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Typical JUNCAO Overwintering Performance and Optimized Cultivation Conditions of *Pennisetum* sp. in Guizhou, Southwest China

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Abstract: JUNCAO technology plays a critical role in managing soil ecology and alleviating contradiction between mushroom and forest, as JUNCAO can partially replace the wood chip as mushroom culture medium. At present, few reports focus on exploring the effects of seeding density, nitrogen fertilizers on JUNCAO growth and their overwintering performance. To close the above-mentioned research gaps, five typical types of JUNCAO were evaluated by investigating their grass yield, overwintering germination rates and nutrient adsorption condition. The results indicated that *Pennisetum* sp. showed the best overwintering performance. In addition, the optimized planting conditions for *Pennisetum* sp. include cultivation density (60 cm × 50 cm), oblique seeding using stem with double nodes, and 800 kg·ha^{-1} nitrogen fertilizer. This study gave good insights into low-temperature resisting performance and their overwintering characteristics of diverse JUNCAO species that favor for promoting the safe and efficient productions of the JUNCAO industry in subtropical areas.

Keywords: ecology; green manure; landscape; mountain; orchard

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Citation: Zhu, S.; Zhang, Q.; Yang, R.; Chen, B.; Zhang, B.; Yang, Z.; Chen, X.; Wang, X.; Du, M.; Tang, L. Typical JUNCAO Overwintering Performance and Optimized Cultivation Conditions of *Pennisetum* sp. in Guizhou, Southwest China. *Sustainability* **2022**, *14*, 4086. https://doi.org/10.3390/su14074086

Academic Editor: Zhihua Xiao

Received: 23 January 2022
Accepted: 28 March 2022
Published: 30 March 2022

1. Introduction

JUNCAO, e.g., *Dicranopteris pedata* [1], *Neyraudia reynaudiana* [2], *Phragmites australis* [3] and *Pennisetum gigantium* z.x.Lin [4], *Pennisetum purpureum* Schumach [5], *Medicago sativa* L. [6], is a kind of herbaceous plant that is able to nourish edible and medicinal fungi [7]. JUNCAO types have several good properties including strong stress resistance, high yield, abundant crude protein and sugar content, and developed root system which can also show good performance in remediating the stony desertification or desertification [8]. The application of JUNCAO to cultivate edible and medicinal fungi is called JUNCAO technology [7]. JUNCAO is also regarded as livestock forage and serves as an economic nutrient source [9]. Therefore, development of the JUNCAO industry favors the nutrient management and safe production of a livestock–fungi–plant system (Figure 1) [7,10]. In addition, the developed root system and strong stress resistance of JUNCAO benefits their function in ecological management [4,8]. Besides, JUNCAO can also serve as a renewable biomass fuel [11].

Most types of JUNCAO were introduced from Africa or tropical areas; therefore, JUNCAO was more adaptive to a tropical climate, and more vulnerable to low temperature in winter [12]. Therefore, JUNCAO was not suitable for being planted in cold-climate areas. It is reported that JUNCAO types planted in subtropical areas were limited in their ecological and physiological functions as these areas have experienced low temperature winter [13]. For example, low temperature can decrease the stubble germination rate [14], yield [15] and nutrient absorption rates [16]. Herein, examining their overwintering characteristics for selecting appropriate JUNCAO species planting in subtropical areas with low temperature in winter are necessary [17]. Nitrogen is one of the key factors affecting the quality and yield of JUNCAO [18]. Jia et al. (2021) used the nitrogen-fixing bacteria to increase the soil nitrogen content, and afterwards the growth of *Pennisetum giganteum* was significantly improved [19]. It is also reported that a suitable nitrogen fertilizer supply can promote the plant cell elongation/division, prolong the growing period overground part and significantly enhance the yield [20]. However, oversupply of nitrogen fertilizer will not only induce fertilizer waste but also reduce the plant nutrient content, damage plant mechanical tissue and reduce stress resistance [21]. Therefore, an investigation is necessary to find a suitable nitrogen application rate on diverse JUNCAO growth, nutrient utilization ratio and quality favors for further development of the JUNCAO technology. In addition to fertilization, planting density was also a critical factor influencing plant yield; suitable planting density can reduce planting cost and increase nutrient utilization rate [22,23]. For example, Lin et al. reported that as the *Pennisetem* spp. planting density increased, the *Pennisetem* spp.'s tiller number and stem diameter decreased [22]. Planting density showed obvious effects on the yield of *Lolium multiflorum* Guicao No.1 [24] and *Pennisetum americanum* × *P*. *purpureum*. [25], the yield of both being above the JUNCAO increase and then remaining stable as the planting density increases.

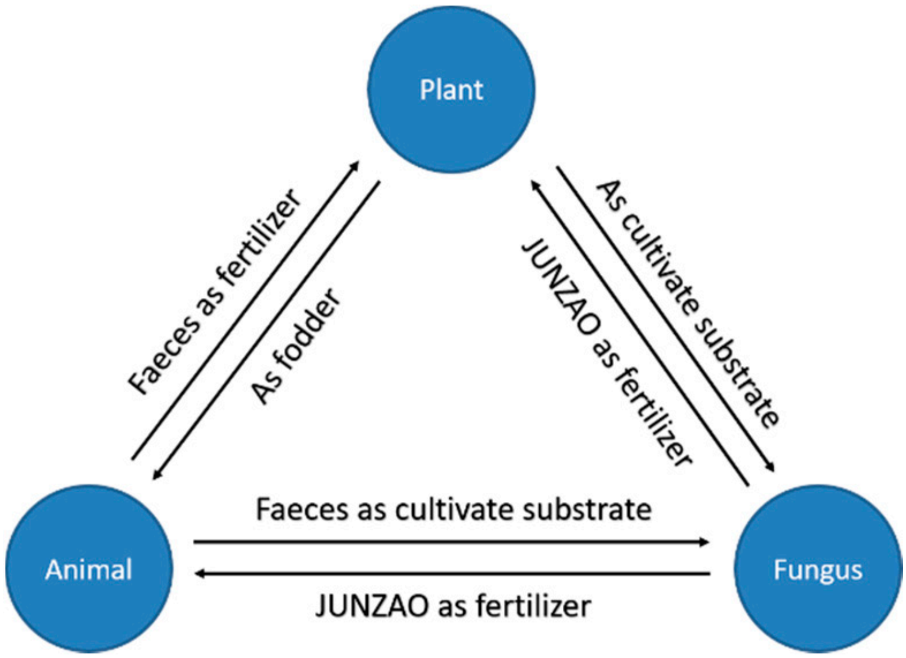

Figure 1. The nutrient recycling system of JUNCAO industry.

In this research, to development the safe and efficient JUNCAO industry and optimize the most suitable JUNCAO species for planting in subtropical areas, a plot experiment was conducted in Bijie city of Guizhou province, which has a typical subtropical monsoon climate, by investigating the effects of cultivation density, seeding methods and fertilizer condition on the overwintering nutrient component, fresh and drought grass yield, and germination rate of five JUNCAs in this research. In addition, the tiller number, plant height, stem diameter and blade number of the JUNCAO types were investigated in order to explore the JUNCAO cultivation measures of high yield and good quality.

2. Materials and Methods

2.1. Experimental Field Description

The JUNCAO field experiment was held in Yayuan village, Chadian town, Zhijin County, Bijie City (26.8603° E, 106.7574° N). This experiment field was a subtropical monsoon climate, with altitude 1350 m, annual average air temperature around 14.7 °C, annual average rainfall of 938.4 mm, annual sunlight reaching 1172 h, and frost-free duration reaching 327 days. The soil of experimental field is loess, and the physiochemical properties of plough layer soil (0–20 cm) are: 15.15 g·kg^{-1} organic matter, 0.85 g·kg^{-1} total nitrogen, 0.71 g·kg^{-1} total phosphorus, 13.29 g·kg^{-1} total potassium, 67.9 mg·kg^{-1} alkali-hydrolyzale nitrogen, 45.9 mg·kg^{-1} available phosphorus, 109.2 mg·kg^{-1} available potassium, and pH value 5.39.

2.2. JUNCAO Resource

Five typical JUNCAO types were studied in this study, including *Pennisetum gigantium* z.x. Lin (*Pennisetum* sp.), *Arundo donax* cv.Lv zhou.No.1 (A. donax. NO.1), *Pennisetum purpureum schumabcv.* Red" (Napiergrass), *Pennisetum pur-pureum* × *P. typhoideum* cv.Reyan.No.4 (Reyan No.4) and *Pennisetum purpureum Schum*. cv. Gui Min Yin (Gui Min Yin). All species were collected from the China National Engineering Research Center of JUNCAO Technology.

2.3. Investigation on Overwintering Characteristics of JUNCAO

The JUNCAOs were planted on 26th April in 2018. Seeding method is double nodes horizontally buried in soil, the area of each plot is 2 m × 5 m. The basal fertilizer used in the experiment was chemical fertilizer (N-P-K = 15:15:15), the application amount is 1500 kg ha^{-1}. The 60% fertilizer was used as a base fertilizer, 20% fertilizer was applied in tillering stage, and 20% fertilizer was used during elongation stage. First harvest was conducted on 23 November 2018 and the stubble height was kept at 3–4 cm, afterwards, the second harvest was performed on 12 December 2019. After JUNCAO sample was collected, the fresh samples were weighed and dried for determination of dry weight/matter, crude protein, crude fat, and crude fiber [26]. Germination rate of the different seeds, viz. overwintering germination rate, was determined on 12 April 2019. The Kjeldahl method (AOAC 2001.11) was used to measure the total nitrogen in JUNCAO samples collected from the total two harvests [27,28]. After the plant samples were digested using aqua regia microwave assisted method [29,30], the contents of total potassium and total phosphorus in the plant samples were determined by flame spectrophotometer (FP6450, Xinyi, Inc., Shanghai, China) [31,32] and molybdenum blue colorimetric method using 700 nm wavelength mode (Shimadzu TNM-1 detector) [26,33], respectively. The dry matter, rude fat and crude fiber were determined by drying method (oven-dried at 70 °C for 3 days) [34,35], Soxhlet extraction method [36] and the traditional Van Soest method [37], respectively. The crude protein content is 4.2 times the total nitrogen [34].

2.4. Optimization of Cultivation Condition

After the overwintering characteristics of the above five JUNCAOs were investigated and compared, the JUNCAO showing the best overwintering performance will be screened for further optimizing the planting conditions, i.e., cultivation density, seeding methods and nitrogen fertilizer amount. This optimization design was performed in 2020. The area of each group was 4.8 m × 10 m (main plots) that each of them was, respectively divided into three subplots (1.6 m × 10 m) as triplicate experiment design. In cultivation density group, three seeding densities including, 40 cm × 50 cm, 60 cm × 50 cm and 80 cm × 50 cm were included. The stem with one or two nodes of the selected JUNCAO was used as the seeding material. In terms of the sowing ways, they were given as follows: (A) stem with one node buried in soil at a 45-degree angle; (B) stem with one node vertically buried in soil; (C) stem with two nodes vertically buried in soil and (D) stem with two nodes buried in soil at a 45-degree angle. Nitrogen fertilizer amount includes four levels: control (0 kg ha^{-1}), less amount (400 kg ha^{-1}), medium amount (800 kg ha^{-1}) and high amount (1600 kg ha^{-1}). All experimental groups were planted on 15 April 2020, harvested on 28 November 2020, then the tiller number, plant height, stem diameter and blade number were analyzed.

2.5. Statistical Analysis

Excel 2007 and SPSS 18.0 were used for a statistical analysis of the data. One-way ANOVA and Duncan's method were used for ANOVA and multiple comparison ($\alpha = 0.05$). Excel 2007 software was used for plotting. Data in the chart are mean ± standard error.

3. Results

3.1. Overwintering Characteristics of Different JUNCAOs

Table 1 revealed that A.donax No.1 showed the highest fresh or dry yield in the first harvest (viz. before winter). The fresh yield of A.donax No.1 reaches 116.1 t ha^{-1} which is higher than *Pennisetum* sp., Napiergrass, ReYan No.4 and Gui Min Yin about 2.5%, 53.6%, 25.5% and 7.9%, respectively, in the first harvest. Dry yield of A.donax NO.1 reaches 13 t ha^{-1}, which is higher than those of *Pennisetum* sp., Napiergrass, Re Yan No.4 and Gui Min Yin by 27.5%, 73.3%, 49.4% and 34.0%, respectively. However, the overwintering fresh yield of *Pennisetum* sp. Reaching 171.4 t ha^{-1} was higher than those of A.donax No.1, Napiergrass, ReYan No.4, Gui Min Yin by 2.2%, 36.6%, 15.6%, 22.7%, respectively. Similar trend was found in dry yield, the overwintering dry yield of *Pennisetum* sp. reaching 42.0 t ha^{-1} was higher than those of A.donax No.1, Napiergrass, ReYan No.4, Gui Min Yin by 0.7%, 34.6%, 39.5%, 59.1%, respectively.

Table 1. Yield of fresh (dry) grass and seedling germination rate in both harvests.

JUNCAO Species	Fresh Yield (t ha^{-1})		Dry Yield (t ha^{-1})		Stubble Germination Rate (%)
	First Harvest	Second Harvest	First Harvest	Second Harvest	
Pennisetum sp.	113 ± 2.64 ^{ab}	171 ± 9.28 ^{a}	10.2 ± 1.52 ^{ab}	42.0 ± 5.71 ^{a}	99.3 ± 0.60 ^{a}
Arundo donax cv. Lv zhou. No. 1 (A.donax No.1)	116 ± 3.25 ^{a}	168 ± 5.51 ^{a}	13.0 ± 1.59 ^{a}	41.7 ± 6.77 ^{a}	99.5 ± 0.15 ^{a}
Pennisetum purpureum schumabcv. Red (Napiergrass)	75.6 ± 5.41 ^{d}	126 ± 6.87 ^{c}	7.50 ± 0.82 ^{c}	31.2 ± 5.63 ^{b}	91.2 ± 2.05 ^{b}
Pennisetum purpureum × *P. typhoideum* cv. Reyan. No.4 (Reyan No.4)	92.5 ± 3.82 ^{c}	148 ± 7.95 ^{b}	8.70 ± 1.57 ^{bc}	30.1 ± 2.50 ^{bc}	93.2 ± 2.76 ^{b}
Pennisetum purpureum Schum. cv. Gui Min Yin (Gui Min Yin)	108 ± 6.27 ^{b}	140 ± 6.54 ^{b}	9.70 ± 0.05 ^{b}	26.4 ± 3.62 ^{c}	83.4 ± 2.51 ^{c}

Note: Different letters of the same parameter among the treatments indicate there is significant difference among them ($p < 0.05$).

The stubble germination rate of *Pennisetum* sp. and A.donax No.1 are 99.3% and 99.5% which are significantly higher than other treatments. The stubble germination rate of Gui Min Yin is the lowest. In addition, the fresh yield of the second harvest was obviously higher than those in the first harvest (Table 1). It is obviously found that the overwintering fresh or dry weights and the stubble germination rates of *Pennisetum* sp. and A.donax No.1 were significantly higher than the other treatments (Table 1).

3.2. The change of Nutrient Content in Diverse JUNCAOs

To uncover the reasons that diverse JUNCAO types showed different overwintering performances from the aspect of JUNCAO utilization ability, the nutrient content (i.e., the total nitrogen, phosphorus, and potassium) in the first and second harvest were respectively determined. As shown in Figure 2, the overwintering nutrient content of all JUNCAO types is clearly higher than those before winter. As for total nitrogen content (Figure 2a), the A.donax No.1 exhibited the best uptake ability for the soil nitrogen. In addition, the overwintering nitrogen content of A.donax No.1 is 174.2% higher than that before winter.

The nitrogen content of the *Pennisetum* sp. is slightly lower than the A.donax No.1, and the overwintering nitrogen content of A.donax No.1 is 242.1% higher than that before winter.

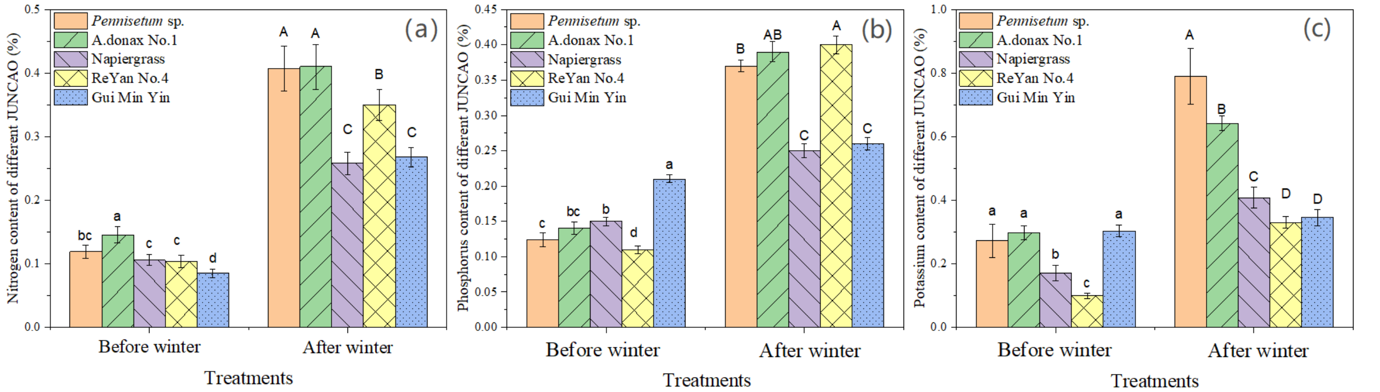

Figure 2. The nutrient contents of diverse JUNCAOs before and after winter. Different letters among the treatments in same group indicate there is significant difference among them ($p < 0.05$). (**a**–**c**) represent the nitrogen, phosphorus and potassium contents of JUNCAOs respectively.

In terms of total content of phosphorus in different JUNCAO species (Figure 2b), the total phosphorus of ReYan No.4 is increased after winter by 263.6%. Meanwhile, the total phosphorus of both the A.donax No.1 and *Pennisetum* sp. were also increased by more than two times. Even though the Gui Min Yin showed the highest total phosphorus content before winter, it slightly increased after winter.

Total potassium of *Pennisetum* sp. was increased by 189.0% after winter (Figure 2c). The A.donax No.1 and *Pennisetum* sp. showed the significant increase in total potassium content ($p < 0.05$). Gui Min Yin still displayed feeble nutrient uptake performance after winter.

3.3. The Change of JUNCAO Quality

That discrepant nutrient quality in different kinds of JUNCAO was showed in Table 2, which demonstrates dry matter, crude fat, crude protein, and crude fiber of diverse JUNCAOs in the first and second harvests. Dry matter in these five grass types (i.e., *Pennisetum* sp., A.donax No.1, Napiergrass, Re Yan No.4, Gui Min Yin) were increased by 348%, 243%, 172%, 121% and 233%, respectively, the crude protein was raised by 355%, 118%, 182%, 427% and 12%, respectively, the crude fat was enlarged by 185%, 17.0%, 83.4%, 97.8% and 130%, respectively, and the crude fiber was enhanced by 7.14%, 3.60%, 9.95%, 19.1% and 26.3%, respectively. From the aspect of overwintering nutrient quality of different species after overwintering, the accumulation of dry matter and crude protein in *Pennisetum* sp., A.donax No.1, Napiergrass are remarkably higher than the other. The crude fat of A.donax No.1 is highest while the crude fiber of Gui Min Yin is lowest.

Table 2. Diverse JUNCAO type quality (%) before and after winter.

JUNCAO Species	First Harvest				Second Harvest			
	Dry Matter (%)	Crude Protein (%)	Crude Fat (g kg^{-1})	Crude Fiber (%)	Dry Matter (%)	Crude Protein (%)	Crude Fat (g kg^{-1})	Crude Fiber (%)
Pennisetum sp.	6.67 ± 0.98 ^{b}	5.58 ± 0.75 ^{b}	1.21 ± 0.14 ^{c}	40.6 ± 2.56 ^{b}	29.9 ± 1.42 ^{a}	25.4 ± 2.65 ^{a}	3.45 ± 0.52 ^{b}	56.6 ± 3.21 ^{a}
A.donax No.1	7.04 ± 1.05 ^{b}	9.63 ± 1.23 ^{a}	7.65 ± 0.75 ^{a}	52.7 ± 3.45 ^{a}	24.2 ± 2.65 ^{b}	23.3 ± 0.65 ^{a}	8.95 ± 0.85 ^{a}	54.6 ± 2.54 ^{a}
Napiergrass	10.8 ± 0.86 ^{a}	9.05 ± 1.14 ^{a}	1.93 ± 0.15 ^{b}	44.2 ± 3.19 ^{b}	29.4 ± 2.12 ^{a}	25.6 ± 2.51 ^{a}	3.54 ± 0.25 ^{b}	48.6 ± 2.48 ^{c}
Re Yan No.4	9.87 ± 0.87 ^{a}	4.15 ± 0.34 ^{c}	1.34 ± 0.15 ^{c}	43.0 ± 1.59 ^{b}	21.9 ± 1.65 ^{b}	21.9 ± 1.28 ^{a}	2.65 ± 0.24 ^{c}	51.2 ± 2.37 ^{b}
Gui Min Yin	6.62 ± 0.78 ^{b}	10.8 ± 0.87 ^{a}	1.41 ± 0.18 ^{c}	44.8 ± 2.68 ^{b}	22.1 ± 1.24 ^{b}	12.1 ± 1.02 ^{b}	3.25 ± 0.29 ^{b}	43.5 ± 2.68 ^{d}

Note: Different letters of the same parameter among the treatments indicate there is significant difference among them ($p < 0.05$).

In addition, the cold-resistance characteristics (e.g., overwintering germination rate, dry/fresh yield, nutrient uptake amount, etc.) of *Pennisetum* sp. and A.donax No.1 showed similar advantages. Thus, *Pennisetum* sp. and A.donax No.1 are more appropriate to be planted in this selected subtropical area. In addition, as the dry matter and crude protein are key factors influencing their application as forage, the *Pennisetum* sp. with higher content of crude protein and less content of crude fat was chosen for the next optimal experiment.

3.4. The Agronomic Trait Changes under Three Optimal Conditions

The results in Table 3 showed that the plant height, stem diameter and fresh yield of sowing density (40 × 50) are lower than the corresponding parameters of the other two sowing densities. The density of sowing density (60 × 50) had the highest fresh yield, being 35.6% higher than that of sowing density (40 × 50). In terms of density sowing density (80 × 50), the stem diameter is lower than the sowing density (40 × 50), and the fresh yield is 5% higher than the sowing density (40 × 50). It is noteworthy that as the sowing density increases, the *Pennisetum* sp. cane becomes thicker and there are more blades. In short, sowing density (60 × 50) showed the best overall agronomic traits during the *Pennisetum* sp.'s growth.

Table 3. The effect of cultivation density on agronomic traits of *Pennisetum* sp.

Optimizing Group	Sowing density (cm)	Tiller Number	Plant Height (mm)	Stem Diameter (mm)	Blade Number	Yield of Fresh Grass ($t{\cdot}ha^{-1}$)
1	40 × 50	15.0 ± 1.00^{a}	194 ± 10.6^{b}	14.6 ± 2.10^{a}	17.0 ± 1.52^{b}	155 ± 12.5^{b}
2	60 × 50	16.0 ± 1.00^{a}	238 ± 15.9^{a}	15.9 ± 1.85^{a}	21.0 ± 2.05^{a}	175 ± 10.8^{a}
3	80 × 50	13.0 ± 1.00^{b}	220 ± 13.2^{a}	16.5 ± 3.02^{a}	24.0 ± 1.95^{a}	176 ± 9.68^{a}

Note: Different letters of the same parameter among the treatments indicate there is significant difference among them ($p < 0.05$).

In terms of seeding method, the *Pennisetum* sp. fresh yield, blade number, stem diameter, plant height and tiller number of A were lower than those of C by 25.4%, 44.0%, 19.0%, 10.0% and 33.3%, respectively. The *Pennisetum* sp. fresh yield of B is lower than that of D by 34.1% (Table 4). The *Pennisetum* sp. fresh yield of B was 3.9% higher than A. The five agronomic traits of C and D were similar, but the fresh yield of C was 3.8% lower than D (Table 4). Obviously, the seeding using the stem with two nodes (treatment C and treatment D) is more suitable for planting the *Pennisetum* sp. after winter.

Table 4. The overwintering agronomic traits of *Pennisetum* sp. planted different seeding methods.

Seeding Method	Tiller Number	Plant Height (mm)	Stem Diameter (cm)	Blade Number	Yield of Fresh Grass ($t\ ha^{-1}$)
A (Stem with one node buried in soil at a 45-degree angle)	14.0 ± 1.00^{b}	207 ± 5.68^{b}	14.1 ± 1.51^{b}	14.0 ± 1.00^{c}	141 ± 8.65^{b}
B (Stem with one node vertically buried in soil)	13.0 ± 1.00^{b}	212 ± 8.51^{b}	14.2 ± 1.05^{b}	16.0 ± 1.00^{b}	147 ± 5.64^{b}
C (Stem with two nodes vertically buried in soil)	21.0 ± 2.52^{a}	230 ± 12.1^{a}	17.4 ± 1.98^{a}	25.0 ± 2.00^{a}	189 ± 10.8^{a}
D (Stem with two nodes buried in soil at a 45-degree angle)	22.0 ± 1.00^{a}	243 ± 10.8^{a}	18.2 ± 1.68^{a}	24.0 ± 2.00^{a}	196 ± 13.4^{a}

Note: Different letters of the same parameter among the treatments indicate there is significant difference among them ($p < 0.05$).

The effect of nitrogen fertilizer amount on agronomic traits of *Pennisetum* sp. was shown in Table 5. The data of agronomic traits gradually increased as the nitrogen fertilizer amount increased. The yield of fresh grass in blank group was lower than the normal amount group by 21.4%. The tiller number, plant height, stem diameter, blade number and fresh grass yield of high amount group has a significant increase by 33.3%, 4.10%, 6.70%, 31.8%, and 35.9%, respectively, when contrasted with normal amount group ($p < 0.05$). However, there is no significance ($p > 0.05$) between medium and high amount groups in fresh grass yield, which implies that an excessive application of nitrogen fertilizer is not conducive to significant increase of the JUNCAO fresh yield.

Table 5. The effect of nitrogen fertilizer amount on agronomic traits of *Pennisetum* sp.

Nitrogen Amount	Tiller Number	Plant Height (mm)	Stem Diameter (mm)	Blade Number	Yield of Fresh Grass ($t\cdot ha^{-1}$)
CK	13 ± 1^{c}	212 ± 7.25^{c}	14.2 ± 0.12^{c}	15 ± 2^{c}	160 ± 10.5^{c}
400 kg ha^{-1} (Normal amount)	18 ± 1^{b}	268 ± 10.5^{b}	17.9 ± 0.12^{b}	22 ± 1^{b}	195 ± 5.82^{b}
800 kg ha^{-1} (Medium amount)	23 ± 2^{a}	277 ± 5.45^{a}	18.8 ± 0.24^{a}	27 ± 2^{a}	253 ± 12.5^{a}
1600 kg ha^{-1} (High amount)	24 ± 1^{a}	279 ± 8.12^{a}	19.1 ± 0.25^{a}	29 ± 2^{a}	265 ± 9.52^{a}

Note: Different letters within the same column indicate significant difference between treatments ($p < 0.05$).

4. Discussion

4.1. Comparisons of the JUNCAOs' Overwintering Characteristics

Pennisetum sp. and A.donax had higher overwintering germination rate, fresh and dry yields in local environment condition. The total yield in the second harvest of *Pennisetum* sp. is higher than the A.donax No.1 despite there has no significant difference which means *Pennisetum* sp. can accumulate more biomass after winter. The overwintering cold resistance of diverse JUNCAOs can be evaluated by comparing their stubble germination rates [38,39]. Table 1 implied that the stubble germination rate of Gui Min Yin is the lowest which indicated the Gui Min Yin showed the worst cold resistance in this subtropical area winter, while the stubble rate of *Pennisetum* sp. (99.3%) and A.donax No.1 (99.5%) are mildly affected by low-temperature stress in winter and remain well root activity. The dry matter accumulation rate and overwintering germination rate of *Pennisetum* sp. were highest among five JUNCAO types, implying that the regrowth ability of *Pennisetum* sp. after mowing is most active. *Pennisetum* sp. as a typical C4 plant was from Africa and that is suitable for growing in tropical zones, the optimum growth temperature was at 25~35 °C, annual yield of fresh grass reaches over 200 $t\cdot ha^{-1}$ [40]. *Pennisetum* sp. was perennating in tropical zone which will wither under 0 °C in winter [41]. The climate condition of winter in the northwest area of Guizhou province is a challenge for *Pennisetum* sp., because its winter temperature was around 5 °C. The nutrient uptake analysis (Figure 2) of five JUNCAOs implied that the low-temperature stress showed the least effect on the nutrient uptake of *Pennisetum* sp., which may be attributed to the developed root system of *Pennisetum* sp. [42]. In brief, *Pennisetum* sp. showed the best resistance to the low-temperature stress among the five JUNCAOs in this subtropical area. More investigation on exploring low-temperature-adapted JUNCAO species will facilitate the development of the JUNCAO industry in subtropical areas.

4.2. Optimization of JUNCAO Cultivation Conditions

The higher cultivation density caused the lower tiller number, plant height and stem diameter, ultimately causing the lower *Pennisetum* sp. yield. On the contrary, the lower cultivation density caused the higher tiller number, plant height and stem diameter as well as the higher grass yield of *Pennisetum* sp. The reasonable cultivation density of JUNCAO can supplement enough illumination and reduce the nutrition competition for JUNCAO planting [43]. It can be speculated from the results that both the sparse and tight density are unsuitable for JUNCAO growth (Table 3). If cultivation density is too sparse, it will limit the initial seeding number, which leads to low grass yield under the same cultivation condition and environment [44]. The small density creates a sparser space for each plot, which ensures there are plenty of nutrients for plant growth, but it decreases their yield over the whole growth period [45]. On the other hand, the initial seeding number will be very large if the cultivation density is too tight. In addition, the blocking of the sunlight by upper blades is such that when the plant enters a quick growth period, the small tillers and bottom blades cannot acquire enough sunlight to support photosynthesis.

In addition, the fresh yield of the second harvest was obviously higher than that of the first harvest (Table 1), which suggested that all the selected JUNCAO types can overcome low-temperature stress to some extent in winter, while showing higher overwintering fresh and dry yields. It is obviously found that the overwintering fresh or dry weights and the stubble germination rates of *Pennisetum* sp. and A.donax No.1 were significantly higher than in the other treatments (Table 1). Therefore, *Pennisetum* sp. and A.donax No.1 showed the best overwintering performance in this subtropical area.

There are two methods of JUNCAO seeding, including vertical and oblique bury [17]. In fact, these methods provide different germination conditions for JUNCAO planting. Compared with the seeding method of vertical bury, the method of oblique bury at a 45-degree angle showed no significant difference in yield of fresh grass [46]. Obvious differences of JUNCAO agronomy characteristics were found between the two seeding ways. For example, seeding using stems with two nodes can significantly enhance the typical agronomic traits (yield, plant height, tiller number, etc.) of JUNCAO [9]. In addition, the germination rate has a positive correlation with the JUNCAO yields based on the data of one-node and two-node stems. The real conditions of JUNCAO planting must consider the JUNCAO planting requirements, soil nutrient, fertilizer amount and weather environment [17].

To examine if the nitrogen application amount was the limiting factor on *Pennisetum* sp.'s growth, the *Pennisetum* sp.'s growth of diverse treatments with various N application amounts were investigated. Compared with the yield increase extent (by 30.4%) of a medium amount of nitrogen, the yield increase extent (by 4.34%) of 1600 kg ha^{-1} was lower (Table 5), which implied that the nitrogen fertilizer does not necessarily increase the JUNCAO yield, and excessive nitrogen fertilizer cannot further promote the JUNCAO growth. In addition, oversupplying nitrogen may cause a waste of workforce and production feedstocks, and more emissions of greenhouse gases [26]. Furthermore, an over-dose of nitrogen fertilizer can cause plant stem cell expansion, which leads to a decrease in the cane strength at the medium growth stage of JUNCAO [47]. The blade area increase caused by excessive nitrogen fertilizer is another problem that can hamper the photosynthesis process in middle blades [26,32]. Thus, the over-dose of nitrogen fertilizer was not favorable for the plantation purpose of JUNCAO as feedstock [31,48].

5. Conclusions

In this research, five JUNCAOs were compared to select the most suitable JUNCAO species for planting in this typical subtropical area by comprehensively assessing their agronomic traits before and after winter, such as germination rate, nutrient acceleration, dry and fresh yields. Among the five selected JUNCAO types, *Pennisetum* sp. showed highest stable metabolism ability and overwintering germination rate, which makes it a propagable JUNCAO species in northwest Guizhou province in China. In addition, cultivation density (60 cm × 50 cm), oblique seeding using stems with double nodes, 800 kg·ha^{-1} nitrogen fertilizer were the recommended dosage for *Pennisetum* sp. planting in this typical subtropical area. To expand the JUNCAO industry in subtropical areas, more effort should be paid to explore those JUNCAO species showing good low-temperature-resistant performance. This research is conducive to facilitating the economic, efficient and safe production of the JUNCAO industry in subtropical areas. Future works should more focus on how the other environmental influence factors affect the JUNCAO growth.

Author Contributions: S.Z., Q.Z., X.C., B.C., Z.Y. and X.W. collected the required materials and prepared the manuscript; B.Z. contributed the design and edition of the manuscript, M.D., L.T., B.Z. and R.Y. reviewed and prepared the revised manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the science and technology project of Guizhou Province ([2019]3006-4), Guizhou Province Science and technology planning project ([2018]4008, [2020]1Y073) and China edible fungus industry technology system (CARS-20).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We gratefully acknowledge the financial support of the youth funding of Guizhou Academy of Agricultural Sciences ([2019] No.20), the science and technology project of Guizhou Province ([2019]3006-4), Guizhou Province Science and technology planning project ([2018]4008, [2020]1Y073) and China edible fungus industry technology system (CARS-20).

Conflicts of Interest: The authors declare no conflict of interest.

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