

Addition *P. massoniana* fallen wood improved the growth of *Plagiomnium acutum* in a substrate cultivation

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Abstract

The main purpose of this study was to evaluate the effects of *Pinus massoniana* fallen wood as a soilless culture substrate on the growth and physiology of the ornamental bryophyte - *Plagiomnium acutum*. The results showed that V Ⅴ decomposition grade *Pinus massoniana* fallen log: V grass charcoal soil =1:1 (SW8) substrate had the highest overall evaluation index, which meant the growth condition of *P. acutum* in SW8 was the best. The total porosity, water-holding pore space, and water-holding capacity of the mixed substrate containing fallen wood of *P. massoniana* were significantly higher than those of other cultivated substrates. Pearson's correlation analysis showed that the average cover of the *P. acutum* was significantly and positively correlated with the substrate's total porosity and water-holding porosity. The average length was significantly and negatively correlated with the total potassium content of the substrate. Chlorophyll content was highly significantly and positively correlated with the water holding capacity and total nitrogen content of the substrate and highly significantly and negatively correlated with the total potassium content of the substrate. The soluble protein content was highly significantly and positively correlated with the total phosphorus content of the substrate. In summary, SW8 treatment could be used as the best substrate for cultivating *P. acutum*. The addition of *P. massoniana* fallen wood to the soil substrate increased the total porosity, water-holding pore space, and water-holding capacity of the substrate, which was conducive to the growth of *P. acutum* and the increase of chlorophyll content.

Introduction

Soilless growing systems are characterized by dense plants roots confined in a small volume of growth medium, as well as high rates of metabolic activity, respiration and growth. [1] In recent years, soilless culture has been widely used in vegetable and flower production. Compared to soil cultivation, the physicochemical properties of soilless substrates are more homogeneous, have low pH buffering capacity, saving water and nutrients and reducing the waste of resources [2]. Soilless culture was mainly divided into three types: substrate culture, hydroponics, and aerosol culture, among which substrate culture was the most widely used in practical production [3]. According to the different substrate, cultivation could be divided into three types: inorganic substrate cultivation, organic substrate cultivation, and mixed substrate cultivation. Among them, the mixed substrate was made by mixing materials with different structure and properties, and the advantages of physical and chemical properties can be complemented between each material component, which made the mixed substrate better than inorganic and organic substrate in terms of water, gas and fertilizer coordination [4]. Soilless culture technology had many advantages, but there were fewer studies of their application in the cultivation of bryophytes plants. Selecting a new high-quality cultivation substrate suitable for the growth of bryophytes was the key to the industrial cultivation of bryophytes.

Plagiomnium acutum (Lindb.) T. Kop has long branches and strong covering ability, and beautiful appearance, so it was often used in garden landscaping, bonsai, micro landscape, and as moss jade ball. It has hemostatic function[5] and was also a common plant material for making ecological tanks, which meant high medicinal value and market application value. At present, there were few studies on the propagation and cultivation of *P. acutum*, and the huge market demand has led to severe harvesting of *P. acutum* in the wild, which has caused severe damage to the natural ecological environment. Therefore, rapid and effective propagation and cultivation technique is a critical way to address the market demand of *P. acutum*.

Pinus massoniana was a significant forest species and occupies first place in terms of forest area and accumulation in Guizhou Province. With the natural regeneration and succession of forests, there are a large number of *P. massoniana* fallen wood and abundant epiphytic bryophytes growing on them. Bryophytes have high water storage capacity [6] and nitrogen fixation capacity, which can promote the decomposition of fallen wood and increase the availability of soil nitrogen, which is conducive to the nutrient cycling and growth of forest and plays a non-negligible role in the material cycling of forest ecosystem. In recent years, a large number of studies have proved that bryophytes play an important role in forest regeneration and nutrient cycling [7-8], and other studies have found that bryophytes grow better on fallen forest wood [9-10], the degree of decaying wood affects the life shape and gametophyte growth of bryophytes [11-12]. It can be seen that bryophytes interact with forest fallen wood in a mutualistic way, but what is the mechanism by which forest fallen wood affect the growth of bryophytes? It is urgent to study whether it can be used as a new excellent culture substrate to cultivate moss.

Based on this, this study selected the present high market application value of *P. acutum* as the cultivation material, and mixtures of four kinds of soils and different decomposition grades of fallen *P. massoniana* for moss cultivation was used as the substrate material to evaluate whether the mixed substrate of *P. massoniana* fallen wood could be used as a new soilless culture substrate to promote the growth of *P. acutum*. By studying the formulation of suitable substrate and related influencing factors, we aim to provide theoretical basis and technical support for the propagation and cultivation of *P. acutum*.

Results

Analysis of physical and chemical properties of different cultivation substrates

From Table 1 and Table 2, it can be seen that the total porosity, water holding capacity and water holding capacity of soil-wood mixture substrate (SW) are significantly increased after the fallen wood was added into the substrate, while the bulk density of SW decreases. Treatments with charcoal soil had significantly more total nitrogen, while with granular soil had more total phosphorus, and with garden soil or general nutrient soil component had more total potassium in the substrates.

Table 1

Basic physicochemical properties of soil-soil mixed substrates

Treatment	BD	TP/%	AFP	WFP	AFP/WFP	θf	TN	TP	TK
	g.cm ⁻³	%	%	%		%	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹
SS1	0.81±0.03 a	45.02±6.63 de	13.70±2.33 b	31.33±6.99 de	0.46±0.14 b	138.92±9.86 e	0.26±0.01 d	0.83±0.01 c	3.33±0.07 e
SS2	0.70±0.04 b	65.18±4.58 a	17.18±2.29 b	48.01±4.08 ab	0.36±0.06 b	169.07±10.00 cd	0.64±0.24 c	0.63±0.01 d	4.76±0.08 b
SS3	0.35±0.03 d	53.89±3.78 bc	14.58±0.86 b	39.31±2.99 bcd	0.37±0.01 b	214.12±13.97 a	1.17±0.11 b	0.70±0.03 d	5.03±0.21 a
SS4	0.84±0.03 a	48.48±2.68 cd	5.76±3.36 d	42.72±5.58 bc	0.14±0.01 c	150.52±5.55 de	0.16±0.02 d	1.09±0.05 b	4.05±0.05 d
SS5	0.43±0.03 c	39.08±6.91 e	9.70±1.29 c	29.37±5.88 e	0.33±0.04 b	169.39±9.15 cd	1.99±0.31 a	1.43±0.09 a	1.96±0.03 f
SS6	0.38±0.03 cd	60.54±3.20 ab	25.61±0.86 a	34.93±2.70 cde	0.74±0.05 a	192.11±13.91 b	1.76±0.21 a	0.53±0.03 e	4.32±0.09 c
SS7	0.65±0.02 b	62.70±3.59 a	8.93±1.07 cd	53.76±3.90 a	0.17±0.03 c	183.11±8.90 bc	0.65±0.06 c	0.89±0.02 c	4.26±0.09 c

Means in columns followed by different lowercase letters are significantly different at $P < 0.05$. BD–Bulk density; TP–Total porosity; AFP–Aeration porosity; WFP–Water-holding porosity; AFP/WFP–The void ratio; θf–Water holding capacity; TN– total nitrogen; TP– total phosphorus; TK– total potassium

Table 2

Basic physicochemical properties of soil-wood mixed substrates

Treatment	BD	TP/%	AFP	WFP	AFP/WFP	θf	TN	TP	TK
	g.cm ⁻³	%	%	%		%	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹
SW1	0.48±0.03 bc	71.99±0.17 c	5.79±1.32 fgh	66.20±1.46 bc	0.09±0.02 ef	238.31±7.82 ef	0.68±0.09 c	0.51±0.01 d	4.11±0.13 c
SW2	0.51±0.03 b	67.32±9.14 c	5.06±1.13 gh	62.26±8.20 bc	0.08±0.01 ef	220.85±13.63 efg	0.20±0.02 c	1.12±0.07 b	1.89±0.06 e
SW3	0.38±0.01 d	74.52±4.03 c	7.97±1.01 def	66.55±5.04 bc	0.12±0.02 def	275.39±10.82 d	0.32±0.01 c	0.63±0.02 c	4.46±0.19 b
SW4	0.13±0.01 e	84.64±1.95 ab	14.53±1.50 b	70.11±3.26 b	0.21±0.03 b	655.43±20.70 b	2.45±0.47 b	0.34±0.01 e	0.47±0.02 g
SW5	0.49±0.02 bc	76.56±3.53 bc	8.55±1.42 de	68.01±4.78 bc	0.13±0.03 cde	238.24±8.97 ef	0.59±0.12 c	0.49±0.01 e	3.94±0.19 c
SW6	0.58±0.03 a	75.26±1.82 c	10.59±0.57 cd	64.67±1.92 bc	0.16±0.01 bcd	211.70±2.71 fg	0.09±0.01 c	1.30±0.16 a	1.91±0.06 e
SW7	0.42±0.00 d	69.83±5.93 c	10.50±1.49 cd	59.33±4.58 c	0.18±0.01 bc	242.18±9.68 e	0.29±0.05 c	0.58±0.01 cd	4.16±0.20 c
SW8	0.14±0.01 e	73.08±6.51 c	4.78±0.69 h	68.30±6.14 bc	0.07±0.01 f	586.14±17.01 c	4.15±1.47 a	0.38±0.00 e	0.62±0.01 g
SW9	0.47±0.03 c	74.67±5.26 c	12.64±1.25 bc	62.03±5.37 bc	0.20±0.03 b	232.73±20.12 ef	0.54±0.10 c	0.49±0.01 d	3.62±0.19 d
SW10	0.51±0.03 bc	70.98±4.60 c	22.81±1.08 a	48.17±5.15 d	0.48±0.06 a	194.63±12.32 g	0.24±0.06 c	1.06±0.05 b	1.73±0.12 e
SW11	0.41±0.02 d	83.96±5.35 ab	12.78±3036 bc	71.18±4.88 b	0.18±0.05 bc	273.17±4.66 d	0.37±0.15 c	0.61±0.01 c	4.89±0.10 a
SW12	0.14±0.01 e	88.62±0.08 a	7.48±0.13 efg	81.13±0.22 a	0.09±0.00 ef	690.38±33.79 a	2.93±0.54 b	0.39±0.01 e	0.86±0.01 f

Means in columns followed by different lowercase letters are significantly different at $P < 0.05$. BD–Bulk density; TP–Total porosity; AFP–Aeration porosity; WFP–Water-holding porosity; AFP/WFP–The void ratio; θf–Water holding capacity; TN– total nitrogen; TP– total phosphorus; TK– total potassium

Effects of different substrate treatments on the growth and morphological indexes of *P. acutum*

There were significant differences in the growth length and coverage under different mixed substrates in different periods (Fig 1). As shown in Figure 1 (A and B), at 30 days of cultivation, the length of *P. acutum* grown on the SW12 substrate was the longest, 0.80 cm, while the length on SW3 substrate was the shortest, 0.39 cm. The growth rate of *P. acutum* was the fastest during 30 days-50 days, and slowed down during 50 days-60 days, which may be because the air humidity was higher in 30 days-50 days than that in 50 days -60 days, which led to a slowdown in the growth rate(Fig 2). The maximum growth length reached 2.87 cm among all treatments at 90 days under SW4 substrate treatment.

It can be seen from Figure 1 (C and D) that the growth coverage under SW8 substrate was the highest (4.67%), while that under SS3 substrate was the lowest (0.96%). In all treatments, the growth coverage of *P. acutum* increased from 30d to 90d, but decreased from 90d to 120d, which was possibly because the temperature rose and air humidity decreased significantly during the period, and then affected the growth and spread of the moss (Fig 2). During the whole experiment period, the maximum growth coverage reached 18.83% at 100d under SW5 treatment in all treatments.

To precisely determine the differences in the growth conditions of *P. acutum* in different cultivation substrates, the average growth length and coverage under different treatments were taken for each period within 120 days (Fig. 1). As shown in Figure 1 (E and F), among all the treatments, the average growth length of *P. acutum* was the largest in SW4, and the average coverage was the largest under SW5, while the average length and coverage of SS3 were the smallest.

Effects of different substrate treatments on some physiological indexes of *P. acutum*

It could be seen from Tables 3 and 4 that different mixed substrates havd significant effects on some physiological indexes of *P. acutum*. Chlorophyll can reflect the photosynthetic ability of plants [13], and photosynthesis of appropriate intensity is conducive to the accumulation of organic matter in the plant [14]. The content of chlorophyll a, chlorophyll b, and total chlorophyll of *P. acutum* in SS1 was the highest, which was significantly different from the other treatments in the soil-soil mixed substrate treatment. In the soil-wood mixed substrate treatment, the chlorophyll a, chlorophyll b, chlorophyll a/b value, and total chlorophyll content of of *P. acutum* with SW8 were the most abundan, while those in SW7 were the least, which showed that the growth and metabolic capacity of *P. acutum* under SW8 was the most robust performance among all treatments. Soluble sugars provide most of the energy needed by plant growth, and soluble protein content reflects the level of enzyme activity in the plant. In higher plants, soluble sugars and soluble proteins maintain the osmotic balance of the plant body cells and improve plant resistance [15]. Soluble sugars and soluble protein content contributes to the improvement of plants resistance. Among all the treatments, the soluble sugar content of *P. acutum* in SW1 treatment was the highest, and SS2 was second only to SW1. The soluble protein content in SS5 was the highest. Those showed that SW1 and SS5 substrate treatments could increase the growth resistance of *P. acutum*.

Table 3

Effect of soil-soil mixed substrate treatment on some physiological indexes of *P. acutum*

Treatment	Chlorophyll A content	Chlorophyll B content	Chlorophyll a/b	Total chlorophyll content	Total soluble sugar content	Total soluble protein content
	mg.g ⁻¹	mg.g ⁻¹		mg.g ⁻¹	%	mg.g ⁻¹
SS1	0.79±0.05 a	0.51±0.03 a	1.53±0.03 a	1.30±0.08 a	0.85±0.10 bcd	6.22±0.69 abc
SS2	0.42±0.01c	0.28±0.01 c	1.52±0.09 a	0.70±0.01 c	1.16±0.01 a	6.00±0.69 bcd
SS3	0.51±0.07 bc	0.35±0.05 bc	1.49±0.02 ab	0.86±0.12 bc	0.72±0.11 cd	5.34±0.20 d
SS4	0.47±0.05 bc	0.32±0.04 bc	1.47±0.03 ab	0.79±0.08 bc	0.64±0.02 d	5.49±0.26 cd
SS5	0.45±0.06 bc	0.32±0.03 bc	1.40±0.07 b	0.77±0.10 bc	0.96±0.04 abc	6.88±0.24 a
SS6	0.52±0.09 bc	0.36±0.06 bc	1.47±0.03 ab	0.88±0.15 bc	1.00±0.31 ab	5.18±0.29 d
SS7	0.58±0.11 b	0.38±0.07 b	1.55±0.03 a	0.96±0.18 b	0.93±0.08 abc	6.71±0.44 ab

Means in columns followed by different lowercase letters are significantly different at P< 0.05.

Table 4

Effect of soil-wood mixed substrate treatment on some physiological indexes of *P. acutum*

Treatment	Chlorophyll A content	Chlorophyll B content	Chlorophyll a/b	Total chlorophyll content	Total soluble sugar content	Total soluble protein content
	mg.g ⁻¹	mg.g ⁻¹		mg.g ⁻¹	%	mg.g ⁻¹
SW1	0.55±0.03 bcd	0.35±0.02 bcde	1.55±0.02 b	0.90±0.05 bcde	1.18±0.30 a	5.34±0.25 ab
SW2	0.50±0.07 cd	0.33±0.02 cde	1.49±0.11 b	0.83±0.08 cde	0.80±0.12 bcd	6.04±0.22 a
SW3	0.46±0.05 cd	0.30±0.04 e	1.50±0.04 b	0.76±0.09 de	0.74±0.05 bcd	5.06±0.48 b
SW4	0.56±0.10 bcd	0.38±0.08 bcd	1.48±0.08 b	0.94±0.17 bcd	0.93±0.18 bc	5.55±0.44 ab
SW5	0.62±0.04 bc	0.41±0.01 bc	1.51±0.07 b	1.04±0.05 bc	0.90±0.03 bc	5.07±0.10 b
SW6	0.55±0.07 bcd	0.36±0.04 bcde	1.54±0.05 b	0.91±0.11 bcde	0.62±0.03 d	5.03±0.36 b
SW7	0.39±0.07 d	0.26±0.05 e	1.50±0.06 b	0.65±0.12 e	0.73±0.08 bcd	5.00±0.52 b
SW8	0.90±0.16 a	0.52±0.08 a	1.72±0.03 a	1.42±0.24 a	0.75±0.04 bcd	5.52±0.55 ab
SW9	0.53±0.09 bcd	0.35±0.05 bcde	1.48±0.10 b	0.88±0.14 bcde	0.97±0.03 ab	5.27±0.49 b
SW10	0.56±0.07 bcd	0.37±0.04 bcd	1.51±0.01 b	0.93±0.11 bcd	0.84±0.09 bcd	5.25±0.29 b
SW11	0.45±0.09 cd	0.30±0.06 de	1.52±0.08 b	0.75±0.15 de	0.68±0.09 cd	5.52±0.25 ab
SW12	0.69±0.14 b	0.44±0.08 ab	1.56±0.05 b	1.12±0.22 b	0.73±0.19 bcd	5.43±0.25 ab

Means in columns followed by different lowercase letters are significantly different at P< 0.05.

Effects of different cultivation substrate treatments on the growth and development of the *Pacutum*

Pearson's correlation analysis among the physiological growth indicators of *Pacutum* showed that there was a highly significant positive correlation between chlorophyll a, chlorophyll b, chlorophyll a/b values and total chlorophyll content of *Pacutum*. The total soluble protein content was significantly positively correlated with the total soluble sugar content, and the average growth length was also significantly positively related to the total soluble sugar content of *Pacutum* (Table 5). The average length and coverage of growth of the moss were proportional to its physiological indexes, which could show the growth and development of the moss. The results showed that among the soil-wood mixed substrate (SW) treatments, SW8 had the highest overall evaluation index and also ranked 1st among all treatments with an overall evaluation index of 0.7906, and among the soil-soil mixed substrate (SS) treatments, SS1 had the highest overall evaluation index of 0.6377 and ranked 2nd among all treatments (Table 6).

Table 5

Pearson correlation analysis among the growth and physiological indexes of *Pacutum*

Index	Chlorophyll A content	Chlorophyll B content	Chlorophyll a/b	Total chlorophyll content	Total soluble sugar content	Total soluble protein content	Mean length of growth	Mean growth coverage
Chlorophyll A content	1.000							
Chlorophyll B content	0.979**	1.000						
Chlorophyll a/b	0.620**	0.454**	1.000					
Total chlorophyll content	0.997**	0.991**	0.563**	1.000				
Total soluble sugar content	-0.060	-0.043	-0.080	-0.054	1.000			
Total soluble protein content	-0.025	-0.009	-0.073	-0.019	0.266*	1.000		
Mean length of growth	0.164	0.164	0.100	0.165	0.251	0.133	1.000	
Mean growth coverage	0.172	0.150	0.163	0.165	0.277*	0.012	0.752**	1.000

** indicates extremely significant correlation at the P < 0.01 level, * indicates significant correlation at the P < 0.05 level

Table 6

Comprehensive evaluation of the growth and development of *P. acutum* with different cultivation substrate treatments

Treatment	Each indicator belongs to the function value								Comprehensive evaluation index	Rank
	Chlorophyll A content	Chlorophyll B content	Chlorophyll a/b	Total chlorophyll content	Total soluble protein content	Total soluble sugar content	Mean length of growth	Mean growth coverage		
SS1	0.7849	0.9785	0.4011	0.8503	0.6460	0.4041	0.4722	0.5646	0.6377	2
SS2	0.0664	0.0724	0.3562	0.0683	0.5318	0.9653	0.5835	0.7170	0.4201	11
SS3	0.2427	0.3270	0.2601	0.2713	0.1787	0.1728	0.0000	0.0000	0.1816	19
SS4	0.1610	0.2317	0.2141	0.1849	0.2586	0.0395	0.1994	0.2358	0.1906	17
SS5	0.1227	0.2336	0.0000	0.1603	1.0000	0.5976	0.5612	0.6778	0.4192	8
SS6	0.2655	0.3676	0.2096	0.3001	0.0909	0.6725	0.3942	0.4916	0.3490	13
SS7	0.3859	0.4473	0.4703	0.4067	0.9084	0.5602	0.6549	0.7846	0.5773	4
SW1	0.3116	0.3598	0.4529	0.3279	0.1808	1.0000	0.7444	0.9118	0.5362	6
SW2	0.2106	0.2747	0.2786	0.2323	0.5519	0.3136	0.5742	0.6839	0.3900	10
SW3	0.1341	0.1693	0.3177	0.1460	0.0275	0.2054	0.5135	0.6427	0.2695	16
SW4	0.3393	0.4668	0.2301	0.3823	0.2890	0.5488	0.8052	0.9794	0.5051	5
SW5	0.4608	0.5910	0.3194	0.5048	0.0366	0.5044	1.0000	1.2175	0.5793	3
SW6	0.3222	0.3729	0.4430	0.3393	0.0116	0.0000	0.6285	0.7573	0.3593	14
SW7	0.0000	0.0000	0.2963	0.0000	0.0000	0.1860	0.4685	0.5798	0.1913	18
SW8	1.0000	1.0000	1.0000	1.0000	0.2757	0.2235	0.8256	1.0000	0.7906	1
SW9	0.2743	0.3643	0.2538	0.3047	0.1431	0.6257	0.6071	0.7464	0.4149	9
SW10	0.3359	0.4210	0.3459	0.3647	0.1291	0.3820	0.4661	0.5761	0.3776	12
SW11	0.1278	0.1489	0.3576	0.1348	0.2758	0.1052	0.4559	0.5586	0.2706	15
SW12	0.5856	0.6819	0.5059	0.6182	0.2278	0.2016	0.3168	0.3970	0.4418	7

Discussion

Plant morphological indicators can reflect the strength of plant growth and can visually determine the differences in the effects of different cultivation substrates on plant growth, thus judging the superiority or inferiority of that cultivation substrate [16], while the material, structure, and nature of the substrate are the key factors affecting the plant growth status [17]. In this study, the total porosity and water-holding porosity of the substrate were significantly positively correlated with the average growth coverage, and the water-holding capacity of the substrate was highly significantly and positively correlated with the chlorophyll content (Table 9). The bulk density of SW8, the best treatment for the growth of moss, was 0.14 g/cm³, the total porosity was 73.08%, and the void ratio was 0.07, of which except the total porosity, the other two indicator were lower than the Industry Standard for Floriculture Substrates (LY/T2700-2016) (in which the three indicators were 0.30-1.80 g.cm⁻³, 50%-95%, and 0.25-0.67) [18]. Some studies also pointed out that good substrate structure utilized fixation and growth of plant roots [19]. Because the moss was small in size and light in weight and difficult to collapse of moss, this indicated the Industry Standard for Floriculture Substrates (LY/T2700-2016) needs to be improved for moss cultivation. Comparing the soil-wood mixed substrate (SW) treatment and the soil-soil mixed substrate (SS) treatment, it was found that the overall growth of *P. acutum* was better under the soil-wood mixed substrate (SW) treatment, so using the fallen wood could increase the porosity and water-holding capacity of the substrate and reduce the substrate capacity, which would be beneficial to the growth and spread of *P. acutum*. The overall growth was better under the SW treatment, so using the fallen wood could increase the porosity and water-holding capacity and reduce the substrate capacity, which would be beneficial to the growth of *P. acutum*.

In plant physiology, nitrogen is of essential importance. It plays a vital role in the process of photosynthesis as it is important for plant protein production and is very basic in any other aspect of plant physiology [20]. In this study, the chlorophyll content of *P. acutum* was highly significantly and positively correlated with the total nitrogen content, and the soluble protein content was significantly positively correlated with the total phosphorus content of the substrate (Table 7). This indicated that substrate nitrogen content had no significant effect on the growth of *P. acutum*,

while had a certain effect on leaf color, and phosphorus content was beneficial to improve the resilience of the moss, which was similar to the findings of Tahir et al. [21] and Yang H et al. [22].

Table 7

Pearson correlation analysis of cultivation substrate property indexes and physiological indexes of growth of *P. acutum*

Index	Chlorophyll A content	Chlorophyll B content	Chlorophyll a/b	Total chlorophyll content	Total soluble sugar content	Total soluble protein content	Mean length of growth	Mean growth coverage
Bulk density	-0.197	-0.156	-0.215	-0.183	0.078	0.252	-0.103	-0.118
Total porosity	0.076	0.029	0.259	0.059	-0.097	-0.415**	0.208	0.292*
Aeration porosity	-0.151	-0.100	-0.240	-0.133	0.177	-0.099	-0.033	-0.149
Water-holding porosity	0.125	0.063	0.323*	0.103	-0.154	-0.337*	0.199	0.319*
The void ratio	-0.075	-0.009	-0.277*	-0.051	0.203	0.056	-0.091	-0.238
Water holding capacity	0.408**	0.364**	0.362**	0.394**	-0.137	-0.163	0.164	0.173
Total nitrogen content	0.519**	0.467**	0.392**	0.503**	0.018	0.087	0.140	0.109
Total phosphorus content	-0.248	-0.212	-0.286*	-0.236	-0.193	0.375**	-0.018	-0.192
Total potassium content	-0.440**	-0.431**	-0.230	-0.438**	0.127	-0.091	-0.437**	-0.247

** indicates extremely significant correlation at the $P < 0.01$ level, * indicates significant correlation at the $P < 0.05$ level

Conclusions

Different cultivation substrates had specific effects on the growth and physiological traits of *P. acutum*. The substrate containing *P. massoniana* fallen wood had higher total porosity, water-holding porosity and water-holding capacity, which could effectively improve the reproduction and growth efficiency of *P. acutum*. The nutrient composition of the substrate had an effect on the leaf color traits and stress resistance of *P. acutum*. Among all the substrate treatments, the growth and development of *P. acutum* showed the best under V_{decomposition grade Pinus massoniana fallen log}: V_{grass charcoal soil}=1:1 (SW8) treatment, which could be used as the best substrate for cultivating *P. acutum*.

As a scarce ornamental plant in the market, the urgent problem need to be solved was the effective and rapid propagation and cultivation technology of *P. acutum*. The substrate formulation in this study has a high market development potential as a moss breeding substrate, and also provides a new model for soilless culture of bryophytes.

Materials And Methods

Experimental site

The experiment was carried out from March to July 2021 in West Campus of Guizhou University located at Guiyang, Guizhou Province, China(106°65'E, 27°22'N), which has a typical subtropical, humid and mild climate , with plateau and monsoonal characteristics. The mean annual precipitation, annual average temperature, annual average relative humidity and annual average sunshine hours in Guiyang were 1129.5 mm, 15.3 °C, 77%, and 1148.3 hours respectively. Temperature and humidity changes were recorded with a thermohygrometer at 8:00-9:00, 12:00-13:00, and 17:00-18:00 each day during the experiment (Fig. 2). *P. acutum* preferred a shady and the suitable light range for it is 70% to 90% shading [23], so the cultivation area were treated with three layers of shading net to ensure that the shading rate was above 70%. Spray and moisturize daily according to the actual situation.

Cultivation substrate and plant material

Soil substrate include Granular soil, general-purpose nutrient soil, grass charcoal soil and garden soil. *P. massoniana* fallen wood was collected from the West Campus of Guizhou University, and divided into different decay grades (Table 8) regarding the classification and determination criteria of forest coarse woody residue decay grades by Yan Enrong et al. [24], Ma Haoxia et al. [25] and Liu Ling et al. [9]. The mosses were harvested from Guanshan Lake Park in Guiyang City, Guizhou Province.

Table 8

Fallen log classification table

Decision criteria	The decomposing level		
	I	II	III
Needle	Absent	Absent	Absent
Bark	Partial shedding	Partly present only on large branches	Absent
Branches and twig	Branches present	Large branches present	Only large branches stub present
The trunk shape	Round	Round	Round to oval
The indirect method	Cambium decayed, knife blade penetrates 3 mm to 1 cm	Knife blade penetrates less than 2 cm	Severely decay, knife penetrates 2–5 cm
Structural integrity	Sapwood rotten heartwood intact	Sapwood missing heartwood intact	The heartwood has rotted
wooden	solid	Semi solid	Part of the tender
Wooden color	Primary colors	Primary color to fade	Primary color to fade

Experimental design

The soils were disinfected by exposure to sunlight for seven days and the bark, sapwood and heartwood of the fallen woods were sampled and then crushed and mixed separately before planting. Two groups of treatments were set up in the cultivation trials (Table 9), namely the soil-soil mixed substrate equal volume mixed homogeneous (SS) treatment and the soil-wood mixed substrate equal volume mixed homogeneous (SW) treatment. After laying the non-woven fabric in the planting pots, put the same volume of mixed substrate in the pots and watered thoroughly before transplanting. The moss cultivation was done by gametophyte cultivation, borrowing from the mixed seeding method (area method + weight method) of Sun [26], which is that the moss crushing was weighed by a crusher, and then it was evenly laid flat on the substrate with 4cm*4 cm area, and each treatment was repeated three times.

Table 9

Cultivation substrate treatment

Cultivation type	Treatment	Substrate ratio(V:V)
Soil-soil mixed substrate(SS)	SS1	The garden soil:Granular soil=1:1
	SS2	The garden soil:General-purpose nutrient soil=1:1
	SS3	General-purpose nutrient soil:Grass charcoal soil=1:1
	SS4	General-purpose nutrient soil:Granular soil=1:1
	SS5	Granular soil:Grass charcoal soil=1:1
	SS6	The garden soil:Grass charcoal soil=1:1
	SS7	Grass charcoal soil:Granular soil:General-purpose nutrient soil:The garden soil=1:1:1:1
Soil-wood mixed substrate(SW)	SW1	decomposition grade <i>Pinus massoniana</i> fallen log:The garden soil=1:1
	SW2	decomposition grade <i>Pinus massoniana</i> fallen log:Granular soil=1:1
	SW3	decomposition grade <i>Pinus massoniana</i> fallen log:General-purpose nutrient soil=1:1
	SW4	decomposition grade <i>Pinus massoniana</i> fallen log:Grass charcoal soil=1:1
	SW5	decomposition grade <i>Pinus massoniana</i> fallen log:The garden soil=1:1
	SW6	decomposition grade <i>Pinus massoniana</i> fallen log:Granular soil=1:1
	SW7	decomposition grade <i>Pinus massoniana</i> fallen log:General-purpose nutrient soil=1:1
	SW8	decomposition grade <i>Pinus massoniana</i> fallen log:Grass charcoal soil=1:1
	SW9	decomposition grade <i>Pinus massoniana</i> fallen log:The garden soil=1:1
	SW10	decomposition grade <i>Pinus massoniana</i> fallen log:Granular soil=1:1
	SW11	decomposition grade <i>Pinus massoniana</i> fallen log:General-purpose nutrient soil=1:1
	SW12	decomposition grade <i>Pinus massoniana</i> fallen log:Grass charcoal soil=1:1
	SW12	decomposition grade <i>Pinus massoniana</i> fallen log:Grass charcoal soil=1:1

Indicator measurement method

Cultivation substrate index determination

The physical and chemical property indexes of the substrate include capacity, water holding capacity, total porosity, aeration pore, water holding porosity, and the void ratio, which were measured before cultivation, drawing on the determination methods of Li et al. [27] and Zhao et al. [28]. Nutrient property indicators include total nitrogen, total phosphorus, and total potassium. The solution to be measured was prepared by H₂SO₄-HClO₄ decoction method, the total nitrogen content was determined by CleverChem automatic interrupted chemical analyzer, the phosphorus content was determined by the molybdenum yellow colorimetric method, and the total potassium content was determined by the flame photometric method.

Determination of growth and physiological indexes of *Pacutum*

Growth length measurement: After 30 days of cultivation, ten mosses were randomly selected for each treatment every 10 days and their growth length was measured (accuracy 0.1 cm).

Growth coverage measurement : After 30 days of cultivation, take a picture every 10 days, and then measure the moss area with AutoCAD 2016 tracing, and the moss cover calculation formula was as follows.

$$C=(A/B)*100\%$$

Formula :C-moss coverage;A-moss area; B-planting area

Determination of physiological indicators

Determination of chlorophyll content in *Pacutum* referred to the determination method of Bao[29] , with slight modifications. After 120 days of growth in different cultivation substrates, the moss was taken out, washed and drained for later use, 0.2 g of new branches were weighed and put into a 10 ml centrifuge tube. Subsequently, 8 ml of 95% ethanol was added to the centrifuge tube. After 24 h of extraction under dark conditions, the liquid in the centrifuge tube and the moss residue were filtered several times and fixed into a 25 ml brown volumetric flask, and then the extract was poured into a 1 cm colorimetric cup of optical diameter. Finally, the absorbance at wavelengths of 665 nm and 649 nm was measured with a spectrophotometer with 95% ethanol as a blank control and then chlorophyll a content, chlorophyll b content, chlorophyll a/b, and total chlorophyll

content was calculated according to the Arnon method. Soluble sugar content was determined by the anthrone colorimetric method, and soluble protein content was determined by the Komar Brilliant Blue G-250 (Bradford method).

A comprehensive evaluation of the growth and development status of the mosses

The fuzzy affiliation function evaluation method was used to comprehensively evaluate the growth and development status of the *Pacutum*. The calculation equation is as follows.

$$F_1(X_i) = (X_i - X_{\min}) / (X_{\max} - X_{\min})$$

$$F_2(X_i) = 1 - (X_i - X_{\min}) / (X_{\max} - X_{\min})$$

Formula: X denotes the measured value of the i th index, $X_{\min}$ denotes the measured minimum value of the i th index, and $X_{\max}$ denotes the maximum measured value of the i th index. Pearson correlation analysis was performed on the growth and physiological indexes of the *Pacutum*. If the indexes were opposite to the growth indexes of the *Pacutum*, the value of its affiliation function was calculated by using the inverse affiliation function formula F_2 . The values of the subordinate functions of the growth and development indexes of *Pacutum* under different cultivation substrates were accumulated and averaged to obtain the comprehensive evaluation index of plant morphology. The larger the index value is, the better the plant growth under the cultivation treatment of the substrate [30–31].

Statistical analysis

The data were analyzed by one-way ANOVA with Excel 2010 and SPSS19.0 software. Multiple comparisons were performed by Duncan's ($P < 0.05$), and correlations between indicators were analyzed by Pearson correlation coefficients and plotted with Excel 2010 and Origin 2021.

Declarations

Author Contributions: Conceptualization, Bingyang Shi. Data curation, Bingyang Shi. Funding acquisition, Xiurong Wang. Investigation, Shuoyuan Yang, Hongmei Chen and Yang Zhao. Methodology, Bingyang Shi. Resources, Bingyang Shi, Shuoyuan Yang and Hongmei Chen. Writing—original draft, Bingyang Shi. Writing—review & editing, Xiurong Wang.

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Competing interests: The authors declare no competing interests.

Data availability: The datasets generated during or analysed during the current study are available from the corresponding author on reasonable request.

Licences for collection of plant: The collection of plant material are comply with relevant institutional, national, and international guidelines and legislation.

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Figures

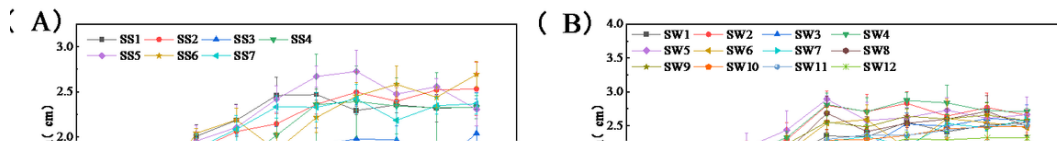


Figure 1

Different substrate treatments on the growth and morphological indices of *Plagiomnium acutum*, including growth length(A and B), growth coverage(C and D), mean growth length(E), mean growth coverage(F)

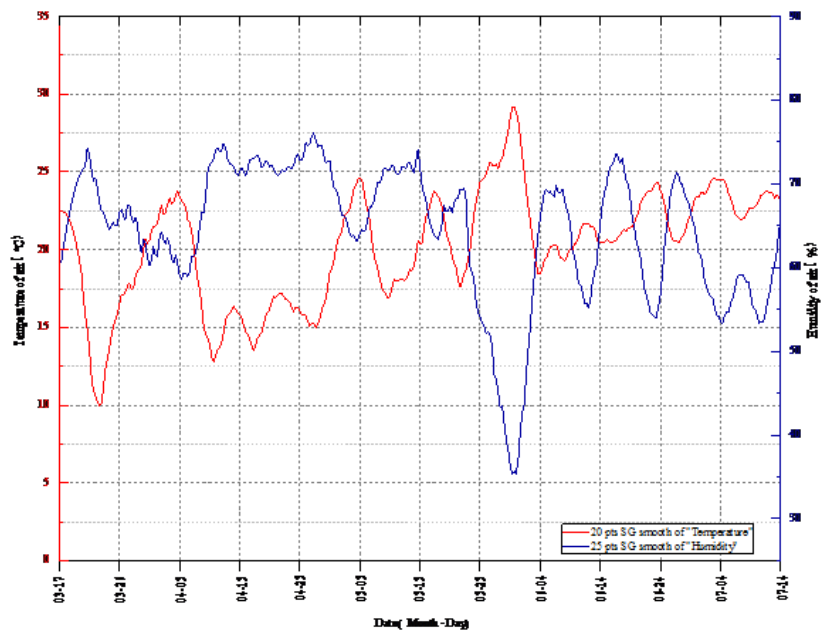


Figure 2

Dynamic change of air temperature and humidity during the experimental period(March 2021- July 2021)