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Species of associated bryophyte and their effect on the yield and quality of *Dendrobium nobile*

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Abstract

Background *Dendrobium nobile* demanding growing environment, unstable yield and high management cost are the key factors restricting the development of its imitation wild cultivation industry. Through the study, it was found that the growth of *Dendrobium nobile* is closely related to the microenvironment of Danxia stone, in which the different associated bryophytes have different effects on the growth of *Dendrobium nobile*. The present study aimed to explore the effects of different associated bryophyte species on the yield and quality of *Dendrobium nobile*, and to clarify the dominant bryophyte species associated with *Dendrobium nobile*, and to provide a scientific basis for the rational cultivation and quality evaluation of *Dendrobium nobile*.

Results It is a rich variety of associated bryophyte with *Dendrobium nobile*. There are identified a total of 15 families, 24 genera and 31 species of bryophyte in the study area, including 13 families, 22 genera and 29 species of mosses and 2 families, 2 genera and 2 species of liverworts, and mosses predominated in the association with *Dendrobium nobile*; Usually 3-9 species of bryophyte were mixed with *Dendrobium nobile*, of which 5-6 species of bryophyte were more common, and the associated bryophyte with *Dendrobium nobile* were only related to the species to which they belonged; The dry matter accumulation, quality and mineral content of *Dendrobium nobile* differed significantly among different species of bryophyte. The coefficients of variation of dry matter accumulation, dendrobine and content of 11 mineral elements of *Dendrobium nobile* in the 35 quadrats were 25.00%, 21.08%, and 11.33%-57.96%, respectively. The biomass, dendrobine and mineral content of *Dendrobium nobile* were analysed by principal component analysis and affiliation function. The results showed that each evaluation method initially screened *Trachycystis microphylla*

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and *Leucobryum juniperoideum* as the preliminary identification of dominant associated bryophytes, and the frequency and coverage of the two bryophytes were significantly higher than that of the rest of the bryophytes, and it was determined that *Trachycystis microphylla* and *Leucobryum juniperoideum* were the dominant associated bryophytes.

Conclusions It is a rich variety of associated bryophyte with *Dendrobium nobile*. The yield and quality of *Dendrobium nobile* differed significantly among different species of bryophyte. It was determined that *Trachycystis microphylla* and *Leucobryum juniperoideum* were the dominant associated bryophytes, which were the two bryophytes associated with *Dendrobium nobile* through mixed growth.

Keywords *Dendrobium nobile*, Bryophyte, Associative relationships, Yield and quality

1. Background

Dendrobium nobile is a perennial epiphytic herb of the genus *Dendrobium* in the family Orchidaceae, which is used as medicine by dried stems [1], and is a rare and valuable Chinese medicinal herb under secondary protection in China. *Dendrobium nobile* is rich in polysaccharides, alkaloids and other active substances, with the effect of improving immunity, lowering blood sugar, anti-tumour, etc., the medicinal value is very high [2, 3]. *Dendrobium nobile* has a long history of artificial cultivation in Chishui City, Guizhou Province, China. Chishui City, relying on the unique geological conditions of *Dendrobium nobile* and the high temperature and high humidity of the climate, has gradually becomed the main source of raw herbs for the market. Chishui City has been used *Dendrobium nobile* in imitation of wild cultivation since the early 1980s to pilot artificial cultivation. In 1998, the State Administration of Traditional Chinese Medicine (State Administration of Traditional Chinese Medicine Sheng [1998] No. 17 document) approved the establishment of *Dendrobium nobile* production base in Chishui City, Guizhou Province, planning to develop the original ecological *Dendrobium nobile* planting, is China's only national *Dendrobium nobile* base. In 2006, Chishui *Dendrobium nobile* was awarded National Geographical Indication Products, and in 2019, it was selected for China's Agricultural Brand Catalogue. At present, the original cultivated area of *Dendrobium nobile* planted in Chishui on Danxia stone under the forest reaches 64 320 hectares, accounting for more than 90% of the national *Dendrobium nobile* cultivation area and

Product quantity.

Dendrobium nobile has high requirements for the growing environment, preferring high-temperature, high- humidity and cool environment, and suitable for growing at an altitude of 300-1 000 m. It is often attached to rocks or tree trunks with humus and bryophyte aggregates. The terrain is mostly cliffs, usually shaded by trees, near water sources, and requires sufficient oblique sunlight, and grows more slowly [4, 5, 6]. *Dendrobium nobile* planted on Danxia stone in imitation of wild conditions from planting to harvesting generally takes more than 3 years, the management cycle is longer and the yield is unstable, which need to invest a lot of manpower, material and financial costs. It is the key factor restricting the development of *Dendrobium nobile* industry at present. Our preliminary research has found that the growth of *Dendrobium nobile* planted on Danxia stone imitating wild conditions was closely related to its microenvironment. The different conditions of shade, direction and surrounding water environment of each Danxia stone scattered in the planting area led to differences in light, temperature, water, fertiliser and bryophyte species in its surface layer. At the same time, there were large differences in the growth of *Dendrobium nobile*, associated bryophyte species and their biomass in different microenvironments. Therefore, we hypothesised that there were differences in the degree of association between different bryophytes and *Dendrobium nobile*. Based on the results of previous studies it is known that bryophyte have many different morphological and physiological characteristics [7, 8, 9] that can have different effects on plant growth. The effect of bryophyte on plant growth may depend on a variety of external factors, and bryophyte can indirectly affect plants positively or negatively by regulating water effectiveness [10, 11], reducing temperature fluctuations [7, 9], increasing the rate of apoplastic decomposition to provide nutrients [12, 13], and potentially contributing to soil nitrogen levels through symbiotic relationships with nitrogen-fixing cyanobacteria [14, 15].

So far, studies on the factors influencing the yield and quality of *Dendrobium nobile* have focused on the harvesting period [4, 16], cultivation substrate [17, 18, 19] climatic conditions [20, 21,22] etc. However, the critical role of microenvironment on the growth of *Dendrobium nobile* has been neglected, while the investigation of *Dendrobium nobile* microenvironment, associated bryophyte species, and the effect of bryophyte on the formation of yield and quality of *Dendrobium nobile* have not been reported yet. So, this study was conducted to identify the associated bryophyte species with *Dendrobium nobile* through field survey sampling, to analyse the effects of different bryophytes on the yield and quality of *Dendrobium nobile*, and to clarify the dominant associated bryophytes with *Dendrobium nobile*. To investigate the formation and interaction of microenvironment of Danxia stone, *Dendrobium nobile* and bryophyte, aiming to provide reference for the rational cultivation and quality evaluation of *Dendrobium nobile*.

2 Results and analyses

2.1 Survey of associated bryophyte species in the study area

Associated bryophyte with *Dendrobium nobile* were identified as 31 species in 15 families and 24 genera (Fig. 1), and their species composition is shown in Table 1. As can be seen from the table, it can be seen that among the associated bryophyte with *Dendrobium nobile*, there are 29 species in 13 families and 22 genera of mosses, and 2 species in 2 families and 2 genera of liverworts. The proportions of mosses in the total families, genera, and species reached 86.67%, 91.67%, and 93.55%, respectively, indicating that associated mosses with *Dendrobium nobile* are better adapted to the local lithophytic environment than liverworts.

Analysing the composition of the 31 bryophytes families (Table 2), the only family containing five genera is the family Bryophyta; There were one and three families containing three and two genera, respectively, with nine species of plants, accounting for 26.67% of the total number of families; The remaining families all contain only one genus of plants, accounting for 66.67% of the total number of families, indicating a high degree of richness, although not many species of associated bryophytes with *Dendrobium nobile* in the region.

Analysing the composition of 31 bryophyte genera (Table 3), there were one genus containing three species of plants, five genera in two species of plants and 18 genera in one species of plants, which accounted for 75.00% of the total number of genera and 58.06% of the total number of species. It indicates that the bryophyte composition monotypic is more in this region and the degree of species diversity of bryophytes is higher.

Fig. 1 31 species of bryophyte

Table 1 Composition of 31 bryophytes species

Species	Genus	Family
Leucobryum juniperoideum	*Leucobryum*	Leucobryaceae
Trachycystis microphylla	*Trachycystis*	Mniaceae
Plagiomnium maximoviczii	*Plagiomnium*	Mniaceae
Fissidens dubius	*Fissidens*	Fissidentaceae
Thuidium kanedae	*Thuidium*	Thuidiaceae
Calohypnum plumiforme	*Hypnum*	Hypnaceae
Hypnum calcicolum	*Hypnum*	Hypnaceae
Eurohypnum leptothallum	*Eurohypnum*	Hypnaceae
Brachythecium glaciale	*Brachythecium*	Brachytheciaceae
Eurhynchium laxirete	*Eurhynchium*	Brachytheciaceae
Eurhynchium longirameum	*Eurhynchium*	Brachytheciaceae
Palamocladium euchloron	*Palamocladium*	Brachytheciaceae
Trichostomum platyphyllum	*Trichostomum*	Pottiaceae
Trichostomum involutum	*Trichostomum*	Pottiaceae
Trichostomum aristatulum	*Trichostomum*	Pottiaceae
Tortella humilis	*Tortella*	Pottiaceae
Tortella tortuosa	*Tortella*	Pottiaceae
Hyophila involuta	*HyopHila*	Pottiaceae
Weissia controversa	*Weissia*	Pottiaceae
Didymodon rufidulus	*Didymodon*	Pottiaceae
Campylopus subfragilis	*Campylopus*	Dicranaceae
Campylopus umbellatus	*Campylopus*	Dicranaceae
Fauriella tenerrima	*Fauriella*	Theliaceae
Racopilum cuspidigerum	*Racopilum*	Racopilaceae
Bryum caespiticium	*Bryum*	Bryaceae
Bryum billarderi	*Bryum*	Bryaceae
Brachymenium capitulatum	*Brachymenium*	Bryaceae
Plagiothecium euryphyllum	*Plagiothecium*	Plagiotheciaceae
Entodon macropodus	*Entodon*	Entodontaceae
Porella densifolia	*Porella*	Porellaceae
Heteroscyphus argutus	*HeteroscypHus*	Geocalycaceae

Table 2 Composition of 31 bryophytes families

Number of genera included in a single family	Number of families	Proportion of total families /%	Number of genus	Proportion of total genus/%	Number of species	Proportion of total number of species/%
5	1	6.25	5	20.83	8	25.81
3	1	6.25	3	12.50	4	12.90
2	3	18.75	6	25.00	5	16.13
1	11	68.75	11	45.83	14	45.16
合计	16	100.00	23	100.00	31	100.00

Table 3 Composition of 31 bryophytes generas

Number of species in a single genus	Number of genus	Proportion of total genus/%	Number of species	Proportion of total species/%
3	1	4.17	3	9.68
2	5	20.83	10	32.26
1	18	75.00	18	58.06
Total	24	100.00	31	100.00

2.2 Analysis of associated bryophyte species on 35 Danxia stones

The species, Coverage, moisture content and biomass of bryophyte were counted of 35 Danxia stones (Table 4 and Fig. 2). From Table 4, a total of 28 bryophyte species were obtained from the 35 quadrats, and only three bryophytes were missing compared to the bryophyte species collected during the previous ecological surveys in the study area, suggesting that the 35 Danxia stones are representative of the distribution of associsted bryophyte in the study area. Three bryophytes, *Eurhynchium laxirete*, *Hypnum calcicolum*, and *Porella densifolia*, were missing from the 35 quadrats, suggesting that these three bryophytes have a relatively weak associative relationship with *Dendrobium nobile*. According to the results of the survey, the associated bryophyte with *Dendrobium nobile* were all a mixture of multiple bryophytes, and unfound no single species of bryophyte. There are obvious differences in bryophyte species between different Danxia stones, with 3-9 species of bryophyte epiphytic to one Danxia stone. Attachment of 5-6 bryophyte species to one Danxia stone was more common (Fig. 3), accounting for 60.00% of the total number of quadrats, followed by attachment of 3, 4, and 7 bryophyte species, accounting for 34.29%, and attachment of 8-9 bryophyte species, accounting for only 5.71%. In addition, bryophyte in 35 quadrats showed large differences in moisture content and biomass accumulation (Fig. 2). Moisture content of 35 quadrats bryophyte ranged from 8.81%-238.78% with a coefficient of variation of 76.27%, and bryophyte biomass ranged from 0.38 g/clump-5.60 g/clump with a coefficient of variation of 48.05%.

Table 4 Bryophyte species and their coverage of 35 quadrats

Number	Bryophyte species	Coverage /%	Number	Bryophyte species	Coverage /%	Number	Bryophyte species	Coverage /%
D1	*Brachythecium glaciale*	5	D13	*Leucobryum juniperoideum*	10	D24	*Trachycystis microphylla*	90
	Bryum caespiticium	5		*Campylopus umbellatus*	5		*Leucobryum juniperoideum*	20
	Leucobryum juniperoideum	5		*Brachythecium glaciale*	5		*Bryum caespiticium*	5
	Fauriella tenerrima	5		*Calohypnum plumiforme*	5		*Campylopus subfragilis*	1
D2	*Trachycystis microphylla*	50		*Plagiothecium euryphyllum*	5		*Heteroscyphus argutus*	1
	Campylopus umbellatus	1	D14	*Thuidium kanedae*	1	D25	*Tortella tortuosa*	80
	Eurohypnum leptothallum	90		*Leucobryum juniperoideum*	50		*Racopilum cuspidigerum*	50
	Brachymenium capitulatum	5		*Trachycystis microphylla*	50		*Fissidens dubius*	50
	Fissidens dubius	5		*Plagiothecium euryphyllum*	5		*Eurhynchium longirameum*	30
D3	*Bryum billarderi*	1		*Racopilum cuspidigerum*	5		*Bryum billarderi*	1
	Eurohypnum leptothallum	90	D15	*Thuidium kanedae*	1	D26	*Thuidium kanedae*	1
	Fauriella tenerrima	1		*Racopilum cuspidigerum*	50		*Trachycystis microphylla*	90
	Brachythecium glaciale	10		*Thuidium kanedae*	10		*Tortella tortuosa*	30
	Thuidium kanedae	1		*Brachythecium glaciale*	5		*Bryum caespiticium*	10
D4	*Trachycystis microphylla*	10		*Fissidens dubius*	5		*Leucobryum juniperoideum*	5
	Leucobryum juniperoideum	1		*Entodon macropodus*	1		*Palamocladium euchloron*	5
	Heteroscyphus argutus	20	D16	*Leucobryum juniperoideum*	60		*Racopilum cuspidigerum*	1
	Racopilum cuspidigerum	10		*Trachycystis microphylla*	30	D27	*Trachycystis microphylla*	90
	Trichostomum involutum	50		*Fissidens dubius*	5		*Tortella tortuosa*	10
	Trachycystis microphylla	5		*Heteroscyphus argutus*	5		*Bryum billarderi*	10
	Bryum billarderi	5		*Campylopus subfragilis*	5		*Brachythecium glaciale*	10
D5	*Leucobryum juniperoideum*	20		*Racopilum cuspidigerum*	5		*Racopilum cuspidigerum*	5
	Bryum billarderi	1		*Thuidium kanedae*	1		*Leucobryum juniperoideum*	1
	Trachycystis microphylla	50	D17	*Brachythecium glaciale*	50	D28	*Racopilum cuspidigerum*	70
D6	*Leucobryum juniperoideum*	50		*Bryum caespiticium*	20		*Bryum caespiticium*	60
	Racopilum cuspidigerum	10		*Thuidium kanedae*	1		*Hyophila involuta*	50
	Brachymenium capitulatum	80		*Campylopus umbellatus*	1		*Fissidens dubius*	5
	Thuidium kanedae	30		*Leucobryum juniperoideum*	1		*Bryum billarderi*	1
	Calohypnum plumiforme	5	D18	*Trachycystis microphylla*	80		*Weissia controversa*	1
	Brachythecium glaciale	1		*Campylopus umbellatus*	5	D29	*Bryum caespiticium*	50
D7	*Trachycystis microphylla*	70		*Leucobryum juniperoideum*	5		*Trachycystis microphylla*	50
	Thuidium kanedae	1		*Trichostomum platyphyllum*	5		*Leucobryum juniperoideum*	10
	Fissidens dubius	5		*Didymodon rufidulus*	5		*Fauriella tenerrima*	5
	Eurohypnum leptothallum	5	D19	*Leucobryum juniperoideum*	10	D30	*Racopilum cuspidigerum*	90
	Trichostomum aristatulum	1		*Trichostomum platyphyllum*	10		*Tortella tortuosa*	50
D8	*Bryum caespiticium*	5		*Fissidens dubius*	5		*Leucobryum juniperoideum*	10
	Calohypnum plumiforme	1		*Trachycystis microphylla*	5	D31	*Trachycystis microphylla*	80
	Thuidium kanedae	1		*Racopilum cuspidigerum*	1		*Thuidium kanedae*	30
	Racopilum cuspidigerum	10	D20	*Trachycystis microphylla*	70		*Bryum billarderi*	10
	Brachythecium glaciale	5		*Leucobryum juniperoideum*	10		*Campylopus umbellatus*	5
D9	*Trachycystis microphylla*	95		*Fauriella tenerrima*	10	D32	*Brachythecium glaciale*	80
	Eurohypnum leptothallum	5		*Calohypnum plumiforme*	1		*Trachycystis microphylla*	50
	Calohypnum plumiforme	1		*Thuidium kanedae*	1		*Fauriella tenerrima*	10
D10	*Leucobryum juniperoideum*	5	D21	*Trachycystis microphylla*	90		*Tortella tortuosa*	10
	Trachycystis microphylla	90		*Leucobryum juniperoideum*	10		*Campylopus umbellatus*	50

	Fissidens dubius	5
	Tortella tortuosa	3
	Fauriella tenerrima	2
	Leucobryum juniperoideum	1
D11	*Trachycystis microphylla*	90
	Leucobryum juniperoideum	1
	Heteroscyphus argutus	1
D12	*Trachycystis microphylla*	90
	Brachythecium glaciale	1
	Campylopus umbellatus	1
	Leucobryum juniperoideum	1
	Calohypnum plumiforme	1
	Heteroscyphus argutus	1
	Bryum billarderi	1
	Bryum caespiticium	10
	Tortella tortuosa	1
D22	*Trachycystis microphylla*	90
	Leucobryum juniperoideum	10
	Tortella tortuosa	5
	Plagiomnium maximoviczii	5
	Fissidens dubius	5
	Bryum billarderi	1
	Racopilum cuspidigerum	1
	Trichostomum platyphyllum	1
	Brachymenium capitulatum	1
D23	*Trachycystis microphylla*	80
	Tortella tortuosa	50
	Bryum caespiticium	10
	Racopilum cuspidigerum	10
	Leucobryum juniperoideum	1
	Tortella humilis	1
D33	*Trachycystis microphylla*	90
	Trichostomum platyphyllum	5
	Tortella tortuosa	5
	Leucobryum juniperoideum	5
D34	*Racopilum cuspidigerum*	90
	Bryum caespiticium	10
	Bryum billarderi	5
	Weissia controversa	10
	Eurohypnum leptothallum	5
	Trichostomum involutum	70
D35	*Campylopus subfragilis*	50
	Bryum billarderi	50
	Fissidens dubius	5
	Leucobryum juniperoideum	1
	Heteroscyphus argutus	1
	Tortella tortuosa	1

Note: Since each bryophyte grows mixed within the same quadrat, the sum of coverage is not 100%.

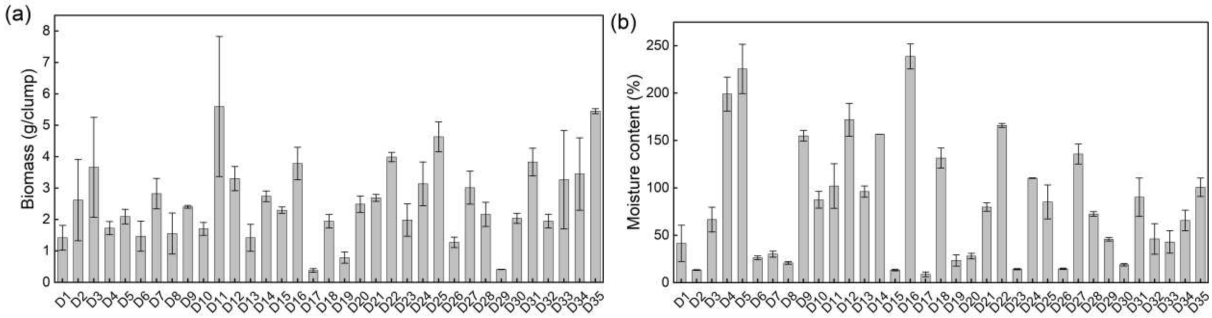


Fig. 2 bryophyte biomass and moisture content of 35 quadrats

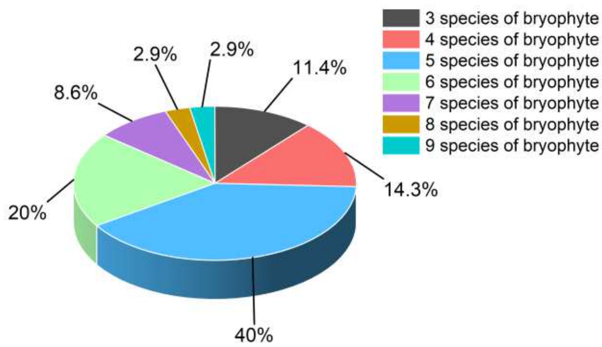


Fig. 3 Differences in 35 quadrats of bryophyte species

2.3 Frequency and coverage analyses of different bryophytes

Frequency and coverage of different species of bryophyte within the 35 quadrats were counted (Fig. 4). There are large differences in the frequency of different species of bryophyte. Among them, *Leucobryum juniperoideum* and *Trachycystis microphylla* were the most common, with both bryophytes growing within 70% of the quadrat at a frequency of 71.43% and 68.57%, respectively; The frequency of 7 bryophytes that is *Racopilum cuspidigerum*, *Thuidium kanedae*, *Bryum billarderi*, *Tortella tortuosa*, *Brachythecium glaciale*, *Bryum caespiticium*, and *Fissidens dubius* was 28.57%-42.86%; 14 bryophytes, *Trichostomum platyphyllum*, *Brachymenium capitulatum*, *Campylopus subfragilis*, *Trichostomum involutum*, *Plagiothecium euryphyllum*, *Weissia controversa*, *Plagiomnium maximoviczii*, *Entodon macropodus*, *Tortella humilis*, *Trichostomum aristatulum*, *Eurhynchium longirameum*, *Weissia controversa*, *Plagiomnium maximoviczii*, *Entodon macropodus*, *Tortella humilis*, *Trichostomum aristatulum*, *Eurhynchium longirameum*, *Didymodon rufidulus*, *Eurhynchium longirameum*, *Didymodon rufidulus*, *Palamocladium euchloron*, *and Hyophila involuta* , had the lowest frequency, ranging from 2.86%-11.43%. Besides, the coverage of different species of bryophyte varied considerably, ranging from 1.00%-65.87%. The large coverage of 3 bryophytes, *Trachycystis microphylla*, *Trichostomum involutum*, *Hyophila involuta*, is more than 50%; The coverage of 6 bryophytes, *Eurohypnum leptothallum*, *Eurhynchium longirameum*, *Brachymenium capitulatum*, *Racopilum cuspidigerum*, *Tortella tortuosa*, *Bryum caespiticium*, was the next highest, ranging from 20.00%-39.00%; The coverage of three bryophytes, *Entodon macropodus*, *Tortella humilis* and *Trichostomum aristatulum*, is the smallest of 1.00%.

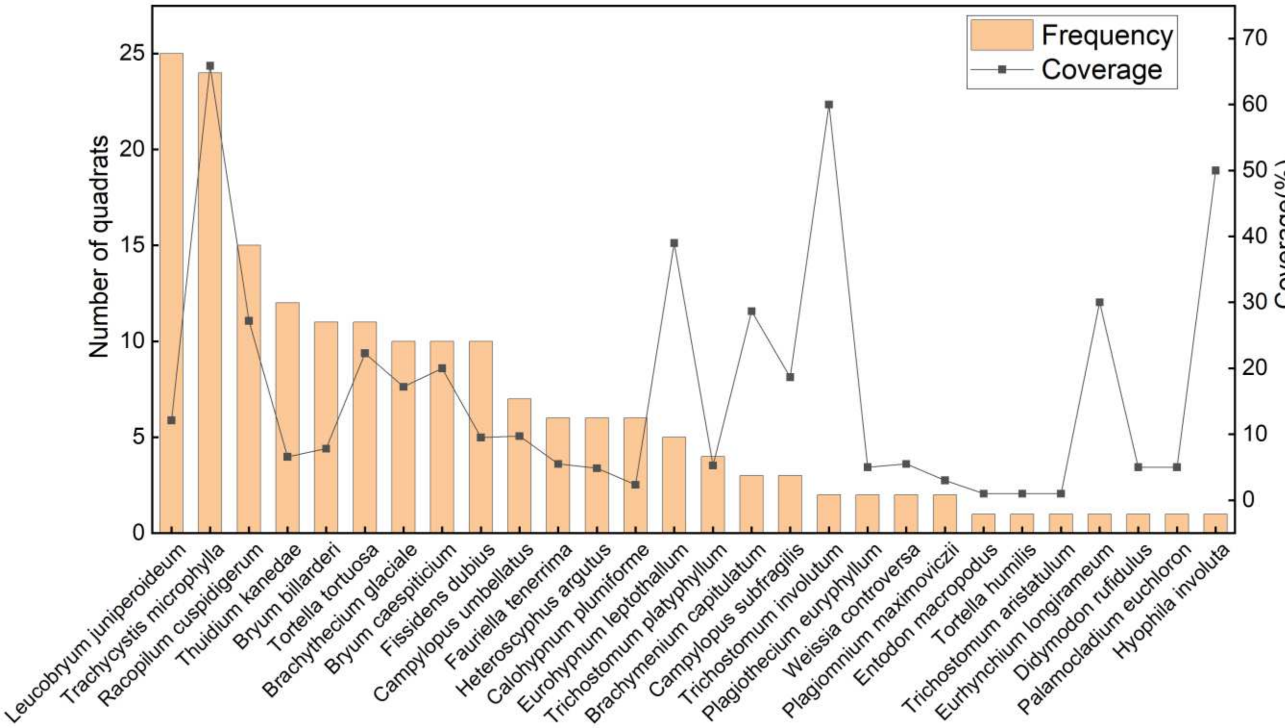


Fig. 4 Frequency and coverage of 28 species of bryophyte

To clarify the relationship between mixed growth among different species of bryophyte. We counted the number of quadrats of all species of bryophyte growing in mixture with each other, and classified the two species of bryophyte with a number of mixed growth quadrats greater than 5 as the preliminary identification of dominant associated bryophytes (Fig. 5). The results showed that only 9 species of bryophyte, *Leucobryum juniperoideum*, *Trachycystis microphylla*, *Racopilum cuspidigerum*, *Thuidium kanedae*, *Bryum billarderi*, *Tortella tortuosa*, *Brachythecium glaciale*, *Bryum caespiticium*, *Fissidens dubius*, grow in mixed quadrats with each other in numbers greater than 5. Among them, *Leucobryum juniperoideum* had the highest frequency of mixed growth with *Trachycystis microphylla* (20 times), which was significantly higher than the rest of the bryophytes. The frequency of mixed growth among the bryophytes was mostly 6 times, with a total of 9 pairs of bryophyte.

We compared the frequency of each bryophyte with coverage and found a weak correlation between them. Of these, only 9 species of bryophyte, *Trachycystis microphylla*, *Plagiothecium euryphyllum*, *Weissia controversa*, *Plagiomnium maximoviczii*, *Entodon macropodus*, *Tortella humilis*, *Trichostomum aristatulum*, *Didymodon rufidulus*, and *Palamocladium euchloron*, had a high correlation between frequency and coverage. In addition, the frequency and coverage of each bryophyte was compared with its family/genus, and it was found that the frequency of occurrence and coverage of bryophyte were not strongly correlated with their family/genus. It suggests that the bryophyte associated with *Dendrobium nobile* are related only to the species to which they belong.

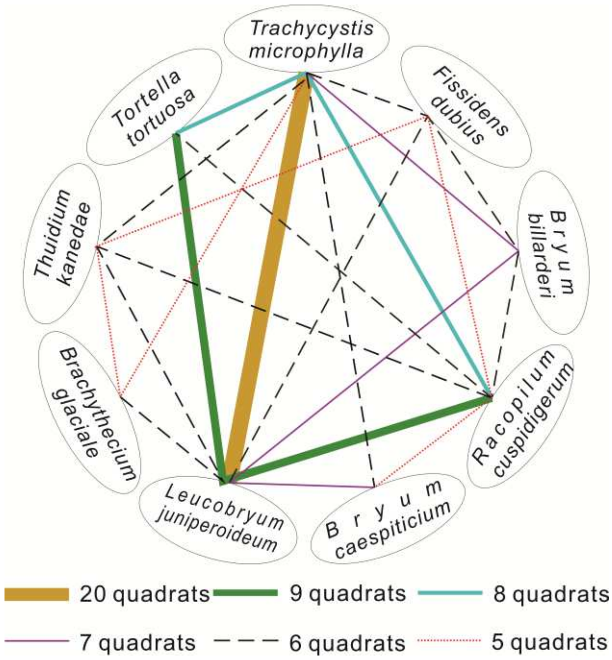


Fig. 5 Number of quadrats of 9 bryophytes growing in mixture with each other

2.4 Effect of different species of bryophyte associated on the biomass of *Dendrobium nobile*

There were differences in the accumulation of dry matter in *Dendrobium nobile* by different species of bryophyte associated (Fig. 6). The dry matter accumulation of 1-, 2- and 3-year-old stems of 35 quadrats was 0.056-0.301 g/branch, 0.338-1.065 g/branch, and 0.147-0.75 g/branch, with coefficients of variation of 35.98%, 24.50%, and 40.13%, respectively.

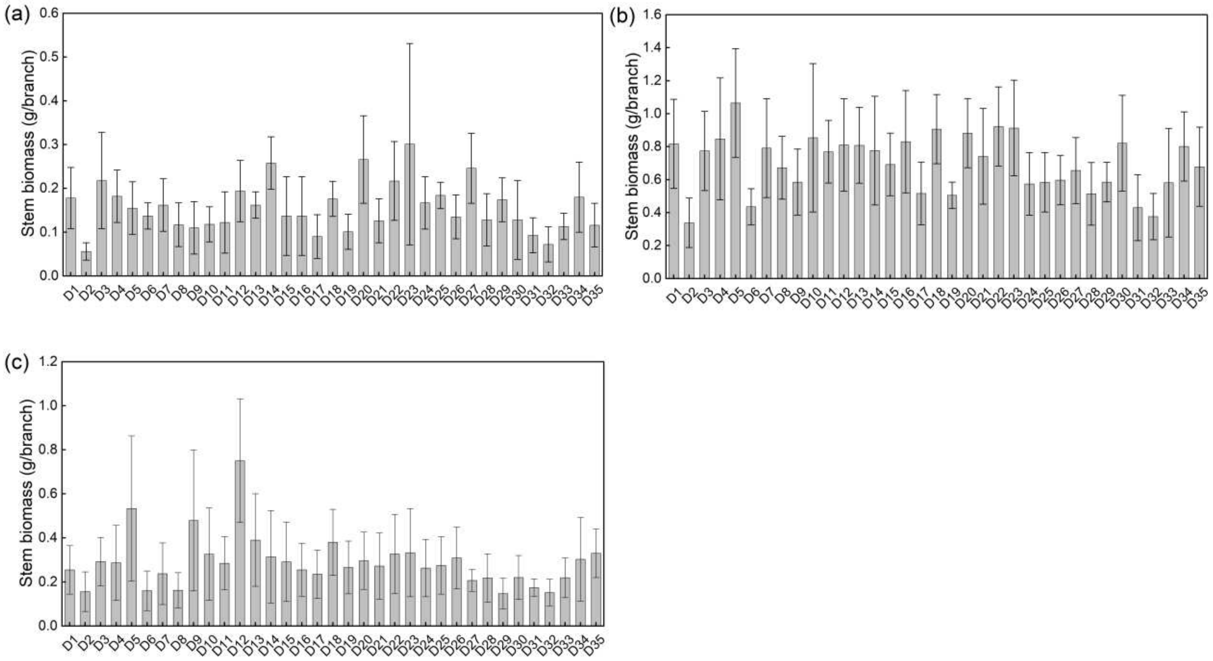


Fig. 6 Dry matter accumulation of *Dendrobium nobile* stems in 35 quadrats. (a)Dry matter accumulation of 1-year-old stems, (b)Dry matter accumulation of 2-year-old stems, (c)Dry matter accumulation of 3-year-old stems

2.5 Effect of different species of bryophyte associated on the quality of *Dendrobium nobile*

The statistical results of the leachate and dendrobine content of the 3-year-old stems of *Dendrobium nobile* from 35 quadrats are shown in the graph (Fig. 7). The leachate content of the 3-year-old stems of *Dendrobium nobile* from 35 quadrats ranged from 23.41%-28.74%, with little variation in content and a coefficient of variation of 5.46%. In contrast, the dendrobine content is significant differences, ranging from 0.30%-0.81%, and a coefficient of variation of 21.08%.

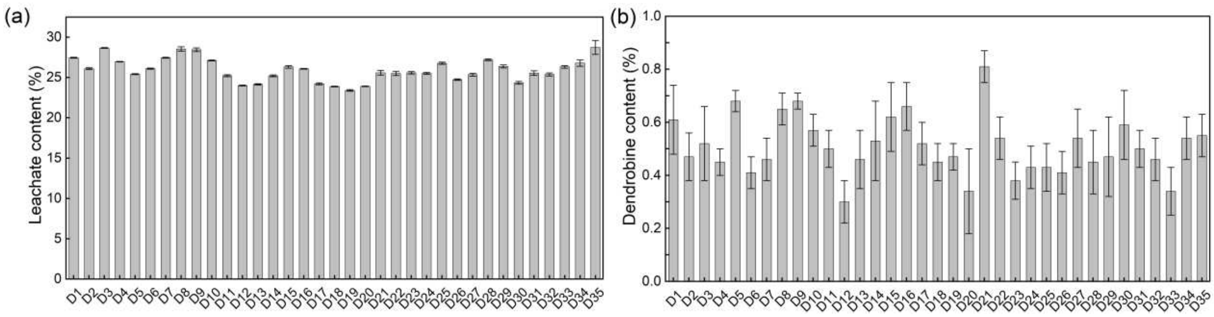
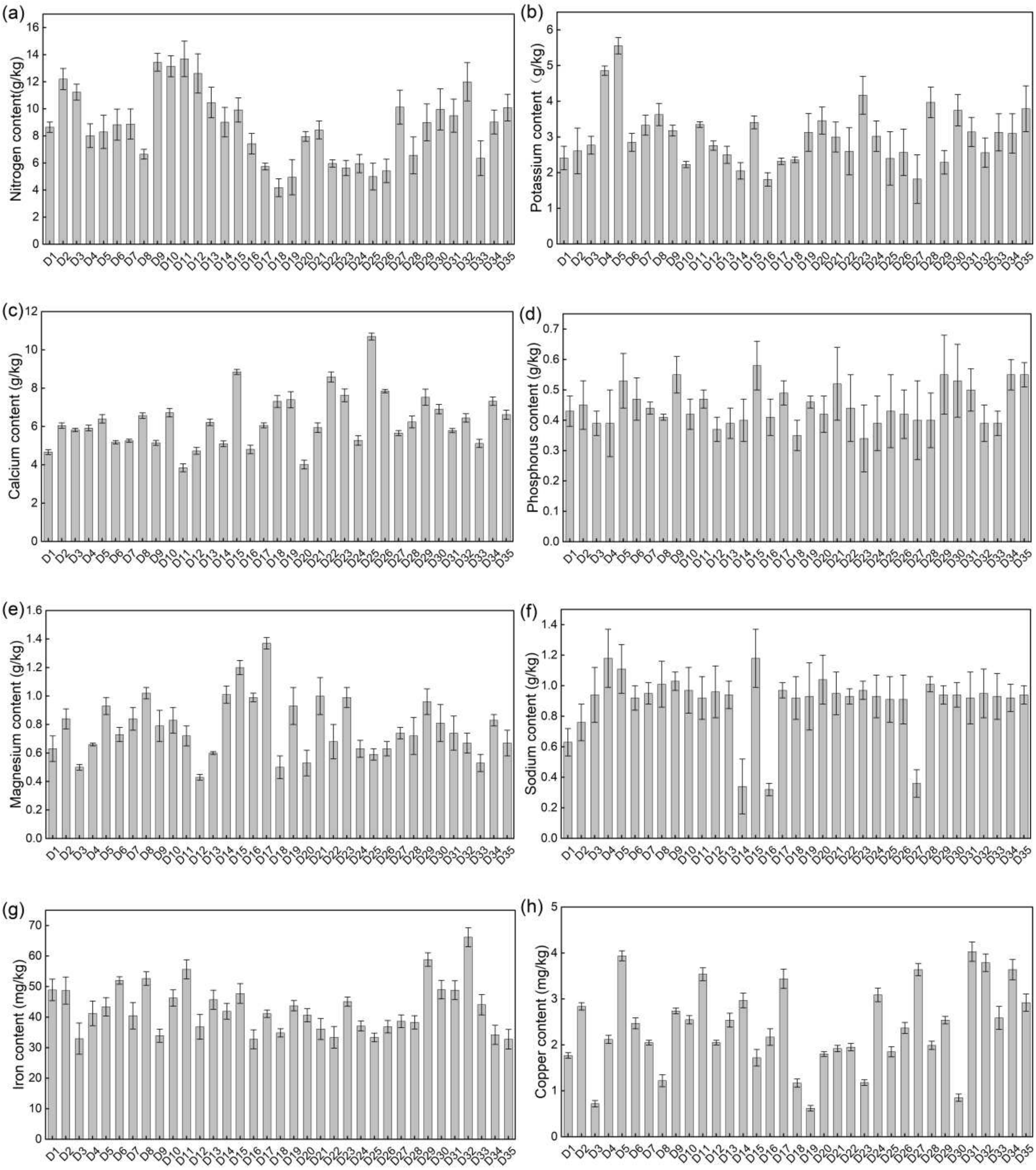


Fig. 7 Leachate and dendrobine content of *Dendrobium nobile* 3-year-old stems in 35 quadrats

2.6 Effect of different species of bryophyte associated on the mineral element content of *Dendrobium nobile*

The content of 11 mineral elements in the 3-year-old stems of *Dendrobium nobile* varied to different degrees among the 35 quadrats (Fig. 8). The content of the 11 elements, N, K, Ca, P, Mg, Na, Fe, Cu, Zn, Mn, B, were 4.18-13.69 g·kg^{-1}, 1.81-5.56 g·kg^{-1}, 3.85-10.70 g·kg^{-1}, 0.34-0.58 g·kg^{-1}, 0.43-1.37 g·kg^{-1}, 0.32-1.18 g·kg^{-1}, 32.75-66.18 mg·kg^{-1}, 0.62-4.03 mg·kg^{-1}, 14.27-58. 44 mg·kg^{-1}, 3.26-23.54 mg·kg^{-1}, 8.29-15.83 mg·kg^{-1}, respectively. The content of each element changed the area of ranging, which is Mn>Cu>Zn>N>K>Mg>Ca>Na>Fe>P>B with coefficients of variation ranging from 11.33% to 57.96%.



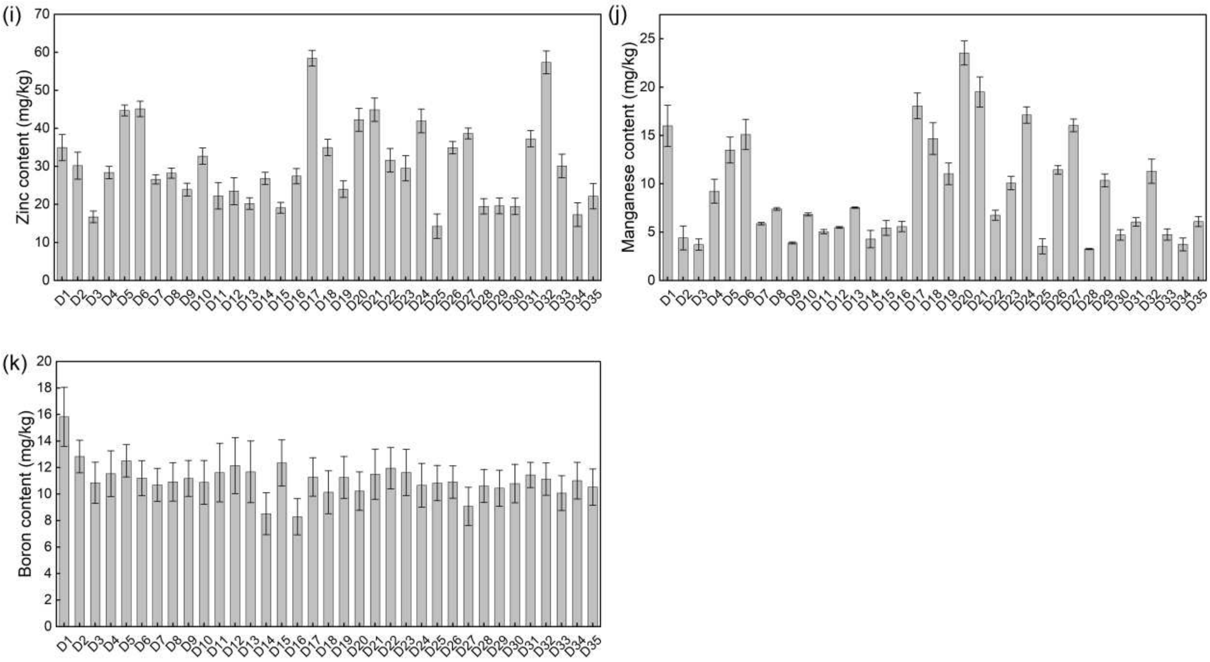


Fig. 8 11 mineral elements content of 3-year-old stems of *Dendrobium nobile* in 35 quadrats

2.7 Correlation analysis

The results of correlation analysis between environmental factors and dry matter, quality and mineral element content of *Dendrobium nobile* are shown in the figure (Fig. 9). The results of correlation analyses of five environmental factors, direction, slope, moisture content of Danxia stone and moisture content and biomass of bryophyte, showed that the moisture content of Danxia stone was significantly and positively correlated with moisture content and biomass of bryophyte (P<0.05), but the correlations among the rest of the environmental factors were not significant.

Correlation analyses of dry matter accumulation, quality and mineral content of different species of bryophyte-associated *Dendrobium nobile* showed that 13 pairs of traits were significantly or highly significantly correlated among 14 indicators. Among them, dry matter showed significant negative correlation (P<0.05) only with elemental Fe; leachate showed significant positive correlation (P<0.05) with dendrobine and significant negative correlation (P<0.05) with Mn. Dendrobine was significantly and positively correlated (P<0.05) with P and Mg; there were 7 pairs of traits that were significantly or highly significantly correlated among the 11 mineral elements. 6 pairs of elements, N-Cu, K-Na, P-Mg, Mg-B, Cu-Zn, and Zn-Mn, were significantly positively correlated(P<0.05) and N-Ca was significantly negatively correlated (P<0.05).

The results of correlation analysis between environmental factors and the dry matter accumulation, quality and mineral element content of *Dendrobium nobile* showed that the moisture content of Danxia stone was significantly negatively correlated with Fe and B ($P<0.05$); the moisture content of bryophyte was significantly positively correlated with dry matter accumulation of *Dendrobium nobile* ($P<0.05$), and significantly negatively correlated with Fe ($P<0.05$); and biomass of bryophyte was significantly negatively correlated with the Mg, Fe and Mn ($P<0.05$).

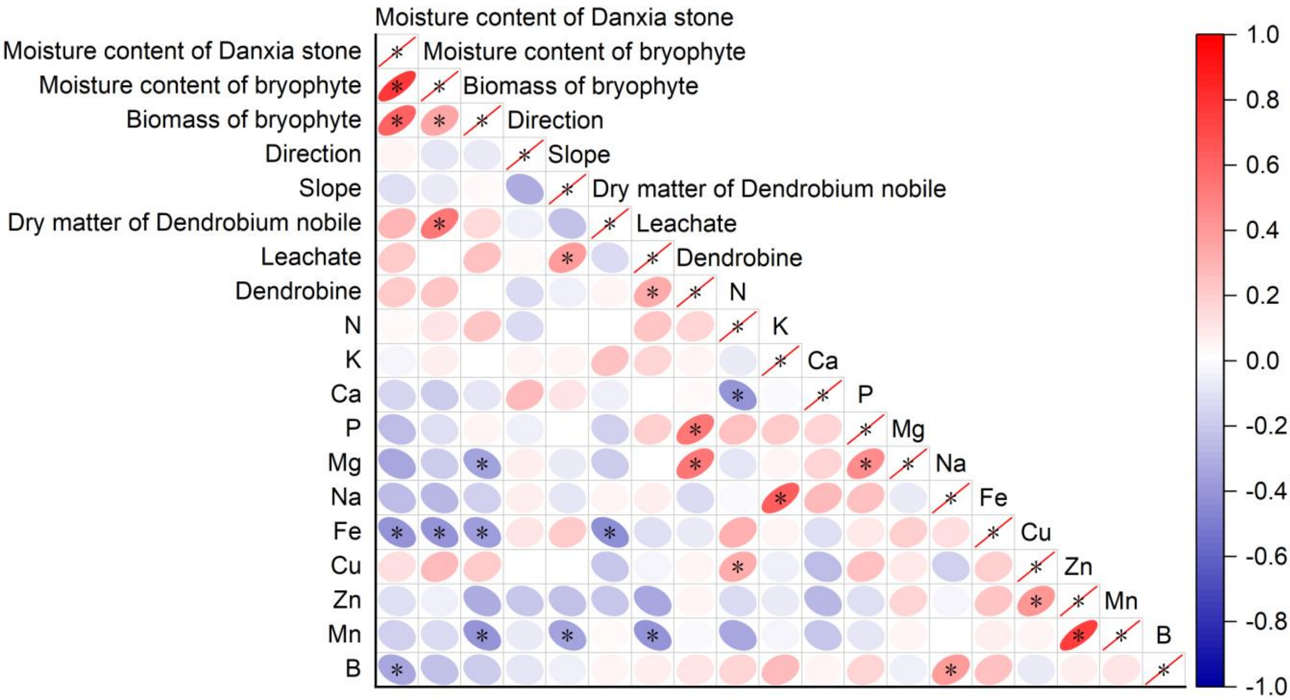


Fig. 9 Correlation analysis of environmental factors with dry matter, quality and mineral element content of *Dendrobium nobile*

2.8 Comprehensive evaluation of yield, quality and mineral element content of *Dendrobium nobile* in 35 quadrats

According to the correlation analysis, the results showed that the correlation was weak among the biomass, quality and mineral content of associated *Dendrobium nobile* of differnent species bryophyte. Due to the non-significant correlation between the indicators, when we evaluate the yield and quality of the 35 quadrats in a comprehensive manner, it may lead to differences between the results of the comprehensive evaluation of multiple indicators and a single indicator. Therefore, in order to clarify the influence of different indexes on the comprehensive evaluation of *Dendrobium nobile*, principal component analysis and affiliation function were used to comprehensively evaluate the biomass, quality and mineral content of *Dendrobium nobile*, and to explore the influence of different species of associated bryophyte on the yield and quality of *Dendrobium nobile*. It is varied greatly that the dry matter accumulation of 1-, 2- and 3-year-old stems of *Dendrobium nobile* in this experiment among all 35 quadrats. So, the biomass of each quadrat was calculated as the total dry matter accumulation of 1-, 2- and 3-year-old stems. Among the quality indicators, the coefficient of variation of leachate content was 5.46%, which was smaller, while the coefficient of variation of dendrobine content was 21.08%, which was larger, so dendrobine content was used as an indicator for quality evaluation of the 35 quadrats. However, it is all large that the coefficient of variation for the content of all 11 mineral elements. So, all elements were used as evaluation indicators. We ranked each quadrat according to its C and D values, and the larger the value, the higher the evaluation. In addition, the single-indicator evaluations of dry matter and quality were ranked according to the size of their measured values.

The scores and ranking results, single indicator evaluation and multi-indicator comprehensive evaluation of dry matter, quality and mineral element content of *Dendrobium nobile* in 35 quadrats, are shown in Table 5. There were four common quadrats in the top 10 of the principal component analysis and affiliation function for multiple indicators (dry matter, quality, and mineral elements), namely D5, D15, D17, and D21, and all were ranked relatively high. The similarity, between the principal component analysis and the affiliation function of the mineral elements and the evaluation results of their corresponding multi-indicators, were high. There are seven identical quadrats in the top 10 of the two principal component analyses, D4, D5, D8, D15, D17, D21, and D30, and also seven identical quadrats in the top 10 of the two affiliation functions, but differences are shown in the numbering of the quadrats, D5, D6, D15, D17, D20, D31, and D32, respectively. It indicates that large differences are gat between different evaluation methods for the comprehensive evaluation of yield and quality of *Dendrobium nobile*. In the evaluation of dry matter and the comprehensive evaluation of principal component analysis and affiliation function of multiple indicators, the top 10 common quadrats were 2 and 3, respectively, with principal component analysis of D4 and D5 and affiliation function of D5, D10 and D20. In the evaluation of dendrobine and the comprehensive evaluation of principal component analysis and affiliation function of multiple indicators, the top 10 common quadrats were 7 and 4, respectively, with principal component analysis of D1, D5, D8, D9, D15, D21, D30, and affiliation function of D5, D10, D15, D21. In the evaluation of dry matter and the comprehensive evaluation of principal component analysis and affiliation function of mineral elements, the top 10 common quadrats were three and two, respectively, with principal component analyses of D4, D5, and D23, and affiliation function of D5 and D20. The evaluation of dendrobine and the comprehensive evaluation of principal component analysis and affiliation function of mineral elements, the top 10 common quadrats were five and three, respectively, with principal component analyses of D5, D8, D15, D21 and D30, and affiliation function of D5, D15 and D21.

In summary, the similarity of the results was obtained when evaluating the principal component analysis and the affiliation function is not high, with only 40% of the top 10 in the same number of quadrats. The order of similarity, between the results of dry matter, dendrobine, and mineral elements when evaluated individually and the comperhensive results of multiple indicators, was mineral elements > dendrobine > dry matter. This indicates that mineral elements are given more weight than dry matter and dendrobine in making a comprehensive evaluation.

Table 5 The top 10 quadrats ranked in the comprehensive evaluation of dry matter, dendrobine and mineral elements in *Dendrobium nobile*

Ranking	Dry matter		Dendrobine		Multiple mineral elements				Multiple indicators (dry matter, dendrobine and mineral elements)			
					Principal component analysis		Affiliation function		Principal component analysis		Affiliation function	
1	D12	1.754	D21	0.81	D15	1.159	D5	0.554	D5	1.411	D5	0.668
2	D5	1.753	D9	0.68	D5	0.943	D17	0.526	D21	1.006	D17	0.569
3	D23	1.547	D5	0.68	D17	0.546	D32	0.509	D15	0.884	D21	0.555
4	D22	1.466	D16	0.66	D19	0.533	D21	0.461	D17	0.747	D32	0.531
5	D18	1.462	D8	0.65	D23	0.521	D6	0.430	D8	0.357	D20	0.484
6	D20	1.444	D15	0.62	D8	0.433	D20	0.426	D9	0.295	D15	0.466
7	D13	1.36	D1	0.61	D21	0.417	D31	0.407	D4	0.256	D6	0.454
8	D14	1.348	D30	0.59	D30	0.396	D15	0.396	D30	0.253	D10	0.445
9	D4	1.316	D10	0.57	D29	0.355	D24	0.391	D1	0.217	D31	0.439
10	D10	1.298	D35	0.55	D4	0.353	D29	0.388	D23	0.185	D11	0.439

The top 10 quadrats were obtained according to each evaluation method, and then the frequency of each bryophyte and its coverage in these 10 quadrats were counted. We identified the bryophyte species with a frequency of occurrence greater than 5 as the preliminary identification of dominant associated bryophytes with *Dendrobium nobile*, and the statistical results are shown in Table 6. As can be seen from the table, the preliminary identification of dominant associated bryophytes screened by all four evaluation methods included *Leucobryum juniperoideum* and *Trachycystis microphylla*. Among them, *Trachycystis microphylla* (9 times) and *Leucobryum juniperoideum* (10 times), which had the strongest correlation with dry matter accumulation, were significantly more frequent than dendrobine, mineral elements, and multiple indicators in the evaluation methods. By comparing the coverage of each preliminary identification of dominant bryophyte, the results showed that the coverage of *Trachycystis microphylla* in dry matter and dendrobine was 67.22% and 67.50%, respectively, which was significantly higher than that of the comprehensive evaluation of mineral elements and multiple indicators, while the difference was not obvious, which is the coverage of *Leucobryum juniperoideum* among different evaluation methods. In summary, *Trachycystis microphylla* and *Leucobryum juniperoideum* were identified as the dominant bryophytes associated with *Dendrobium nobile*.

Table 6 Frequency and coverage of preliminary identification of dominant associated bryophytes (frequency > 5)

Dry matter		Dendrobine		Mineral elements		Comprehensive evaluation of dry matter, dendrobine and mineral elements	
Moss species	Frequency (coverage/%)	Moss species	Frequency (coverage/%)	Moss species	Frequency (coverage/%)	Moss species	Frequency (coverage/%)
Trachycystis microphylla	9（67.22）	*Trachycystis microphylla*	6（67.50）	*Trachycystis microphylla*	6（57.50）	*Trachycystis microphylla*	6（60.00）
Leucobryum juniperoideum	10（15.80）	*Leucobryum juniperoideum*	7（19.57）	*Leucobryum juniperoideum*	6（13.67）	*Leucobryum juniperoideum*	6（16.00）
				Racopilum cuspidigerum	5（16.20）	*Brachythecium glaciale*	5（28.20）
				Brachythecium glaciale	5（28.20）	*Thuidium kanedae*	5（8.60）
				Thuidium kanedae	5（8.60）		

3 Discussion

3.1 Effects of microenvironmental differences in Danxia stone on bryophyte species composition

The distribution of different species is related to biotic and abiotic characteristics of the environment [23, 24], including forest structure, substrate, microorganisms and climatic conditions [25, 26]. The existence and persistence of bryophyte depends largely on the availability of nutrients, water and shade [27, 28, 29]. Among these, human productive activities (deforestation, cultivation, agriculture, and urban expansion, etc.) are one of the main drivers of biodiversity change [30]. In the study area, the planting technique of *Dendrobium nobile* attached Danxia stone was used based on its unique requirements for the growing environment, which requires cleaning of the Danxia stone and its surroundings prior to planting. Firstly, use a brush to clean the surface of the Danxia stone of soil, deadfall and bryophyte to ensure that its roots can attach smoothly; Secondly, if the trees are too dense, we need to cut down some of the trees around the Danxia stone to ensure that it receives sufficient sunlight. For no trees around the Danxia stone, in order to avoid direct sunlight, when summer comes, to pull a shade net above the Danxia stone is needed; Finally, to cope with extreme drought conditions, the study area is set up with a sprinkler system using pipes for proper hydration in drought. Under the interference of these anthropogenic measures, the microenvironment (temperature, light intensity, sunshine hours, moisture, nutrients) of the surface layer of Danxia stone is altered, which provides the possibility of survival for bryophyte to colonise or turnover, e.g., *Trachycystis microphylla* and *Leucobryum juniperoideum* can only survive in the Danxia stone that has a high degree of shading and moisture content. Besides, the successful colonisation of *Dendrobium nobile* provided a more favourable environment for the growth of bryophyte. The root system of *Dendrobium nobile* is attached to the rock surface, and its root system is a rare spongy tissue with better aeration, adsorption and water retention [31], which improves the microenvironment of the surface layer of Danxia stone, which is more conducive to the attachment of bryophyte, and the two interact with each other to improve each other's adaptive ability on Danxia stone. In this study, we found that differences in the microenvironments (moisture content, light intensity and temperature, etc.) of Danxia stone may be the determining factor for the different species compositions of bryophyte. The moisture content of the 35 Danxia stones ranged from 0.91%-10.47%, with a coefficient of variation of 59.61%, which was significantly and positively correlated with the moisture content of the bryophytes (P<0.05). In addition, we found significant differences in shade, light intensity, hours of sunshine and surface temperature for each Danxia stone through field observations. The bryophyte species (3-9 species), coverage (1%-95%), and biomass accumulation (0.38 g/clump-5.60 g/clump) of the 35 Danxia stones also varied considerably, and the moisture content of Danxia stone was significantly and positively correlated with the moisture content and biomass of bryophyte (P<0.05). So, we suggest that the microenvironment of Danxia stone plays an important role in bryophyte species composition.

3.2 Effect of species differences in bryophyte on the growth of *Dendrobium nobile*

Even though research on how bryophyte influence vascular plants has attracted increasing attention during the last decade, much is still unknown of the driving processes behind these effects [12]. The aim of this study was to understand the effect of different species of associated bryophyte on the yield and quality of *Dendrobium nobile* and to identify the dominant bryophyte species associated with it. The results showed that the coefficients of variation of biomass, dendrobine and mineral content of *Dendrobium nobile* were higher in different species of associated bryophyte, ranging 40.13%, 21.08%, 11.33%-57.96%, respectively. Indicates a strong correlation between different species of associated bryophyte and the growth of *Dendrobium nobile*. This finding is consistent with previous findings that the presence of bryophyte can increase the aboveground biomass and height of plants [32, 33]. Although bryophyte are widespread on rocks, the bryophyte layer is highly variable due to differences in their species composition [34, 35]. Bryophyte have many different morphological and physiological characteristics that can have different effects on plants. The reason for this is that the water retention capacity of different bryophytes varies considerably [36, 37], determining the severity of drought exposure of the bryophyte layer, which shows the same effect on the effectiveness of associated plants in resisting drought [38, 39]. This was also confirmed by the significant positive correlation (P<0.05) between the moisture content of the bryophyte and the biomass accumulation of *Dendrobium nobile* in the present study, demonstating that the water-holding properties of bryophyte play an important role in the biomass accumulation of *Dendrobium nobile*. However, it is worth noting that the correlation was not significant between the biomass of bryophyte and the yield and quality of *Dendrobium nobile*, indicating that the thickness and biomass of bryophyte are not the key factors determining the yield and quality of *Dendrobium nobile* during its growth. In addition, due to differences in morphological and physiological characteristics, bryophyte species also differ in spatial competition [7, 40], in their ability to intercept and retain nitrogen [12], and take up phosphate, among other things [41], which can have different effects on the growth of *Dendrobium nobile*, but quantifying these effects further research is needed.

In this study, *Trachycystis microphylla* and *Leucobryum juniperoideum* were analysed and screened as the dominant associated bryophytes of *Dendrobium nobil*e. Both bryophytes showed morphological characteristics of small plant size, erect stems, and dense clumps [42, 43, 44], and had strong water-holding properties. While the rest of the bryophytes performed poorly in terms of water-holding properties, e.g., *Hypnum*'s plants had long, creeping stems, more branched, and loosely interwoven into sheets; The stems of *Fissidens* are single, rarely branched, and sparsely arranged among the plant bodies; Although small and densely clumped, *Bryum* prefers to be borne on slightly drier rocks [44]. As a result, the morphological characteristics and habits of these bryophytes lead to poor water-holding properties. In addition, by observing the growth of *Dendrobium nobile* root system with bryophyte, we found that *Trachycystis microphylla* and *Leucobryum juniperoideum*, due to the dense clumping and tight arrangement, could better wrap the root system of *Dendrobium nobile*, and keep its moist even under long-time drought conditions, which ensured the normal growth of *Dendrobium nobile* to the maximum extent.

In summary, we make preliminary generalisations about the formation of the microenvironments of Danxia stone, *Dendrobium nobile* and bryophyte. The differences in the microenvironments (shade, direction, water and humidity conditions, etc.) in which the Danxia stone itself are located, as well as the intervention of anthropogenic measures during the planting and management of *Dendrobium nobile*, led to changes in the microenvironments of the Danxia stone. That to say, there are subtle differences in environmental factors such as moisture, nutrients, light intensity, sunshine hours and temperature in the surface layer of Danxia stone, which provide suitable environments for the growth of different species of bryophyte. Secondly, after the successful colonisation of bryophyte and *Dendrobium nobile*, the three interacted with each other and gradually formed a stable mini-ecosystem that continuously influenced the growth of *Dendrobium nobile*.

The present study focuses on the important role of moisture in the microenvironment of *Dendrobium nobile* with respect to the experimental results, but the effects of the remaining microenvironmental factors, such as light, nutrients, temperature, and microbial activity, cannot be ignored. Therefore, rational experiments should be designed for these microenvironmental factors in subsequent studies, to understand comprehensively the effects of associated bryophyte on the growth of *Dendrobium nobile*. To explore the effects of these factors on the growth of *Dendrobium nobile* and to study the interactions between them, so as to reveal more systematically the mechanism of formation of the microenvironment of Danxia stone, *Dendrobium nobile* and bryophyte.

4 Conclusion

In summary, it is a rich variety of associated bryophyte of *Dendrobium nobile*. There are identified a total of 15 families, 24 genera and 31 species of bryophyte in the study area, including 13 families, 22 genera and 29 species of mosses and 2 families, 2 genera and 2 species of liverworts, and mosses predominated in the association with *Dendrobium nobile*. In this case, a mix growth of 5-6 bryophytes is more common. Different associated bryophytes had a greater effect on the dry matter accumulation, quality and mineral content of Dendrobium nobile. We determined the dominant associated bryophytes of *Trachycystis microphylla* and *Leucobryum juniperoideum* based on the Principal Component Analysis and the Affiliation Function, and the two bryophytes associated with *Dendrobium nobile* through mixed growth.

5 Materials and methods

5.1. Study location

The test site selected for this study was the representative Yaling Village, Wanglong Town, Chishui City, Guizhou Province, where *Dendrobium nobile* was cultivated by a centralised and continuous planting method in an arboreal understory. Wanglong Town (105°90′N, 28°52′E) is located in the central part of Chishui City, one of the main production areas of Chishui *Dendrobium nobile*. The *Dendrobium nobile* planting base in Yuling Village (105°91′E, 28°51′N), at an altitude of 500 m, has the largest concentrated and continuous planting area of *Dendrobium nobile* in Chishui City (more than 134 hectare). Wanglong Town is located in the river valley semi-alpine hills, the terrain is high in the southeast and low in the northwest, belonging to the subtropical monsoon climate, with an altitude of 228-1 256 m. According to the Chishui Meteorological Bureau, the average annual rainfall in Wanglong Town from 2012 to 2022 is 785.11-1 658.37 mm, and the average annual temperature is 17.37-18.79°C. The exact location of the study area is shown in Fig. 10.

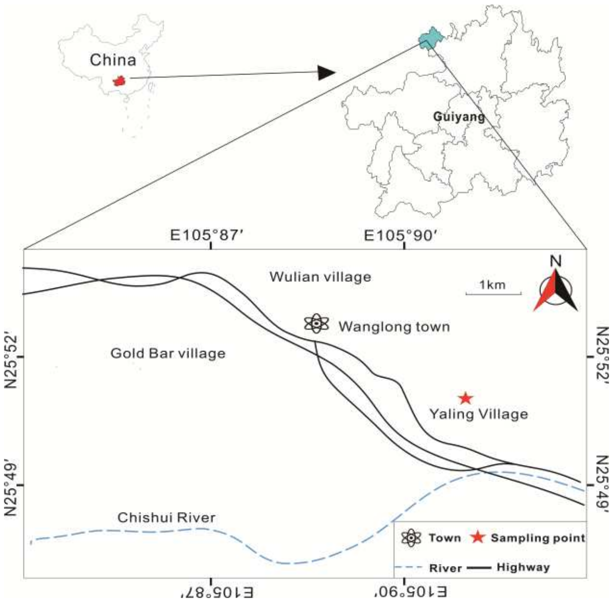


Fig. 10 geographical location of sampling points

5.2 Test materials

Dendrobium nobile: It was identified as *Dendrobium nobile* of the genus *Dendrobium*, family Orchidaceae, by Professor Zhi Zhao of the College of Agriculture, Guizhou University. It is planted for 3 years.

Danxia stone: Rock properties are sandstone.

Bryophyte: associated bryophyte only with the root system of *Dendrobium nobile*.

5.3 Main instrumentation

Plasma Emission Spectrometer (ICP-OES Optima 8000), Fully Automated Kjeldahl Nitrogen Determination (HGK-55), Gas Chromatograph (Agilent 7890A).

5.4 Sample collection

In this experiment, bryophyte specimens were collected twice in the study area, in September 2021 and May 2022. Only the associated bryophyte with *Dendrobium nobile* root system on Danxia stone were collected, so that the collected specimens could represent the status of associated bryophyte with *Dendrobium nobile* in the region, a total of 163 specimens were collected and brought back to the laboratory for drying, and the collected specimens were identified to species. Secondly, 35 Danxia stones were randomly selected in the study area, and the principle of selection was that the area of one face of the Danxia stone was larger than 1.5 m×1.5 m, and the face was set to be one quadrat, with a total of 35 quadrats. In order to ensure that the selected quadrat is representative of *Dendrobium nobile* growth and bryophyte distribution in the study area, the Danxia stone was selected with differences in direction, shade and slope. The 35 quadrats details are shown in Table 7. We collected samples of *Dendrobium nobile*, bryophyte and Danxia stone within quadrat, and chose to collect samples on an overcast day on 5 May 2022, after three consecutive days of sunny days, in order to minimise experimental error. The specific sampling steps were: ①*Dendrobium nobile* sample collection: Each quadrat selected 15 clumps of *Dendrobium nobile* with basically the same growth, and each clump of *Dendrobium nobile* cut off one 1-, 2- and 3-year-old stems with scissors, respectively, and removed the leaves, and the 15 stems were mixed into one sample, which was transported back to the laboratory for the determination of the dry matter accumulation, quality, and mineral element contents of *Dendrobium nobile* stems. ②Bryophyte sample collection: Samples of all species of bryophyte within the quadrat were collected in self-sealing bags and labelled, and the coverage of each bryophyte was counted; 182 samples were collected. Secondly, three clumps of *Dendrobium nobile* were randomly selected from 15 clumps of *Dendrobium nobile* and delimited by 10 cm × 10 cm centred on the base of the stems. Scrape all the bryophyte in the range with a knife, remove the roots and other sundries and seal the collected bryophyte in a self-sealing bag. A total of 105 samples were collected and transported back to the laboratory to determine their fresh and dry weights and to calculate the moisture content and the biomass of the bryophyte. ③Danxia stone sample collection: Three clumps of *Dendrobium nobile* were selected from each quadrat, and Danxia stone samples were collected from the delineated area after the bryophyte samples collected. About 0.5 cm of the surface layer of Danxia stone was chiselled with a geological hammer to obtain lumpy or powdery samples of Danxia stone, removed roots and other sundries and the obtained samples of Danxia stone were sealed in self-sealing bags. A total of 105 samples of Danxia stone were collected, determined their fresh and dry weights and moisture content (Table 7).

Table 7 Basic information of 35 Danxia stones

Number	Direction	Slope/°	moisture content/%	Number	Direction	Slope/°	moisture content/%	Number	Direction	Slope/°	moisture content/%
D1	Northeast	90	2.57	D13	West	90	4.34	D25	Southwest	90	7.89
D2	West	90	1.01	D14	North	90	6.64	D26	Northeast	90	1.77
D3	Northeast	90	6.16	D15	East	90	1.45	D27	Northeast	90	6.37
D4	Northwest	90	7.28	D16	Northeast	90	10.47	D28	West	90	2.77
D5	North	90	6.11	D17	West	45	1.21	D29	Southeast	90	1.07
D6	Towards the top	180	1.51	D18	North	90	7.13	D30	West	90	1.67
D7	Towards the top	180	1.35	D19	East	90	1.10	D31	Northeast	90	3.55
D8	West	80	6.71	D20	North	30	1.98	D32	Northeast	90	4.76
D9	East	80	6.48	D21	North	60	6.08	D33	Northwest	90	6.07
D10	Northwest	90	3.54	D22	Northeast	80	6.94	D34	North	90	2.61
D11	North	90	7.48	D23	West	90	0.91	D35	West	90	8.68
D12	North	30	6.95	D24	Northwest	90	8.35				

5.5 Measurements

5.5.1 Determination of biomass, quality and mineral content of *Dendrobium nobile*

Biomass determination:

The dry weight of 15 stems per sample was determined as follows: Firstly, the fresh weight (g/branch) of the 15 stems was weighed respectively in the natural water content state of the plant and secondly the total fresh weight of the 15 stems. Subsequently, the stems were cut into small sections and baked in an oven at 60°C until constant weight, the dry weight was weighed and the drying rate of the stems was calculated, and finally the dry weight of a single stem was calculated based on the drying rate (g/branch). Drying rate formula:

$$\text{Drying rate (\%)} = \frac{\text{Fresh weight of } \textit{Dendrobium nobile}}{\text{dry weightof } \textit{Dendrobium nobile}} \quad (1)$$

Quality determination:

Measurement of leachate content: Determination of ethanol leachate was carried out according to the hot leaching method under the method for determination of alcohol-soluble leachate in Part IV of the 2020 edition of the Chinese Pharmacopoeia (General rule 2201) [45].

Determination of dendrobine content: The content of dendrobine was determined by gas chromatography (General rule 0521) with reference to the 2020 edition of the Chinese Pharmacopoeia [46]. Dendrobine control solution was prepared at a mass concentration of 52.5 μg·mL^{-1}, aspirated 1 μl and injected into a gas chromatograph, recorded the chromatogram of Dendrobine. The chromatographic column was a DB-1 capillary column with programmed temperature increase: the initial temperature was 80 °C, then the temperature was increased to 250 °C at a rate of 10 °C per minute, and held for 5 min. The temperature of sample inlet and the FID detector was 250 °C; the carrier gas was nitrogen，the split ratio of 5:1，the flow rate of hydrogen of 30 mL·min^{-1}，the flow rate of air of 300 mL·min^{-1}, and the flow rate of the tailblow gas was 30 mL·min^{-1}. Besides Theoretical plate number not less than 10 000. The standard curve was plotted using the concentration as the horizontal coordinate and the peak area ratio (each concentration of the reference solution (s) required/peak area of the internal standard) as the vertical coordinate. The standard curve equation: $y = 0.102x-0.06$ ($R^2 = 0.9997$) indicates that Dendrobine has good linearity in the range of 1.0-20.0 μg·mL^{-1}.

Mineral element content determination:

Elemental N content was determined by using a Kjeldahl nitrogen apparatus, in accordance with the 2020 edition of the Chinese Pharmacopoeia (General rule 0731) in the protein content determination method by Kjeldahl nitrogen determination [45]. The elemental contents of K, Ca, P, Mg, Na, Fe, Cu, Zn, Mn and B were determined by plasma emission spectrometry (ICP-OES), and Reference substance for each element were provided by China Nonferrous Metals and Electronic Materials Analysis and Testing Centre.

5.5.2 Bryophyte species identification, frequency, coverage, biomass and moisture content determination

Species identification: The collected specimens were identified by Professor Yuanxin Xiong and Associate Professor Wei Cao from the School of Life Sciences, Guizhou University, and the specimens were identified to species.

Frequency, coverage and moisture content calculations:

Frequency: The number of occurrences of each bryophyte in all quadrats; Frequency (%): the number of times a bryophyte occurs as a proportion of the number of all quadrats; Coverage (%): estimated using the grid method, i.e. the proportion of the area of one type of bryophyte growth within the quadrat to the area of the Danxia stone, expressed as a percentage; Moisture content (%): 3 clumps of *Dendrobium nobile* were randomly selected from each quadrat, and an area of 10 cm × 10 cm was delineated with the base of *Dendrobium nobile* stems as the centre, and all the bryophyte within the area were scraped with a knife to remove the root system and other sundries. The bryophyte obtained were sealed in self-sealing bags and brought back to the laboratory to be weighed fresh. Subsequently the bryophytes were dried to get weighed dry and calculate their moisture content. Frequency and moisture content are calculated by the formula:

$$\text{Frequency (\%)} = \frac{\text{Frequency of occurrence of bryophyte}}{\text{Total number of quadrats}} \quad (2)$$

$$\text{Moisture content (\%)} = \frac{\text{Moisture weight}}{\text{Dry weight of bryophyte}} \quad (3)$$

5.5.3 The moisture content of Danxia stone

Three clumps of *Dendrobium nobile* selected based on the bryophyte moisture content determination were delineated in a 10 cm × 10 cm area centred on the base of *Dendrobium nobile* stems, and the surface layer was chiseled with a geological hammer for about 0.5 cm from the surface of the Danxia stone, to obtain samples of lumpy and powdered Danxia stone, with the root system and other sundries removed. Samples of the Danxia stone obtained were sealed in self-sealing bags and brought back to the laboratory to be weighed fresh. Subsequently these samples were dried to get weighed dry and calculate their moisture content. Moisture content are calculated by the formula:

Moisture content (%) = $\frac{\text{Moisture weight}}{\text{Dry weight of Danxia stone}}$ (4)

5.6 Data processing and statistical methods

SPSS 25.0 and Excel 2019 were applied to statistically analyse the data and Origin 9.8 with CoreIDRAW 2020 software was used for graphing. We used principal component analysis and an affiliation function conducting comprehensive evaluation.

Principal component analysis: Data were standardised for each indicator using SPSS 25.0 software and factors with eigenvalues greater than 1 were extracted as principal components. The coefficients of each principal component composite indicator were obtained by principal component analysis and the corresponding product was performed to calculate the score (C-value) of each quadrat composite indicator [47].

Affiliation function evaluation: The value of the affiliation function *U (x)* for each quadrat was calculated according to equation (5), the weight of each indicator was calculated according to equation (6), and the value of the composite score for each sample (D-value) was derived using equation (7) [48, 49].

$$U_{(Xij)} = \frac{(X - Xmin)}{(Xmax - Xmin)}; (i=1, 2, 3,\ldots, n) \quad (5)$$

In the formula, *X* is the measured value, *Xmax* is the maximum value and *Xmin* is the minimum value.

$$W_i = \frac{Vi}{Vn} \quad (6)$$

In the formula, *Wi* denotes the weight of the i-th indicator extracted, expressed as a percentage, *Vi* is the coefficient of variation of indicator *i* (*i*=1, 2, 3,…, n), and *Vn* is the sum of the n coefficients of variation sought.

Comprehensive affiliation function value (D-value) = $\frac{\sum[U(Xij)\times Wi]}{Wn}$ (7)

In the formula, *Wn* represents the sum of the indicator percentages.

Abbreviations

N Nitrogen

K Potassium

Ca Calcium

P Phosphorus

Mg Magnesium

Na Sodium

Fe Iron

Cu Copper

Zn Zinc

Mn Manganese

B Boron

C-value Scores from Principal Component Analysis

D-value Score of the affiliation function

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Statement of collect plant samples

The plants involved in the manuscript are not rare and endangered plants and are not included in the IUCN protection list.

The plants were collected at Yaling Village, Wanglong Town, Chishui City, Guizhou Province, China, with the permission of Xintian Traditional Chinese Medicine Industry Development Co. Ltd. The specimens are now deposited in Key Laboratory of Breeding and Cultivation of Medicinal Plants of Guizhou Province.

Authors' contributions

Mingsong Li, Chunli Luo, Fulai Luo, Hualei Wang, and Jiyong Yang were responsible for collecting the samples and testing the physiological indexes. Lujun Deng and Zhi Zhao were responsible for collating the data, and Mingsong Li was responsible for writing the paper. Jinling Li was responsible for revising the original manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

The authors confrm that all methods and plant materials comply with local and national regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no confict of interest.

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