). The coefficient of variation that showed a larger difference within individuals was thickness of medulla, with the largest value (P_3) being 3.7 times higher than the smallest value (P_1), while other indices showed a variation within 2- to 3-fold. Overall evaluation of indices revealed that among the eight traits, bare marginal zone width had the largest coefficient of variation (0.69), followed by rhizine density, algal layer thickness, lower cortex thickness, medulla thickness, rhizine length, and upper cortex thickness. The hyphae diameter still had the smallest coefficient of variation (0.10). Comparison of the index coefficient of variation within an individual with the overall index coefficient of variation of all samples revealed that the index variation within an individual was smaller than the overall index variation. This suggests a certain degree of stability for each index in an individual. In addition, the hyphae diameter showed great stability both within and among individuals.

Table 2. Nine biological indices and their variation of lichen in 12 individuals.

No.	Indices of morphology and chemistry																		*CV*
	Atr. mg/g	Ucor.		Alg.		Med.		Lcor.		Hyp.		Baz.		Ard.		Arl.			
		$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*	$\bar{x}$	*CV*		
P_1	9.76	13.4	0.18	11.8	0.23	60.2	0.09	23.3	0.22	3.2	0.07	5.5	0.26	5.1	0.19	1.78	0.22		0.18
P_2	1.13	17.5	0.26	20.5	0.23	26.6	0.20	20.8	0.19	3.7	0.05	0.2	0.47	7.2	0.19	1.44	0.24		0.23
P_3	4.00	16.5	0.26	19.9	0.40	88.1	0.33	12.3	0.21	3.1	0.08	3.7	0.21	13.5	0.21	1.26	0.23		0.24
P_4	4.94	20.8	0.15	49.3	0.19	97.2	0.11	17.7	0.17	3.4	0.06	2.2	0.41	7.5	0.21	0.94	0.38		0.21
P_5	4.23	20.6	0.23	26.1	0.25	68.1	0.21	25.6	0.13	2.7	0.09	0.2	0.41	5.6	0.23	1.49	0.20		0.22
P_6	2.01	30.2	0.23	29.8	0.22	129.7	0.10	25.1	0.19	3.1	0.07	2.4	0.29	3.1	0.44	2.15	0.14		0.21
P_7	3.44	20.6	0.15	19.4	0.20	70.7	0.16	15.6	0.18	3.5	0.06	3.2	0.17	4.6	0.23	2.01	0.14		0.16
P_8	10.66	21.0	0.19	25.4	0.36	125.5	0.11	21.2	0.27	3.4	0.04	5.4	0.32	4.7	0.28	1.13	0.24		0.23
P_9	1.66	31.4	0.26	27.1	0.54	67.5	0.31	39.0	0.39	3.5	0.06	6.9	0.35	6.8	0.24	1.59	0.23		0.30
P_{10}	2.91	23.3	0.31	17.4	0.25	58.1	0.14	24.8	0.33	3.1	0.04	3.9	0.34	13.1	0.28	1.38	0.33		0.25
P_{11}	4.46	22.0	0.28	29.8	0.24	120.2	0.10	19.8	0.31	3.1	0.06	2.6	0.32	5.7	0.17	0.78	0.32		0.23
P_{12}	3.40	20.9	0.32	17.6	0.20	69.9	0.13	12.4	0.20	3.0	0.08	2.5	0.23	14.9	0.22	0.78	0.38		0.22
Total *CV*		**0.33**		**0.47**		**0.40**		**0.43**		**0.10**		**0.69**		**0.55**		**0.38**			

Note: The means and units of abbreviations in column indices to see 2.3 and 2.4.

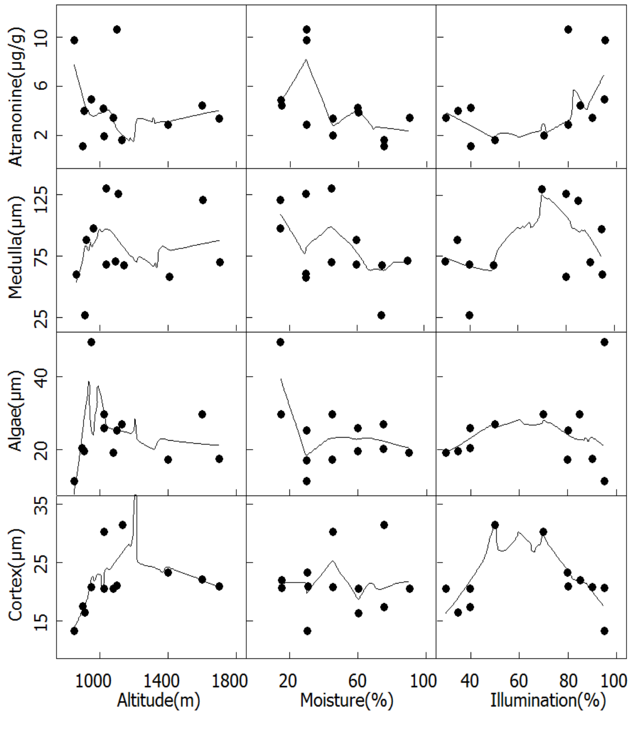

Fig. 2. Scatter maps of altitude, moisture and illumination to four biological characters.

Effects of environmental factors on biological indices: To explore how the differences in lichen morphological structure and chemical substances related to the environmental factors, we selected three environmental indices (altitude, moisture, and illumination) and four biological indices (upper cortex thickness, algal layer thickness, medulla thickness, and atranorin content). The environmental factors were plotted against the biological factors, and the fitting analysis was performed (Fig. 2). As shown in Fig. 2, different biological factors showed a certain pattern of change under different environmental conditions. With the increasing altitude and illumination intensity, three lichen anatomical indices, except for atranorin content, generally showed an increasing trend at mid-altitude and a decreasing trend at high and low altitudes. This may imply that the lichen thallus was thickest in the mid-mountain region and under moderate illumination conditions. The atranorin content was highest in the low-mountain region and, with increasing altitude, its content showed a stable trend, which was supported by the observation that atranorin was a secondary metabolite of lichen and was deposited on the surface of hyphae (Hale, 1983). The results of HPLC analysis (Fig. 4) shows that the changes in atranorin content is related with elevation, as the elevation rises, the content tends to be stable, the highest content appears in lower mountain belt. The electron microscopy images (Fig. 3) supports the results in qualitative research, while the results of HPLC analysis supports in quantitative research. Meanwhile, under conditions of higher illumination, the atranorin level showed a slight upward trend (Figs. 2 & 4). Under different moisture conditions, the thickness of upper cortex was stable, while other indices all showed a decrease with increasing moisture (Fig. 2).

Relationship between environmental and biological variables analyzed by RDA: The above analysis reflected the independent relationship between biological indices (lichen structure and chemical substances) and each environmental factor. However, changes in lichen morphological structure and chemical substances are a response to the combined effects of multiple environmental factors. Therefore, this study three environmental factors (moisture, altitude, and illumination of the lichen environment) were selected as explanatory variables (environmental variables) and eliminated substrate type, a nominal variable. One chemical index, five anatomical indices, and three morphological indices were selected as response variables (species variables) (Table 2). The relationship between the two groups of variables was analyzed using RDA, to study the correlation between the environmental factors and the biological indices and reveal the intrinsic relationship between the two groups of variables. The names and the measurement methods for the above indices are described in sections 2.3 and 2.4, and the designated numbers for each individual are shown in Table 2.

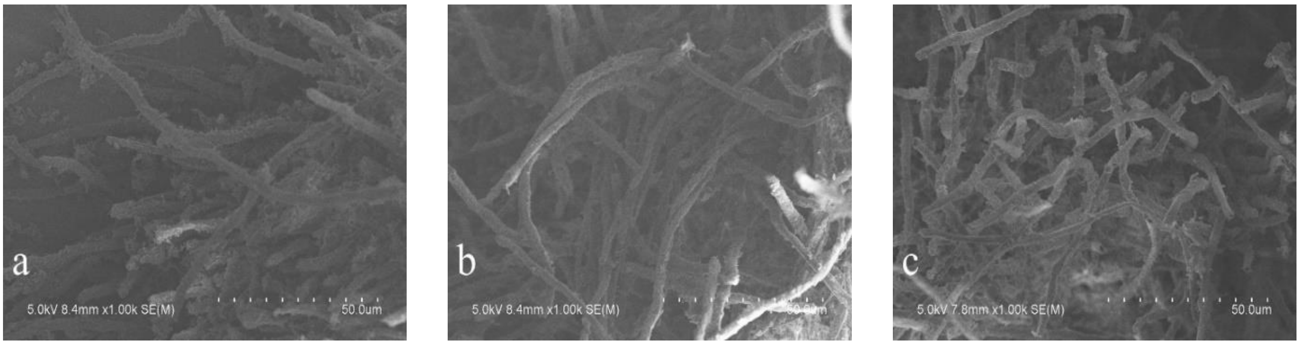

Fig. 3 Secondary metabolite substances on lichen hyphae under SEM. 3a. Hyphae of No.1 individual with more substances, alt.=850 m; 3b. Hyphae of No.9 individual with less substances, alt.=1 130 m; and 3c. Hyphae of No.11 individual with moderate substances, alt.=1 600 m.

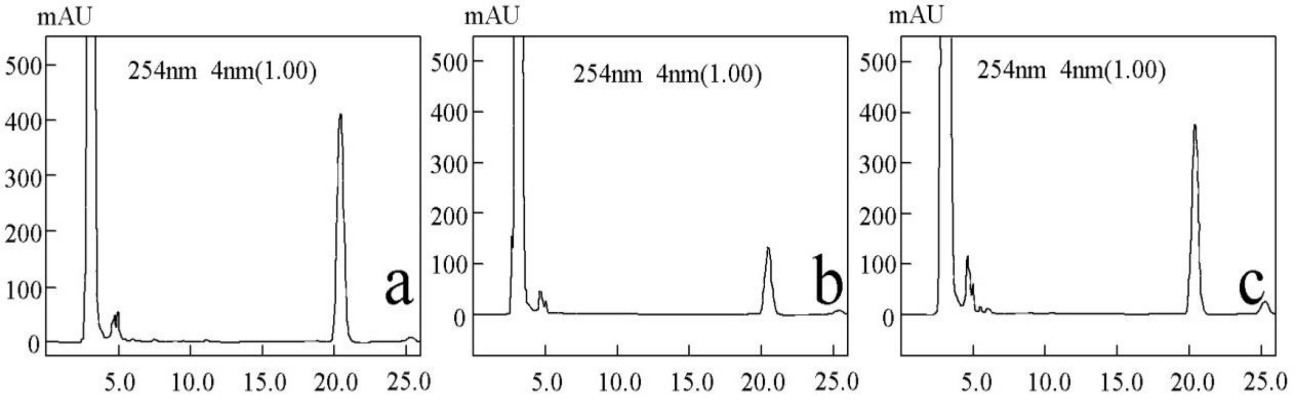

Fig. 4. HPLC results of atranorine in three individuals, λ=254 nm. 4a. Material from No. 1 individual, peak time=20.383 min, content=9.76 mg/g, 850 m; 4b. Material from No. 9 individual, peak time=20.417 min, content=1.66 mg/g, 1 130 m; and 4c. Material from No. 11 individual, peak time=20.38min, content=46 mg/g, 1 600 m.

Table 3. Eigenvalues for RDA axis and species-environment correlations.

	AX1	AX2	AX3
Eigenvalues	0.124	0.083	0.026
Correlations	0.705	0.608	0.386

Table 4. Correlation coefficients between environmental factors and RDA ordination axes.

Environmental factors	AX1	AX2	AX3
Altitude	-0.3293	-0.5556	-0.7635
Illumination	-0.7423	0.4405	-0.5049
Moisture	0.9726	-0.1627	0.1658

RDA is a constrained direct gradient analysis, and the ordination axis is a linear combination of environmental variables involved in the ordination. The effects of explanatory variables on response variables were concentrated on several synthesized canonical axes, which could independently maintain the contribution rate of each environmental variable on the changes in biological communities (TerBraak, 1986). In this study, the species data dimension varied; therefore, we used RDA based on a linear model.

The analysis showed that the eigenvalues of the first and second ordination axes were 0.124 and 0.083, respectively (Fig. 5). The correlation coefficients between species variables and the ordination axes of environmental factors were 0.705 and 0.608, respectively (Table 3), suggesting the ordination could approximately reflect the relationship between the individual and the environmental factors. As shown in Figure 5, biological indices and individuals are well differentiated in the RDA ordination plot. On the right side of ordination plot, there were mostly individuals with a thicker hyphae diameter, a thicker lower cortex, longer rhizines, greater moisture exposure, and under more shade, whereas those on the left side mostly had a thicker medulla, higher atranorin content, less moisture exposure, and abundant illumination.

Correlation coefficients between environmental factors and RDA ordination axes are listed in Table 4. As shown in Table 4 and Figure 5, of the three environmental factors, moisture was positively correlated with the first ordination axis, with a correlation coefficient of 0.9726. Illumination and altitude were negatively correlated with the first ordination axis, with correlation coefficients of -0.7423 and -0.3293, respectively. Altitude showed the strongest negative correlation with the second axis, with a correlation coefficient of -0.5556.

According to the RDA results and Fig. 5, the environmental moisture and illumination showed a clear negative correlation (correlation coefficient=-0.8774). The negative correlation between altitude and moisture was not significant (correlation coefficient=-0.3565). Furthermore, the positive correlation between altitude and illumination was not significant (correlation coefficient=0.3852). The results suggest that in this region, the effects of altitude on moisture and illumination were not obvious, whereas the effect of illumination on moisture was great: the stronger the illumination, the drier the conditions.

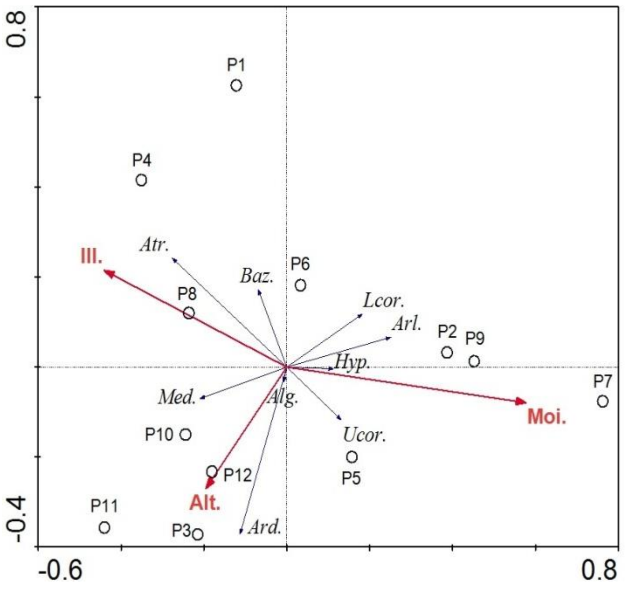

Fig. 5. RDA triplots of biological characters and environmental variables in 12 individuals.

As shown in Fig. 5, the factor that showed the greatest positive correlation with moisture was hyphae diameter, followed by rhizine length, lower cortex thickness, and upper cortex thickness. The factor that showed the greatest negative correlation with moisture was medulla thickness, followed by atranorin content, bare marginal zone width, rhizine density, and algal layer thickness. Factors that showed a stronger positive correlation with illumination were atranorin content, bare marginal zone width, and medulla thickness, whereas factors that showed a greater negative correlation were cortex thickness, hyphae diameter, and rhizine length. Altitude had the greatest positive correlation with rhizine density and the greatest negative correlation with lower cortex thickness. The algal layer thickness was centered between the two ordination axes, suggesting that changes in algal layer thickness had stronger adaptability to several environments.

The plot also shows a different degree of correlation among biological indices, and between biological indices and individuals. For example, there was a stronger positive correlation between rhizine length and lower cortex thickness, and there was a stronger negative correlation between rhizine density and length. However, the relationships between these indices are not discussed in detail.

Discussion

Lichenization, combining algae and fungi, is a successful nutritional strategy (Honegger, 2009). Lichenized fungi are different from other fungi in terms of their thallus structure (Honegger & Bartnicki-Garcia, 1991). Analysis of their coefficients of variation showed that, although the difference in coefficient of variation for individual algal layer thickness was small, when considering the algal layers from all 12 individuals, the total coefficient of variation was largest of all anatomical indicators (0.47). This suggests that the algal layer thickness had a certain degree of stability within an individual, whereas it was more variable between individuals (Table 2). The RDA results showed that the change in algal layer thickness was more affected by altitude, while its correlation with moisture and illumination was very small (Fig. 5). Lichen respiration is controlled by moisture and temperature (Palmqvist, 2000). Photosynthetic products are transported from the photobiont to the mycobiont, which depends on the alternating dry and wet periods of the fungi (Kershaw, 1985). According to the actual conditions, lichen characteristics, and analysis results, illumination and moisture were not the limiting environmental factors for *P. tinctorum* growth in this region. Based on the combined effects of altitude, the change of patterns of the algal layer thickness and other indices might be affected by other factors (such as temperature and inorganic nutrient) not covered in this study. It has been suggested that functional equilibrium models (Brouwer, 1962) can be applied to lichens (Brouwer, 1962; Palmqvist & Sundberg, 2000). Therefore, changes in algal layer thickness might be the response of lichens to environmental changes, like that of the C:N ratio (Grace, 1997). In this respect, algal layer thickness (or the fungi: algae ratio) is the anatomical index that better reflects environmental changes, especially changes in carbon.

One of the significant features of lichens is their duality: photosynthetic algal cells covered by hyphae. Due to the protection by hyphae, algal cells can avoid drought and excessive illumination. The mycobiont can regulate the amount of light entering the algal cells using various methods, one of which is depositing secondary metabolites in the upper cortex (Hale, 1983). Atranorin is one of the common secondary metabolites, water-insoluble and colorless, and is produced by hyphae cells and transported to, and deposited on, the surface of hyphae. Atranorin is localized in the upper cortex of *P. tinctorum* (Zhao *et al*., 1982). As shown in Tables 1 and 2, individuals (P_1 and P_8) that had a higher atranorin content also had stronger illumination in their growth environment. The fitting, SEM, and HPLC analyses also indicated that the atranorin content was higher in environments with stronger illumination (Figs. 2, 3, and 4). Lichens are a poikilohydric organism with poor water-holding capacity, characterized by its environment with frequent wet-dry cycles. Appropriate amounts of atranorin can absorb light and reduce the intensity of light entering the algal cells, therefore increasing photosymbionts' ability to use light of shorter wavelengths (Hale, 1983). As shown in Fig. 5, atranorin content was strongly positively correlated with illumination and strongly negatively correlated with moisture. Therefore, in *P. tinctorum*, the upper cortex atranorin content might be the chemical index that better responds to illumination conditions in the environment.

Rhizines are rhizomorphs composed of hyphae. In *P. tinctorum*, rhizines, upper and lower cortex, and medulla are resource investments of the symbiont in the mycobiont. Results of the analysis of rhizine density and length of the 12 individuals suggested that density showed a greater degree of negative correlation with length (Fig. 5), which indicated that individuals with a higher rhizine density had shorter rhizines, and vice versa. The environmental factor that was more closely associated with rhizine length was moisture (positive correlation, $p<0.05$). Rhizines can transport dissolved inorganic salts and organic metabolites from the substrate to the fungi. However, the extent of this ability has not been experimentally demonstrated. Lichens generally obtain certain substances through passive wet and dry deposition (Nash, 1996), and it is generally believed that lichens have very limited ability in absorbing nutrients from substrates through rhizines (Hale, 1983). Another function of rhizines is to fix the thallus to the substrate (Honegger, 2009), and lichens tend to have minimal contact with the substrate (Will-Wolf *et al*., 2004). Based on the observation of specimens, the patchy distribution of rhizines also support this statement. Lichenization of fungi and algae is a survival strategy (Honegger, 2009). Lichens usually live in harsh habitats, the net assimilation rate is low, and the resources for building the thallus are limited, which is the main reason for its slow growth. The resource investment of the thallus in each structural part is determined by their function and the ecological environment. This study only analyzed the relationships between these structural parts and a limited number of environmental factors. These structural parts included rhizine density, rhizine length, and the relationship between the two. The types of external environmental factors to which they respond to and the response patterns are questions that warrant further exploration.

Conclusions

The Intergovernmental Panel on Climate Change predicted that the pattern of future climate change is global warming, and most subtropical land areas may experience reduced precipitation (Anon., 2007). The response trend of the distribution pattern of different higher plant populations to climate change may be different, possibly including decreased, increased, and unchanged distribution area, and the disappearance of the population (Lv & Wu, 2009). Wu *et al.,* (2012) found that lichen crust was an indicator for changes in environmental heterogeneity in the Gurbantunggut Desert. Jia *et al.,* (2014) believed that soil physical property varies per vegetation types, and the effect of native vegetation on improving soil physical property is stronger than that of non-native vegetation. In the Dabie Mountains, the distribution, concentration, and timing of annual precipitation all showed spatial differences (Shu *et al*., 2005). The effect of this difference on lichen spatial distribution remains to be studied comprehensively. In addition, in terms of species composition and lichen distribution, whether lichens in the Dabie Mountains show features of the south-north transition is a question worth exploring. The Dabie Mountains are important for water and soil conservation in the Yangtze and Huai watersheds. Lichens directly affect maintenance of the function and structure of the forest ecosystem. Therefore, our research on lichens in this region is important because we explored the response pattern of lichens to environmental changes. A broadened understanding of the mechanisms for maintaining ecosystem structure and function under climate change will aid in protecting vegetation resources.

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