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Shifts in Leaf and Branch Elemental Compositions of *Pinus massoniana* (Lamb.) Following Three-Year Rainfall Exclusion

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Received: 18 December 2019; Accepted: 13 January 2020; Published: 16 January 2020



Abstract: We investigated changes in leaf and branch stoichiometry of *Pinus massoniana* caused by seasonal variation and experimental drought in response to a three-year manipulation of the rainfall exclusion. The results showed that (1) in response to rainfall exclusion manipulation, plant capacity to regulate leaf potassium (K) concentrations were notably lower than for leaf nitrogen (N) and phosphorus (P) concentrations. Thus, the plants modulated leaf N and P concentrations to improve water use efficiency, which take part in drought resistance mechanisms. Leaf K concentrations decreased continuously, having additional indirect negative effects on plant fitness. (2) The effects of seasonal variation on both the leaf K and P concentrations were significantly stronger than on leaf N concentrations. High leaf N and P concentrations and a low N:P ratio in the growing season improved the growth rate. (3) Principal component analyses (PCA) revealed that to adapt to drought, the plants regulated nutrient elements and then maintained certain stoichiometries as a capital to resist stress. Our results suggest that, on nutrient-poor soils, a lack of N or P (or both) would probably impede *P. massoniana*'s response to drought.

Keywords: drought; rainfall exclusion; nitrogen; phosphorus; potassium; N:P ratio

1. Introduction

Global climate change is modifying patterns of global precipitation, leading to changes in the frequency and magnitude of extreme climatic events, such as drought [1,2]. Increased drought enhances tree mortality [3,4] and influences ecosystem biodiversity as well as ecosystem structure and function [5–8]. Several studies have shown that terrestrial plants adapted and resistant to drought can alter their physiological characteristics to increase water use efficiencies to prevent death [9–11]. The elemental composition of a species is determined by long-term genetic adaptation and specific ecological strategies to a particular environment [12], which is associated with responses to environmental stress [13,14]. For example, during long-term drought, element concentrations in different organs of plants change, promoting plant adaptation to drought stress. Therefore, the shift in the elemental composition and stoichiometry of different plant organs can reflect the response of plants to forest drought stress.

The main goal of ecological stoichiometry is to understand the relationships among Carbon, Nitrogen, Phosphorus (C:N:P) ratios in organisms and environments [15]. The C:N:P ratios in organisms can be associated with important ecological processes, such as responses to environmental stress [13,16], ecosystem composition and diversity [17,18]. An organism's C:N:P stoichiometry can be influenced

by the environment through two pathways. First, environmental changes can alter the C:N:P ratios of producers, thereby affecting the growth rate and lifestyle through nutrient cycling [19]. Growth rate hypothesis (GRH) posits that to enhance rapid growth influenced by the intensity of the protein synthesis the organism needs more P, which involves a low N:P ratio. It is responsible for the high growth in the environment [20]. Previous studies on planktonic communities reported that low N:P ratios correlate with high growth rates [6]. Similarly, terrestrial plants with low N:P ratios grow rapidly and are more competitive for soil resources [15,21]. However, under normal growth condition, the distributions of the elements in higher plants contribute to the operations of storage, defensive, and anti-stress systems, as well [22]. Many studies reported how foliar stoichiometries vary along environmental gradients [23–25]. All these studies suggested that the main climatic variables (e.g., temperature and precipitation) can influence the actual water content in the plant and intensities of solar radiation arriving at the leaves, which in turn affect foliar elemental stoichiometries.

Leaf elemental compositions and stoichiometries are important in forest research, as they can indicate the nutritional status of soil and the growth rate of plants [6,22], reflecting plant acclimatization to climate change [23,24]. However, most studies of plants stoichiometries considered only foliar N and P; they neglected the other photosynthetic organ, branches, and neglected other elements, as well. The concentrations or ratios of other elements, such as K, magnesium (Mg), and iron (Fe), play more important roles in the physiological functions of organisms [10]. The biogeochemical niche hypothesis, which can reflect species' growth and resources uptake strategies as well as space and time occupation, expresses the main nutrients, such as C, N, P, K, and other essential nutrients, such as calcium (Ca), Mg, and sulfur (S), in different proportions [10,22]. These proportions are called stoichiometrical flexibility in response to environmental changes and competitive situations. For example, Yu et al. [26] showed that species with higher stoichiometrical flexibility tended to have higher concentrations of N and P and lower N:P ratios. It is easier to understand and interpret the plant stoichiometric shift in the multivariate space of the contents N, P, and K in the plant tissues. The alterations in N and P levels separately cannot uncover a relevant explanation of this phenomenon [27].

The tissue concentrations of K are vital in plant biology [28], and particularly in dry environments, because K levels affect several physiological functions in plants, such as controlling the water content of leaves by stomatal control, cell osmosis equilibrium, and water use efficiency [14,29–31]. Shifts in foliar N:P:K ratios are related to the change in plant response to drought [32]. Some plants in dry environments have a higher concentration of K, which is related to metabolome shifts toward increasing concentrations of metabolites linked to osmosis control; these high levels of K help the plants to resist drought [14,16,33]. The concentrations of K, N, and P together help to control water availability [6,34]. Drought is also the driving force of the changes in elemental C and C:nutrient ratios, especially C:N, C:P, and C:K [35]. The published evidence suggests that decreasing water availability in drought conditions differently affects the N and P cycles and increases the C:nutrient ratios in photosynthetic tissues due to increased levels of C-rich compounds, such as phenolics and tannins, to avoid water stress [14]. These are the protective mechanisms associated with slowing growth and increasing leaf N:P ratios [32,36]. Many studies identified the relationship between high N:P stoichiometries and low growth rates in terrestrial plants, determined by water availability [19,37]. Rivas-Ubach et al. [34] observed increasing N:P ratios during the dry season along with the decrease in primary metabolism and secondary anti-stress metabolism. The changes of the element concentrations in plant tissues induced by drought can be considered the indicators of plant physiological state alteration. Thus, we intended to map the relationships of the terrestrial plant stoichiometry to environmental changes.

We investigated the effects of drought on the stoichiometries and compositions of photosynthetic organs (leaves and branches). We used a three-year field-simulated drought test that ran from April 2013 until January 2016 in ChangTing County, Fujian province, China (Figure 1). We aimed to provide evidence of different drought sensitivities of on leaf and branch stoichiometry dynamics of mature *Pinus massoniana* individuals, which are widely used for research in subtropical areas experiencing red soil erosion. We measured leaf and branch C, N, P, and K concentrations and their ratios of P.

massoniana growing in control (natural) conditions and under drought (100% rainfall exclusion). The main aims of this study were (1) to investigate the changes of leaf and branch stoichiometries of *P. massoniana* in response to three-year 100% rainfall exclusion, (2) to investigate the seasonal dynamic characteristics in leaf and branch macro-elemental concentration of *P. massoniana* over the study period, and (3) to investigate which element led to the imbalance of osmotic regulation is the key lethal factor to drought stress.

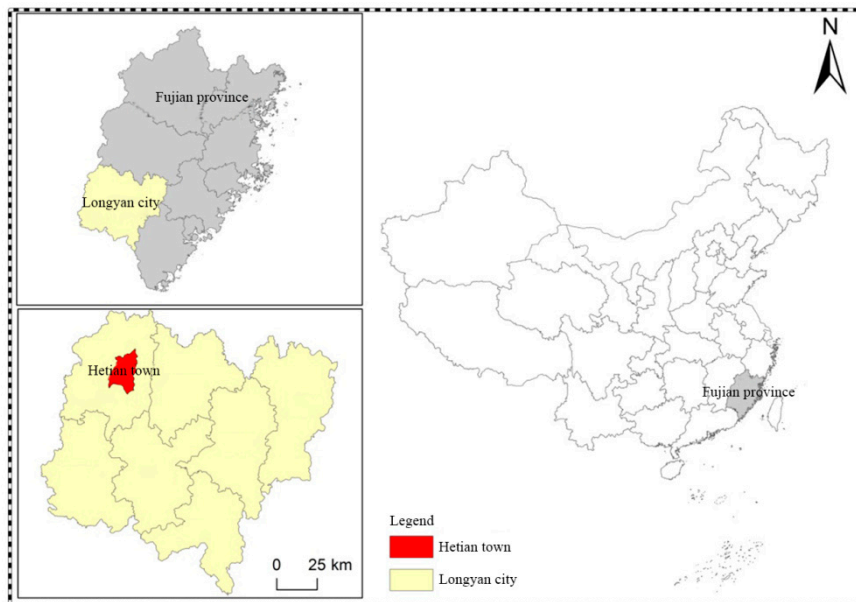


Figure 1. Map of the study area.

2. Materials and Methods

2.1. Study Site

This study was performed in the town of HeTian, southeast of ChangTing County, Fujian province, China ($25^{\circ}33'–25^{\circ}48' N$, $116^{\circ}18'–116^{\circ}31' E$, 310 m above sea level), which experiences some of the most serious soil erosion in the Fujian province [38]. The main soil type is red soil, which is characterized as nutrient-poor with coarse grain granite parent material. Most of the soil is exposed core, or even bedrock. Because of soil depletion, shrub and grass vegetation is scarce, and the dominant plant species are *P. massoniana*, *Dicranopteris dichotoma* (Thunb) Berhn [39]. The integrated mean concentrations of C, N, P, and K in soil (0–30 cm) were $1.06 \text{ mg}\cdot\text{g}^{-1}$, $0.15 \text{ mg}\cdot\text{g}^{-1}$, $0.017 \text{ mg}\cdot\text{g}^{-1}$, and $4.26 \text{ mg}\cdot\text{g}^{-1}$, respectively. The study site belongs to subtropical monsoon climate with the wet seasons typically from March to September and the dry seasons generally from October to February. The study area experienced a mean annual rainfall of 1696 mm in the study period, and a mean annual temperature of 19.85°C , with the variation range from -7.8°C to 39.8°C . Temperature, soil moisture, precipitation, and relative humidity were recorded every 15 min from the installed automatic weather station (Monitor Automatic Weather Station, ICT, NSW, Australia) at the study site (Figure 2). We used the linear relationship of precipitation and temperature to define aridity conditions. The climate was classified as drought when the temperature was 2-fold greater than the precipitation [40]. The calculated results in Figure 2 show that the arid climate happened when the precipitation point was lower than the temperature. As a result, arid conditions occurred for six months between April 2013 and January 2016: October 2013, January 2014, April 2014, September 2014, October 2014, and October 2015. Hence, we concluded that the study site was not arid, so it was used as a control area.

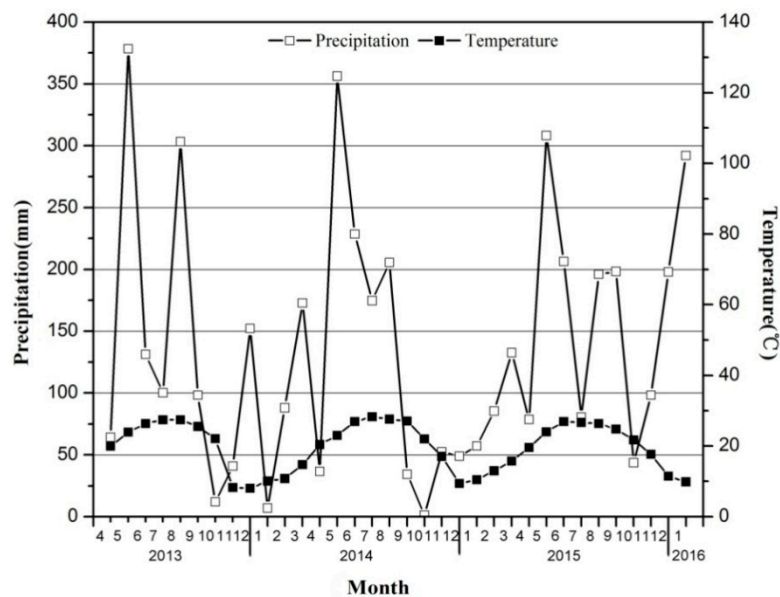


Figure 2. Monthly accumulated precipitation and average temperature from April 2013 to January 2016.

2.2. Experimental Design

The experimental setting involved a three-year experiment of 100% rainfall exclusion. Starting in April 2013, four plots (each 20×20 m) of mature *P. massoniana* were established, on a slope of 30° at 333 m above sea level, facing southwest. In each sample plot, there were 10 or 11 mature *P. massoniana* individuals, which had been sown by plane in 1998. The mean diameter at breast height (DBH) of *P. massoniana* was 4.2 cm, tree height was 2.4 m, and blade length was 8.5 cm (Figure 3). To avoid the impact of rainwater runoff on sampling, two plots (one upslope and one downslope) subject to 100% rainfall exclusion as the drought plot, and the other two were influenced by the rainfall events (Figure 4). In the drought plots, a 4 m transparent wave tile coated with UV paint (light transmittance 90%) was fixed above the canopy. To channel the rainfall flow down the slope, wave tiles were installed parallel to the terrain. Polyvinyl chloride (PVC) strips did not have any effects on ambient temperature and humidity. A ditch 80 cm in depth was dug along the top edge of the drought plots to intercept up-slope runoff, which was channeled to the bottom edge. The remaining plots received no manipulation.



Figure 3. The experimental stand of *Pinus massoniana*.



Figure 4. Actual sample plot.

2.3. Soil Moisture Content

Using a time-domain soil moisture meter (Trime-T3, Moisture Meter, IMKO, Ettlingen, Germany), soil moisture content at depths of 20 and 80 cm in the control and drought groups were measured every 15 min.

2.4. Leaf Water Potential

Leaf water potentials were measured before dawn (Ψ_d) using a WP4 dew-point potential meter (Decagon Device, Pullman, WA, USA). Three sample trees were chosen from each plot approximately every month from April 2013 to March 2014. We collected two south-facing branches from the top and middle parts of each individual canopy, choosing those with healthy leaves. We immediately put the branches into cold storage (0–4 °C), and brought them to the lab.

2.5. Sampling Method

After 115 days of rainfall exclusion, to avoid boundary effects, 6 randomly selected individuals (3 from the upslope and 3 from the downslope plot) were selected from the center of each treatment plot. The date of plant sampling was determined according to the main phenological rhythm of the study area. The specific sampling days were as follows: day 115 (18 August 2013, late summer), day 185 (27 October 2013, autumn), day 256 (6 January 2014, winter), day 332 (23 March 2014, spring), day 467 (1 August 2014, late summer), day 542 (15 October 2014, autumn), day 638 (30 December 2014, winter), day 738 (9 April 2015, spring), day 861 (10 August 2015, late summer), day 941 (29 October 2015, autumn), and day 1032 (28 January 2016, winter).

In the study period, we collected leaf and branch samples from each individual to determine elemental concentrations. To avoid the effects of diurnal variation, four small branches exposed to sunlight were consistently selected from the top and middle parts of each crown, both southeast- and northwest-facing, between 09:00 and 11:00 a.m. Well-developed leaves were collected above the branch samples. The leaf lengths ranged from 8.3 to 8.6 cm, and the branch diameters (with bark removed) ranged from 0.3 to 0.5 cm, which were treated as one leaf sample and one branch sample, respectively. Before laboratory analysis, all samples were placed in cold storage (0–4 °C). To reduce enzymatic activity, all samples were microwave-treated at 800 W for 5 min and then dried at 65 °C for 48 h until the weight became constant [41]. The samples of leaves and branches were ball-milled into a fine powder (Tissuelyser-24, Shanghai, China) for measuring C, N, P, and K.

2.6. Elemental Analyses

To analyze C and N concentrations, 9–11 mg of the powders from each sample was wrapped in tin foil for analysis. The C and N concentrations were determined using a CN elemental analyzer (ElementarVario ELIII, München, Germany).

The plant samples were digested by a $\text{HNO}_3\cdot\text{H}_2\text{O}_2$ mixed system. Firstly, 100 mg dry plant powder was accurately weighed and placed in 50 mL disposable fluorinated ethylene–propylene (FEP) digestion tubes, which are suitable for graphite digestion systems and are characterized by direct volume fixing, which prevents errors in transfer. Then, 8 mL pure HNO_3 (Merck GR 2.5 L) was slowly added along the pipe wall, and finally, 2 mL H_2O_2 was added for overnight treatment. On the second day, after gently shaking the tube, gradient heating digestion in graphite furnace was conducted as follows: temperature increased to 80 °C and maintained for 10 min, and then maintained for 30 min at 100 °C, with continuous heating to 110 °C until thick white smoke was emitted. The final solution was concentrated to 1–2 mL. After cooling, the solution was directly fixed in a fluorinated ethylene–propylene (FEP) tube with a deionizing solution, filtered with a 0.45 microporous membrane, and then placed in the refrigerator for testing.

P concentrations were determined using a continuous flow analyzer (SKALAR SAN++, Breda, Netherlands), and K concentrations were determined using a flame spectrophotometer (Aopu PF640, Shanghai, China). Plant C, N, P, and K concentrations are expressed as $\text{mg}\cdot\text{g}^{-1}$ on a dry weight basis.

2.7. Statistical Analyses

All statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). All results are reported as mean \pm standard deviation (SD) for the six replicates of each treatment. To study the different treatments and seasonality effects, we analyzed data using a general linear model. A repeat measure analysis was used to analyze the effects of different treatments on the element concentrations and ratios. To detect patterns of sample ordination with element concentrations and ratios on seasons and drought, the data were subjected to principal component analysis (PCA). We conducted PCA analyses using all elemental concentrations and important nutrient ratios and selected those that had the highest loading on the main axes. PCA axes were used to detect differences among leaves and branches of different seasons or between control and drought plots. Figures were all drawn using Origin 8.0 software and Canoco 5.0.

3. Results

3.1. Soil Moisture Content and Leaf Water Potential

The initial soil moisture contents in the control group (24.59% at 20 cm and 36.37% at 80 cm) were similar to the drought group (23.05% at 20 cm and 36.47% at 80 cm). In the study period, the soil moisture contents in the drought group decreased continuously. The average soil moisture contents at 20 cm (mean 10.69%) and 80 cm (mean 17.31%) of the drought group were both significantly lower than the corresponding soil moisture at 20 cm (mean 22.56%) and 80 cm (mean 30.85%) in the control group ($p < 0.01$; Figure 5). The decreasing amplitudes were 11.87% and 13.54%, respectively. In this regard, the average soil moisture content in the drought group seems to meet the needs for normal metabolism, and the average soil moisture content decreased by 10%–20% over the study period, which is only defined as mild drought [42]. We tried to explain that 100% rainfall exclusion could only simulate mild drought for the following reasons: (1) groundwater flow caused by moderate slope and (2) stomatal closure of the water stress mechanism initiated by *P. massoniana*. Mild drought was probably a better representation of the real scenario expected under natural conditions in the southeastern coastal areas of China. However, soil moisture contents of 20 and 80 cm depths in the control group presented significant seasonal fluctuations ($p < 0.01$; Figure 5).

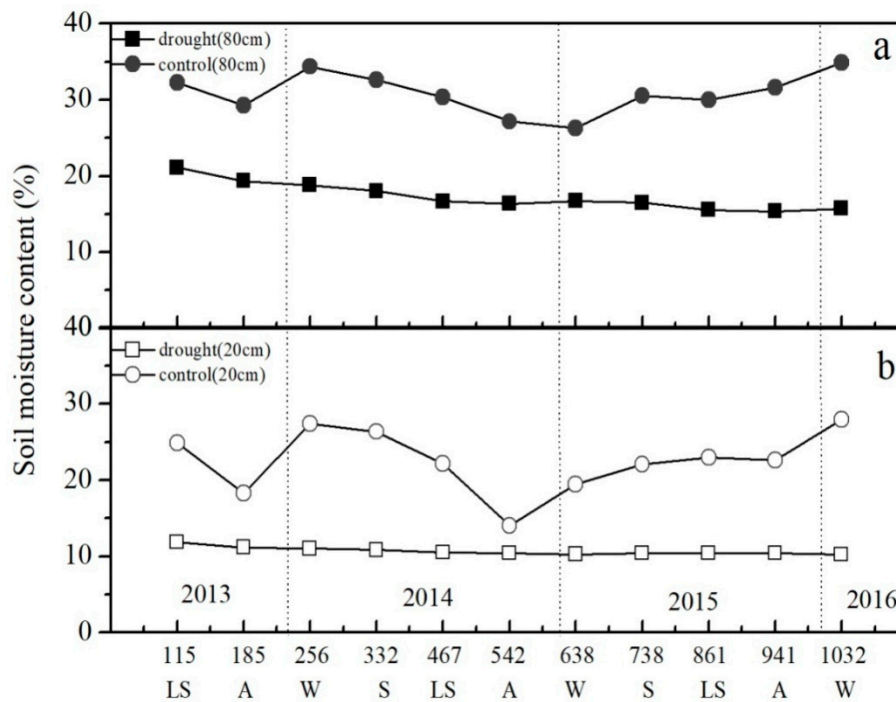


Figure 5. Relative changes in soil moisture content across the study period (from April 2013 to January 2016) in the control and drought plots. Samples were collected in later summer (LS), autumn (A), winter (W), and late spring (S). (a) Soil moisture content at 80 cm in the control and drought plots; (b) Soil moisture content at 20 cm in the control and drought plots.

Then, we determined the leaf water potential of *P. massoniana* to test the effects of rainfall exclusion from April 2013 to March 2014. Continuous drought stress led Ψ_d in the drought group to decrease over the study course. The average value of the drought group (Ψ_d : -3.73 MPa) was significantly lower than that of the control group (Ψ_d : -2.80 MPa, $p < 0.05$) (Figure 6), indicating that differences in soil water could be experienced between the treatments. To further confirm these findings, the correlation between leaf water potential and soil moisture content in different treatments were analyzed. In the control group, we found no significant correlation in the 20 or 80 cm soil layers under normal water supply conditions. Leaf water potential was only affected by abiotic factors. The leaf water potential in the drought group was significantly positively correlated in the 20 cm soil layer ($\Psi_d = 0.077 \text{ smc}^2 - 2.012 \text{ smc} + 8.813$, $R^2 = 0.95286$, $p < 0.01$) and positively correlated in the 80 cm soil layer ($\Psi_d = -0.009 \text{ smc}^2 + 0.577 \text{ smc} - 11.995$, $R^2 = 0.816$, $p < 0.05$). These results showed that when the soil moisture content was limited, the root water uptake of *P. massoniana* could not meet the needs of transpiration, and leaf water potential decreased with decreasing soil moisture content.

3.2. Effect of Drought Stress on Element Concentrations

The 100% rainfall exclusion resulted in a general increase in leaf N and P concentrations over the course of the studied years of 10.88% and 2.81%, respectively (Figure 7). Leaf N and P concentrations shared a common characteristic: leaf N and P concentrations between the control group and the drought group were not statistically different during the early stage of the 1032 days of study (Figure 7). Then, in the later periods, leaf N and P concentrations in the drought group were significantly higher than in the control group ($p < 0.05$), at 23.6% and 10.4%, respectively. Changes appeared on days 542–638 and 467–542 for N and P, respectively (Figure 7). In the simulated drought plots, leaf K concentrations significantly reduced by 14.1% ($p < 0.05$) (Figure 7). Except for day 638, leaf K concentrations in the drought group were all lower than in the control group. We found no significant change in leaf C concentrations. Changes in the concentration of individual elements also contributed to the differences in their ratios. The effects of rainfall decreased leaf C:N ($p < 0.05$) ratio and increased leaf N:P ($p < 0.05$),

N:K ($p < 0.01$), and P:K ($p < 0.01$) ratios. Leaf N:P ratios were similar to N and P concentrations; after divergence on days 542–638, leaf N:P ratios in the drought group (Table 1) were significantly higher than in the control group.

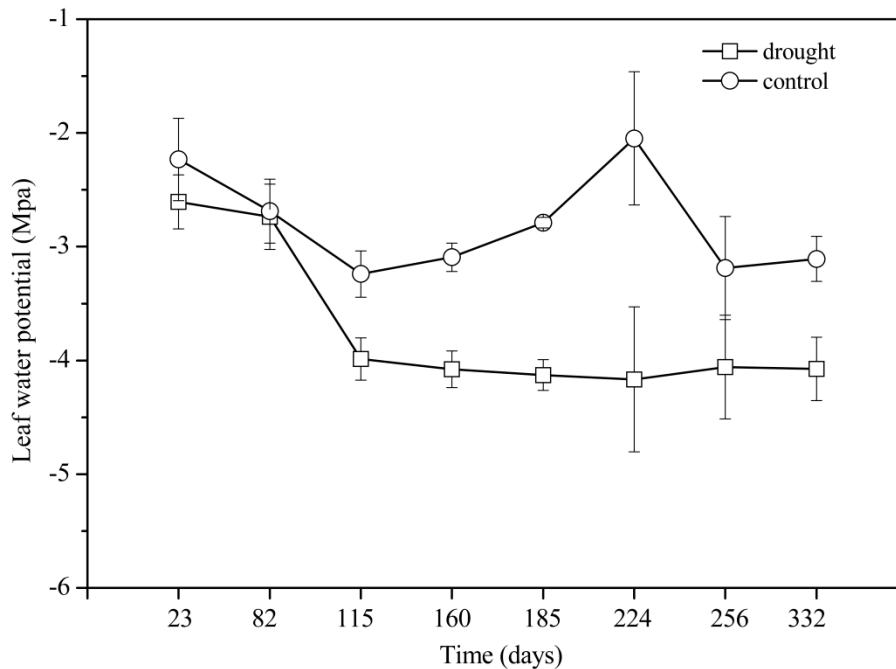


Figure 6. Monthly variation of dawn leaf water potentials between April 2013 and March 2014 (mean ± SD).

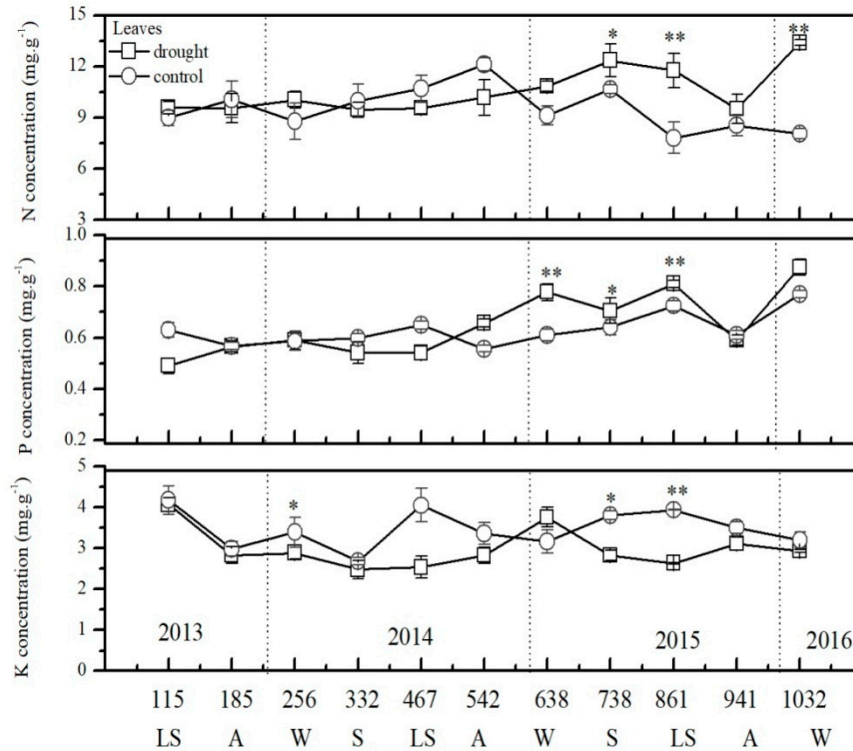


Figure 7. Leaf elemental concentrations of N, P, and K in *P. massoniana* under control and drought treatments (mean ± SD). Samples were selected in late summer (LS), autumn (A), winter (W), and late spring (S) from 2013 to 2016. Different letters indicate significant differences between the control and drought plots; ** $p < 0.01$, * $p < 0.05$.

Table 1. Mean (Standard Deviation, SD) of leaf C: N: P: K ratios of different treatments during the study period.

Element Ratio	Day	115	185	256	332	467	542	638	738	861	941	1032
	Treatment	Mean (SD)										
C:N	drought	52.27 (1.84)	51.81 (4.71)	49.65 (2.19)	53.38 (2.44)	54.24 (3.5)	49.48 (4.25)	48.11 (2.26)	39.82 (2.07)	42.63 (3.25)	52.43 (5.00)	37.47 (0.49)
	control	62.47 (2.47)	49.76 (6.77)	57.28 (3.33)	53.54 (4.95)	46.42 (3.97)	44.58 (2.37)	57.22 (2.79)	46.25 (6.72)	65.25 (4.12)	57.85 (8.47)	63.01 (2.44)
N:P	drought	12.34 (0.79)	16.83 (0.91)	17.5 (0.85)	17.94 (0.34)	17.67 (0.61)	15.23 (0.12)	16.23 (0.86)	18.06 (0.28)	14.52 (0.63)	16.00 (0.26)	15.31 (0.50)
	control	14.3 (0.56)	19.7 (0.78)	15.43 (0.62)	16.65 (0.58)	16.72 (0.56)	22.1 (0.58)	15.86 (0.42)	16.77 (0.46)	10.81 (0.41)	14.2 (0.68)	10.47 (0.77)
N:K	drought	2.48 (0.40)	3.37 (0.10)	3.55 (0.66)	3.92 (0.24)	3.8 (0.13)	3.67 (0.15)	3.274 (0.11)	4.94 (0.42)	4.47 (0.23)	3.31 (0.14)	4.57 (0.23)
	control	1.87 (0.02)	3.77 (0.23)	2.87 (0.16)	4.08 (0.07)	2.87 (0.06)	4.16 (0.29)	3.07 (0.33)	2.8 (0.17)	2.01 (0.28)	2.1 (0.02)	2.51 (0.05)
P:K	drought	0.12 (0.005)	0.20 (0.008)	0.20 (0.007)	0.22 (0.003)	0.22 (0.002)	0.24 (0.005)	0.20 (0.015)	0.27 (0.019)	0.31 (0.005)	0.21 (0.034)	0.30 (0.003)
	control	0.15 (0.017)	0.19 (0.008)	0.19 (0.003)	0.22 (0.005)	0.17 (0.003)	0.17 (0.002)	0.20 (0.002)	0.17 (0.003)	0.18 (0.004)	0.17 (0.009)	0.24 (0.004)

Among tissues, for the different treatments, branches in the drought group had similar trends in the changes in leaves, but the changes throughout the whole process were more diverse (Figure 8, Table 2). Among them, branch N concentrations and C:N, N:P, N:K ratios showed extremely significant differences ($p < 0.01$), but the other differences were not significant (Figure 8, Table 2).

Table 2. Mean (SD) of branch C: N: P: K ratios of different treatments during the study period.

Element Ratio	Day	115	185	256	332	467	542	638	738	861	941	1032
	Treatment	Mean(SD)										
C:N	drought	110.77 (5.45)	91.81 (7.99)	99.02 (3.55)	103.1 (10.38)	88.6 (0.54)	92.59 (9.03)	96.43 (11.77)	91.56 (10.39)	79.81 (7.39)	113.38 (4.11)	85.74 (4.27)
	control	122.39 (1.53)	112.03 (12.41)	120.68 (6.53)	122.98 (7.53)	92.38 (5.99)	110.75 (10.73)	100.88 (9.18)	100.5 (8.78)	105.53 (8.53)	109.05 (4.16)	100.3 (6.21)
N:P	drought	11.48 (0.54)	13.14 (0.28)	11.03 (0.29)	10.96 (0.58)	11.19 (0.53)	11.95 (0.47)	9.59 (0.76)	12.22 (0.17)	11.7 (0.21)	11.52 (0.67)	13.27 (0.34)
	control	13.6 (0.31)	8.35 (0.43)	10.48 (0.93)	12.43 (0.48)	12.48 (0.41)	9.61 (0.46)	8.65 (0.66)	11.2 (0.65)	11.62 (0.41)	11.09 (0.42)	8.12 (0.32)
N:K	drought	1.52 (0.15)	2.54 (0.18)	2.39 (0.14)	2.43 (0.11)	1.82 (0.09)	2.25 (0.12)	2.24 (0.14)	2.04 (0.04)	3.32 (0.20)	3.75 (0.24)	2.63 (0.19)
	control	1.44 (0.12)	1.73 (0.03)	2.03 (0.44)	1.92 (0.14)	1.85 (0.15)	1.62 (0.23)	1.97 (0.09)	1.82 (0.16)	2.03 (0.11)	2.12 (0.11)	2.41 (0.15)
P:K	drought	0.13 (0.030)	0.2 (0.051)	0.21 (0.027)	0.22 (0.003)	0.16 (0.066)	0.19 (0.017)	0.23 (0.014)	0.17 (0.011)	0.28 (0.008)	0.227 (0.014)	0.198 (0.012)
	control	0.11 (0.008)	0.21 (0.017)	0.19 (0.013)	0.17 (0.005)	0.15 (0.016)	0.17 (0.014)	0.23 (0.021)	0.16 (0.007)	0.17 (0.013)	0.19 (0.012)	0.29 (0.016)

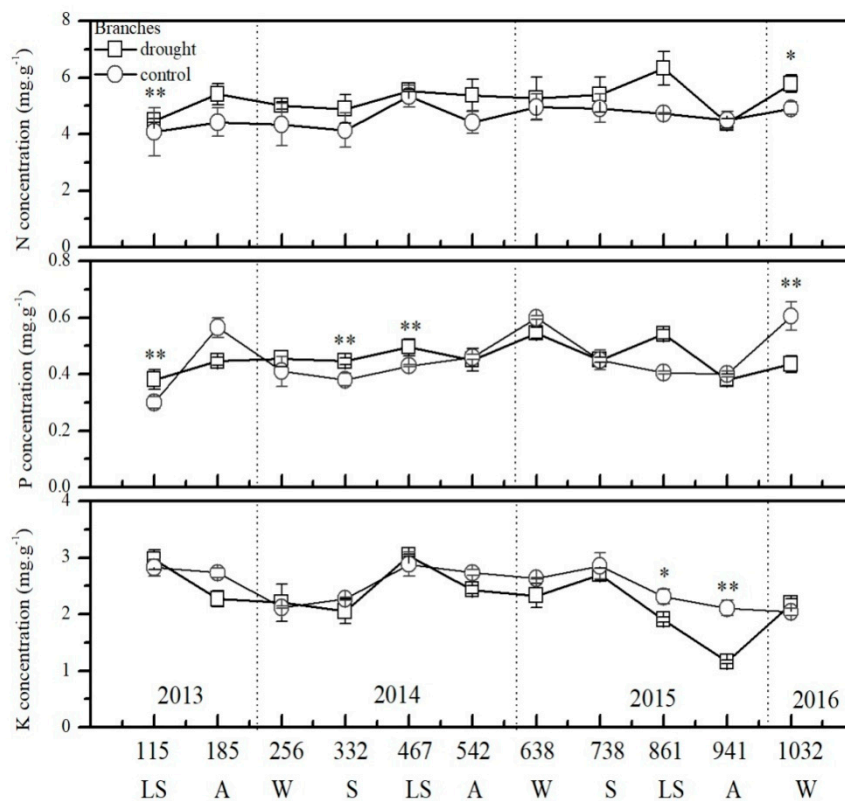


Figure 8. Branch elemental concentrations of N, P, and K in *P. massoniana* under control and drought treatments (mean \pm SD). Samples were selected in late summer (LS), autumn (A), winter (W), and late spring (S) from 2013 to 2016. Different letters indicate significant differences between the control and the drought plots; ** $p < 0.01$, * $p < 0.05$.

3.3. Effect of Season on Concentrations of Elements

In the control group, the concentrations of N, P, and K in leaves showed obvious seasonal dynamics during the study period (Figure 7). In 2013 and 2014, from spring through summer to autumn, leaf N concentrations showed a gradual upward trend in the growing season. The peak value of leaf N concentrations in these two years appeared on day 185 (27 October 2013, autumn) and day 542 (15 October 2014, autumn) at $10.07 \text{ mg}\cdot\text{g}^{-1}$ and $12.12 \text{ mg}\cdot\text{g}^{-1}$, respectively (Figure 7). These two days were also the minima for the soil moisture contents at both depths (Figure 5). However, the seasonal dynamics in 2015 were different from the previous two years (Figure 7); the peak value appeared on day 738 (9 April 2015, spring) at $10.67 \text{ mg}\cdot\text{g}^{-1}$, which was closely related to the rainfall situation (Figure 2). Leaf P concentrations and K concentrations were the highest in summer from 2013 to 2016. The peak value appeared on day 115 (18 August 2013, late summer), day 467 (1 August 2014, late summer), and day 861 (1 August 2014, late summer) (Figure 5). Leaf N:P ratios were the highest in autumn in 2013 and 2014, but in spring in 2015 (Table 1). Leaf N:K and P:K ratios were the lowest in summer, leaf C:N ratios were the lowest in autumn from 2013 to 2014, but in 2015, C:N ratios were lower in spring (Table 1). We further analyzed the relationship between leaf N and P concentrations and N:P ratios. The leaf N:P ratios were significantly positively correlated with leaf N concentrations ($R^2 = 0.8658$, $p < 0.05$) and were significantly negatively correlated with leaf P concentrations ($R^2 = 0.7012$, $p < 0.05$). When leaf P concentrations increased more than N concentrations, leaves had lower N:P ratios.

For branches, C, N, P, and K concentrations and C:N, N:P, N:K, and P:K ratios of the control group changed more diversely than in the leaves. Although we observed certain seasonal dynamics, the annual peak appeared in different seasons during the study period. Then analyzing the branches in

the drought group, we found that both the elements and ratios follow a consistent trend with leaves in the drought group (Figures 7 and 8, Table 2).

3.4. Overall Effects of Drought and Season on Leaf and Branch Nutrient Concentrations

3.4.1. Leaf Nutrient Concentrations

PCA revealed that all concentrations of elements and their ratios generally varied in the drought and control groups. Plants at different sampling times in different treatment groups were separated along the principal component 1 (PC1) axis. In our study, we only used leaf C, N, P, and K concentrations and leaf C:N, N:P, N:K, and P:K ratios in different treatments as variables. In the drought group, leaf N, P concentrations and C:N ratios were the most important factors (highest loading) in PC1 (Figure 9a), showing that leaf N and P concentrations increased and K concentrations decreased over the course of the drought. PCA was able to distinguish between leaves collected at different sampling points on the PC1 axis, which explained 70.26% of the overall variance (Figure 9b), which also expressed the sampling times with longer drought duration that were separated from the shorter duration drought except on day 941.

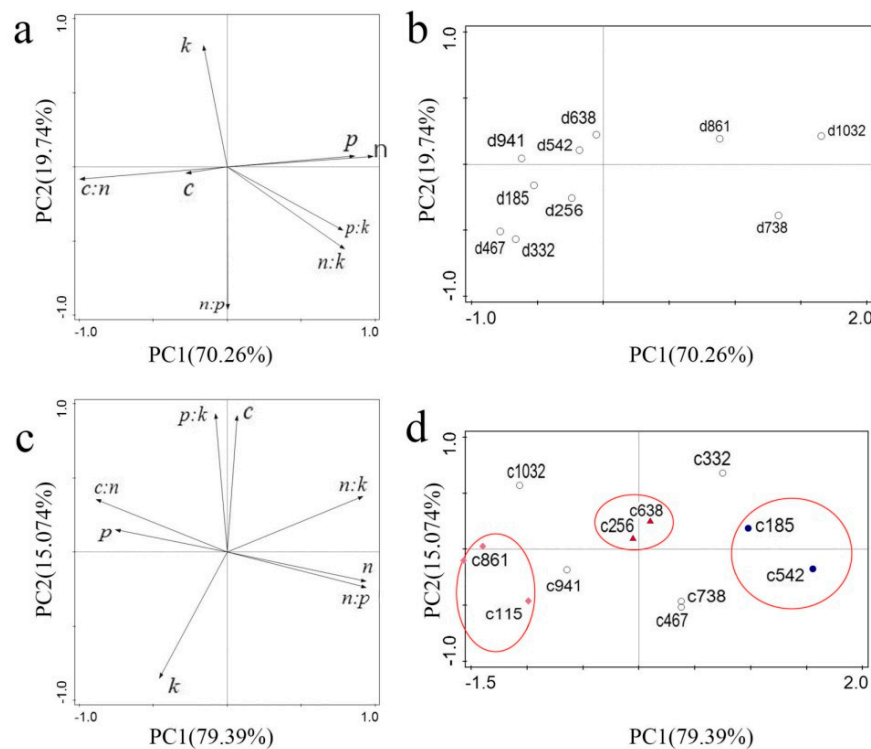


Figure 9. Principal component analysis (PCA) with leaf element concentrations and ratios as variables, and the corresponding variable distribution in (a) and (b) the drought group and (c) and (d) the control group. The significant differences among scores of different sample times are depicted along the PC axes. Note: d256 = day 256 in the drought group; c256 = day 256 in the control group.

In the control group, some of the same seasons were distributed closely, as marked by red circles in Figure 9d and separated along PC1 (explaining 79.39% of the total variance), with leaf N and P concentrations as the main factors (Figure 9c). The results highlighted the negative correlation between the leaf N:P ratios and P concentrations (Figure 9c). Parts of the same seasons were assigned to the same area; the smaller the score distance between in the PCA space, the more similar to the seasonality of the above indicators of leaves. In the drought group, after the three-year 100% rainfall exclusion experiments, the impact of seasonal changes completely disappeared.

3.4.2. Branch Nutrient Concentrations

PCA did not provide effective conclusions for the eight selected indexes for branches in the drought group (Figure 10), which suggests that different tissues have different drought adaptation strategies. In the control group, we were unable to obtain useful information from the PCA.

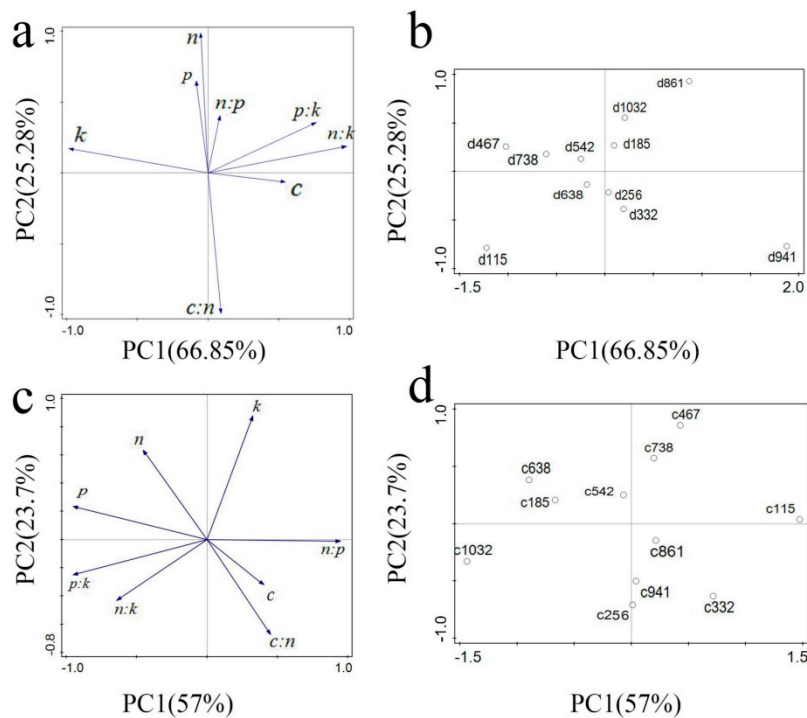


Figure 10. PCA with branch element concentrations and ratios as variables, and the corresponding variable distribution in (a) and (b) the drought group and (c) and (d) the control group. The significant differences among scores of different sample times are depicted along the PC axes. Note: d256 = day 256 in the drought group; c256 = day 256 in the control group.

4. Discussion

4.1. Drought Effect on Nutrient Concentrations

Using PCA, we identified higher leaf N and P concentrations and lower leaf K concentration under water stress. Plants, especially those adapted to harsh environments, usually respond to stress by altering their nutrient storage [16]. Many studies have reported that leaf N concentrations in plants increase due to a decrease in water supply [43,44], which is consistent with our findings. In the absence of water, plant water use tends to be more efficient [45]. Increased leaf N concentrations help plants to achieve maximum photosynthetic volume with minimal moisture content under minimal stomatal conductance [43]. Further increased leaf P concentrations could improve water use efficiency as a drought-resistance mechanism, which has also been widely reported [32,46,47], indicating the positive effects of P on plant growth under drought stress, such as an increase in root growth [48], stomatal conductance and nitrate reductase activity [49], and high cell-membrane permeability [50]. Similar to leaf N concentrations, increasing leaf P concentrations improve photosynthetic efficiency and plant transpiration control capacity [51]. Leaf and branch N:P ratios in the drought group were higher than those in the control group, related to the decrease in growth rate under the condition of lacking water and to the anti-stress mechanism strongly influencing N:P ratio, as plants in anti-stress environments needed higher nutritional investment [14].

Potassium accordingly played a fundamental role under water stress conditions. Most studies suggested that K concentration increases under drought conditions, which could reduce the osmotic

potential of cells, improve the water retention capacity of the cell protoplasm, and enhance the stability of the cell membrane [14,29–31]. Adequate K could help improve water use efficiency (WUE) and hence, plant growth under the water deficiency condition [52]. However, in this study, we found that with increasing water stress, leaf and branch K concentrations decreased considerably, which might lead to the imbalance of osmotic pressure and mineral element concentrations. Concentrations of C, N, and P underwent the self-regulation in the three-year course of 100% isolation from rainfall, with concentrations increasing to adapt to drought conditions, but concentrations of K could not self-regulate. K cannot be synthesized in plant cells; most of it must be absorbed or transferred from the soil [25]. As the transport of K mainly occurs through diffusion, and water is an important factor controlling the diffusion of K, loss of water halts the diffusion. Therefore, we think that the imbalance in osmotic regulation caused by the decrease in K maybe the main reason for the death of *P. massoniana* due to water shortage.

4.2. Seasonal Variation in Element Concentrations

The concentrations of N, P, and K in leaves of *P. massoniana* are closely related to their own structural characteristics and growth rhythm, which all change with the seasons. In the control group, the seasonal variation trend of leaf P concentrations in *P. massoniana* during the 1032 days was consistent with the data published by other studies [24,53,54]. We observed an obvious upward trend from winter through spring to summer, which then decreased significantly in autumn and increased again from autumn to winter. The reasons behind this trend are as follows: In the early growing season (spring to summer), the photosynthetic organs gradually recovered their normal photosynthetic capacity from low temperature in winter while the plant leaves were small; to organize leaves and grow structural tissue continuously, leaf cells are in the stage of rapid division, which requires large amounts of protein and nuclein. Therefore, the selective absorption of P increased strengthened, and the concentrations of P were higher [55]. During the peak growing season (summer to autumn), leaf cells multiply rapidly and expand, so the absorption of nutrient elements by plant roots could not keep pace with the expansion rate of leaf cells, resulting in the gradual dilution of leaf P concentration [56]. From autumn to winter, after the plant stopped growing, leaf P concentration in the three-year study period increased by 3.07%, 9.51%, and 26.23% [18]. The seasonal variations in leaf K concentrations were consistent with those of leaf P, which could be explained using similar reasons. The trend and consistency of the seasonal dynamic change in leaf N were slightly different from leaf P and K concentrations. The peaks in 2013 and 2014 occurred in autumn, and in 2015, the peak occurred in spring. This conclusion is contrary to the findings of other studies, which reported that change process of leaf N was coordinated with that of leaf P. The analysis showed that *P. massoniana*, as a pioneer tree species in eroded red soil areas in ChangTing county, have formed their own adaptive mechanism after years of adversity. They reserve as many nitrogen compounds as possible before winter dormancy to resist severe cold and various stress.

Our research supported GRH in the analysis of the effects of seasonal dynamics on leaf N and P concentrations and N:P ratios. In the control group, leaf N:P ratios were lowest in spring, summer, and winter from 2013 to 2016 and highest in autumn. The lowest leaf N:P ratios occurred in spring and summer [57], which was consistent with the GRH predictions. This indicates that plants often have high leaf N and P concentrations and with low N:P ratio in the growing season to maximize the growth rate capacity [16,24]. The reduction in leaf N:P ratios in the winter is not associated with growth rate, but with the effect of the temperature [23]. With decreasing temperature, leaf N concentrations of plants are basically unchanged, P concentrations increase, and the N:P ratios decrease [24,53,54]. Because at low temperature, plants might activate a temperature-sensitive mechanism that increases leaf P concentration, thus, improving the enzyme efficiency and reducing the rate of RNA synthesis [23]. As a non-photosynthetic organ, the N and P concentrations in branches did not change significantly with the season, so the seasonal variations in branch N:P ratios were inconsistent.

4.3. Effects of Soil Nutrient Availability

The relationship between N and P concentrations in plants was determined by soil nutrient conditions and the plasticity of the plants [58]. Many studies generally reported that the leaf N:P ratio could be used as an indicator of soil nutrient deficit; $N:P < 14$ or $N:P > 16$ were used to indicate N restrictions or P restrictions, respectively [23,56,59]. However, these results were more common in humid areas. These results areas where water is severely deficient are lacking [60]. Our sample plot had red soil that differentially develops from coarse grain granite and characterized by poor nutrient levels and lack of water, which reduce the availability of N and P in the soil [60]. Our results confirm this conclusion. The average concentration of leaf N in the control group was $9.87 \pm 1.35 \text{ mg}\cdot\text{g}^{-1}$, and the leaf P concentration was $0.65 \pm 0.063 \text{ mg}\cdot\text{g}^{-1}$. Both were significantly lower than for terrestrial plants globally (N: $20.1 \pm 8.7 \text{ mg}\cdot\text{g}^{-1}$, P: $1.8 \pm 1.1 \text{ mg}\cdot\text{g}^{-1}$) [15,23]. Because the decrease in soil moisture limits the process of organic matter production and mineralization, the release of P from parent material slowed down, soil organic matter concentration reduced, which was consistent with the availability of N [61]. When the concentration of leaf P decreased more than that of leaf N concentration in the drought group, the average N:P value was 15.73 ± 3.31 , which is significantly higher than that of terrestrial plants globally and, similar to that of desert plants in China [62,63]. Plant P concentrations were closely related to soil P concentration. The concentration of soil P in most parts of China [53] is lower than the global level, which directly affects the P absorbed by plants.

5. Conclusions

In summary, we conducted a continuous study to analyze the element dynamics of *P. massoniana* induced by three-year rainfall exclusion. In the drought group, leaf N and P concentrations increased to improve water use efficiency as a drought resistance mechanism. However, leaf K concentrations decreased continuously and were not regulated in response to water stress, which may be the main reason for death due to water shortages. The changes in elements in leaves and branches produced by ontogeny and seasonality were significantly greater than by experimental drought. In the growing season, high leaf N and P concentrations and low N:P ratios were consistent with the GRH prediction. Leaf N concentrations were highest when the soil moisture content was lowest in the annual cycle. Thus, leaves and branches can regulate the nutrient elements and then maintain certain stoichiometries as a method to adapt to stress. Therefore, our findings suggest that in red soil erosion, a lack of N or P (or both) would probably impede *P. massoniana*'s response to drought, and therefore, one would expect drought-related dieback to occur earlier.

Author Contributions: T.L., H.Z., and J.Z. designed the experiment. T.L. and H.Z. conducted the field experiment. T.L., X.F., and Y.L. conducted the biochemical analyses in the laboratory. T.L. analyzed data and wrote the manuscript. T.L. and H.Z. revised and improved the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Nature Science Foundation of China (General Program: 31270659), the Educational Research Projects for Young and Mid-Aged Teachers in Fujian Province of China (Grant NO. JT180333), the Natural Science Foundation of Fujian Province (2018J05011).

Acknowledgments: This research was supported by We thank W.-Y.L. and X.-H.Z. for their assistance in the field and laboratory.

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

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