# Seasonal variations in leaf and branch trace elements and the influence of a 3-yr 100% rainfall exclusion on *Pinus massoniana* Lamb

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## Abstract

**Background.** Trace elements are essential for the growth and survival of plants, and their concentrations and distributions in plants are effective reflections of ecological adaptation strategies. However, this aspect has seldom been addressed.

**Method.** Changes in the leaf and branch trace elements of *Pinus massoniana* Lamb, induced by seasonal dynamics and in response to a 3-yr 100% rainfall exclusion, were evaluated.

**Results.** The results showed that the concentrations of Fe, Cu, Zn, Cd, Ni and Cr in leaves of *P. massoniana* in the control group had high seasonal resolution. There were three groups according to their patterns over the growing season: (1) nutrient elements (Cu, Zn, Ni and Cr), which continuously decreased in concentration during the growing season, with the highest concentration in spring and the lowest in autumn; (2) accumulating element (Cr), which increased in concentration from spring to autumn; and (3) indifferent element (Fe), which increased in concentration from spring to summer and decreased in concentration from summer to autumn. The concentrations of trace elements in leaves and branches showed no significant differences with mild drought stress, except for Fe and Cr in leaves and Cr in branches, which significantly increased ($p < 0.05$) under the result of self-selection under mild drought stress. Therefore, the resultant seasonal and drought effects on trace element cycling in *P. massoniana* could provide theoretical support to respond to future climate change.

## Introduction

As an important part of the terrestrial ecosystem, the forest ecosystem has the characteristics of strong stability and perfect function (Hisano et al. 2018). Trace elements, defined as elements that are present at low concentrations (mg.kg$^{-1}$ or less) in most soils, plants and living organisms

38  (Phipps 1981) , are widely distributed in the forest ecosystem and essential for the growth and
39  survival of plants. The contamination of terrestrial environments with trace elements is a
40  common environmental stress phenomenon worldwide (Eid & Shaltout 2014). Fe, Cu, Zn, Cd
41  and other trace elements in forest plants come from the soil and atmosphere, and are elements
42  that cannot be degraded by microbial or chemical processes to alter their toxicity. Long-term
43  accumulation of trace elements causes chronic damage to living organisms; for example,
44  increasing the concentration of trace elements in plants affects normal growth and physiological
45  and ecological characteristics (Peñuelas 2002). Plants are sessile organisms that must cope with
46  variable environments (Van Ieperen 2016), both in space and time, and this may induce changes
47  in abiotic factors (light, temperature and water) and biotic factors (bacteria and fungi)(Etienne et
48  al. 2018). For example, plants absorb heavy metals from the atmosphere and soil through
49  respiration, and the root system potentially harbors hazards. To survive, grow and reproduce,
50  plants must develop multiple physiological mechanisms to maintain a reasonable range of trace
51  elements (Philippot et al. 2013). Many studies suggest that environmental fluctuations and
52  features of the ecosystem (such as plant growth strategies, and soil nutrient heterogeneity) affect
53  foliar nutrient elemental concentrations (Han et al. 2005; He et al. 2015; Kerkhoff et al. 2005;
54  Sardans et al. 2011). Changes in the concentrations of nutrient elements during plant growth can
55  reflect the physiological and growth characteristics of plants to a certain extent. In addition, trace
56  elements are involved in all metabolic and cellular functions, have the ability to form stable
57  compounds with biological organic macromolecules, and can remain in plant tissue for a long
58  time and  have seasonal effects (Baycu et al. 2006). Therefore, the study of seasonal variations in
59  plant trace elements can be beneficial to further understanding of the nutrient status and growth
60  trends of the plants.
61      Currently, most research on the characteristics of nutrient elements is mainly focused on the
62  concentrations of C, N, P, K and other macro elements (Güsewell et al. 2005; Sardans et al.
63  2013; Urbina et al. 2015; Wang et al. 2013; Woods et al. 2003). Because of their roles in several
64  physiological functions in plants, such elements correlate with the growth rate (Elser et al. 2010;
65  Peñuelas et al. 2013; Sardans et al. 2013) and with the structure and function of plant
66  communities (Elser et al. 2003; Sardans & Peñuelas 2012). The C:N:P ratio in organisms can be
67  associated with important ecological processes, such as responses to environmental stress
68  (Sardans et al. 2013; Woods et al. 2003), and ecosystem composition and diversity (Güsewell et
69  al. 2005; Roem & Berendse 2000). However, trace elements that help macronutrients improve
70  physiological functions are not well studied (Paiva et al. 2017; Sardans et al. 2007; Waraich EA
71  2011). Some studies of trace elements vary along environmental gradients (Eid & Shaltout 2014;
72  Paiva et al. 2017), and their resistance to stress (Mahdavi S 2016; Urbina et al. 2015). Water is
73  the most limiting factor in ecosystems and regulates plant growth and yield; a lack of water leads
74  to a substantial number of effects on plants and species relationships, including readjustment of
75  transport and metabolic processes, community diversity and ecosystem productivity (Peñuelas et
76  al. 2013). A drought-induced decrease in soil moisture content may reduce the rate at which
77  plants absorb trace elements. If we are to fully understand the impact of climate change on

78    ecosystems, it is necessary to study the interactions between predicted droughts and the changes
79    in trace elements. However, it is uncertain whether and how drought can affect trace element
80    dynamics.
81       The leaf is the main site of photosynthesis and the most active organ in plant metabolism,
82    its chemical element concentrations can reflect plant absorption and accumulation of elements
83    (Anderson & Proctor 1990; Grubb et al. 2009), revealing the plant's demand for nutrient
84    elements and adaptability and feedback to climate and regional changes. The branch is another
85    important photosynthetic organ. when the leaf area is small or the leaves are degenerated, the
86    branch adopts the role of leaves to perform physiological functions, such as photosynthesis and
87    transpiration (Lacointe et al. 1993). Therefore, the concentrations of elements in branches will
88    change with the environmental changes. Trace elements in leaves and branches show different
89    physiological functions. For example,  Fe plays an important role in regulating osmotic pressure,
90    promoting protein synthesis, and improving photosynthetic capacity and plant resistance
91    (Ahanger et al. 2016); Zn is a component of various enzymes in plants and is involved in plant
92    respiration and carbohydrates transformation (Blasco et al. 2015; R Hajiboland 2012); and Cu
93    plays a key role in photosynthetic and respiratory electron transport chains, ethylene sensing, cell
94    wall metabolism, and protection from oxidative stress (Yruela 2009). Changes in environmental
95    factors such as water and temperature have controversial effects on the accumulation of trace
96    element. For example, some scholars believed that under drought conditions, the increased
97    accumulation of trace elements can enhance the negative effects of drought on plant
98    productivity; on the other hand, the decrease in trace element accumulation increases the
99    productive capacity of these ecosystems and improves their capacity to resist drought (Sardans &
100   Peñuelas 2007). Other scholars have come to contrasting conclusions.
101      This study investigated changes in leaf and branch trace elements of *P. massoniana* induced
102   by the annual seasons and in response to a 3-yr manipulated precipitation experiment (100%
103   rainfall exclusion) that began in April 2013 and ended in January 2016 in Changting County,
104   Fujian Province, China. In this experiment, the local pioneer *P. massoniana* was taken as the
105   research object to study the seasonal dynamic of the trace elements and the influence of drought
106   on it, to provide theoretical support for the future response of forest species to climate change.
107   Here, the concentrations of six selected trace elements (Fe, Cu, Zn, Cd, Ni, and Cr) in the leaves
108   and branches of *P. massoniana* growing in the control group (natural conditions) and the drought
109   group (100% rainfall exclusion) were determined. The present research was performed to
110   investigate (1) the seasonal dynamics characteristics of leaf and branch trace element
111   concentrations in *P. massoniana* over the study period; (2) whether drought increases or
112   decreases trace element concentrations and accumulations in leaves and branches of *P.
113   massoniana*; and (3) the interaction between trace element concentrations and seasonal variations
114   or drought.

115   **Materials & Methods**
116   **Research Area**

117    The sampling site was located in Hetian Town, Changting County, Western Fujian Province,
118    China (116°18′-116°31′ E, 25°33′-25°48′ N, 310 asl) (Figure 1). This site was one of the most
119    serious red soil erosionsites in China. Because of soil depletion, the dominant species were *P.*
120    *massoniana, Schima superba, Castanopsis fargesii and Lithocarpus sp.*, and there were only a
121    few *Dicranopteris dichotoma (Thumb) Berhn* under the forest. This area had a humid monsoon
122    climate in the middle subtropical zone, with distinct dry and wet seasons. The rainfall was
123    mainly concentrated from May to July every year, with an average annual rainfall of 1 737 mm,
124    which was relatively abundant. The annual average temperature in this area was 17.5°C ～
125    18.8°C, the lowest temperature recorded was -7.8°C and the highest temperature recorded was
126    39.8°C. An automatic meteorological station was installed to collect temperature, soil moisture,
127    precipitation, and relative humidity measurements every 15 min at the study site. According to
128    the linear relationship between rainfall and temperature recorded by the automatic weather
129    station, A Bagnouls-Gaussen bioclimatic diagram (Figure 2) was made to evaluate the drought
130    situation under natural conditions in the study area. The method considered that when the total
131    monthly precipitation was less than twice the mean temperature in that month defined as arid.
132    The results in Figure 2 show only six months with a soil water deficiency during the 34 study
133    months (April 2013 to January 2016). Therefore, we considered that the study site without aridity
134    could be used as a control group.

**Experimental Design and Sampling**

136    The sample plot was set up on a 30º slope in April 2013 (Field experiments were approved
137    by the farmer Ping Xiu who had the ownership of the field), which had a total 42 *P. massoniana*
138    individuals nearly 25 years old. On average, those similar *P. massoniana* individuals were 4.2
139    cm in diameter at breast height (DBH), 2.4 m in height and 8.5 cm in blade length. The sample
140    plot were divided into four small plots (20 × 20 m)(Urbina et al. 2015). In order to avoid the
141    influence of slope on sampling, two small plots with 20 *P. massoniana* individuals (one uphill
142    and one downhill) received 100% rainfall exclusion as drought plots, and the other two plots
143    with 22 *P. massoniana* individuals in the natural environment did not receive any treatment
144    served as control plots. In the drought group, a top rain reduction device was installed.
145    Transparent tiles were fixed above the study plot, and all rainfall was intercepted above the
146    whole canopy. The light transmittance of the wave pattern can reach 90%. Because the device
147    was built only above the top of the canopy, the air convection around was sufficient, and the
148    temperature and humidity of the two group were consistent. At the same time, to ensure the
149    rainfall could flow out of the isolation area, the wave tiles were parallel to the plots (Sardans et al.
150    2007; Sardans et al. 2013) . Then, a soil lateral water control device was built around the study
151    site. According to the sampling of *P. massoniana,* it was found that the main root was 2 m deep
152    and the lateral root was less than 80 cm. Therefore, an 80 cm deep channel was dug around the
153    study site, and aluminum plates were installed in the channel to avoid water infiltration. The
154    control group maintained the natural state without any treatment. Finally, to prevent small
155    animals from destroying the plots, the four plots were surrounded by wire to ensure that the test
156    ran without interference.

157    On August 18, 2013 (115 days after 100% isolated rainfall), 3 *P. massoniana* individuals
158    with relatively consistent height and diameter were randomly selected in the middle of 4 plots
159    (total n=12). The dates of plant sampling were selected based on the major climatic factors of the
160    study site. In the study period, leaves and branches were collected from each sample individual
161    one a quarterly basis to measure trace elemental concentrations. The 11 sampling times were as
162    follows: day 115 (2013.8.18, late summer), day 185 (2013.10.27, autumn), day 256 (2014.1.6
163    winter), day 332 (2014.3.23 spring), day 467 (2014.8.1, late summer), day 542 (2014.10.15,
164    autumn), day 638 (2014.12.30, winter), day 738 (2015.4.9, spring), day 861 (2015.8.10, late
165    summer), day 941 (2015.10.29, autumn) and day 1032 (2016.1.28, winter) (Anderson 1964).
166    To minimize the impact of diurnal variability, one branch with healthy leaves completely
167    exposed to the sun was collected from the east、south、west、north directions in the middle
168    and upper parts of the crown of the selected sample individual between 9:00 am and 11:00 am on
169    the sampling day, and then the healthy leaves with lengths of 8.3～8.6 cm and the branches with
170    diameters of 0.3～0.5 cm were selected to mix as one leaf sample and one branch sample,
171    respectively. All samples were immediately stored in an ice box (0-4°C) and brought back to the
172    laboratory. First, the enzymatic activity of the sample was inhibited by microwave treatment at
173    800 W for 5 min and then the sample was dried in an oven at 65°C for 48 h to obtain a constant
174    weight (Körner 2002). The samples were ball milled to a fine powder (Tissuelyser-24, CHN),
175    which served to measure trace elements (Lin et al. 2020).
176    **Chemical analyses**
177    The concentrations of Fe, Cu, Zn, Cd, Ni, and Cr were measured in leaves and branches
178    using ICP-MS (Mass Spectroscopy with Inductively Couple Plasma, Thermo iCAP Q, USA).
179    Before ICP-MS analyses, the samples was acid digested with an $HNO_3$~$H_2O_2$ mixed acid system
180    (Martínez-Fernández et al. 2015). First, 100 mg of dried leaf and branch powders were
181    accurately weighed and placed in a 50 ml disposable polyperfluoroethylene (FEP) digestion tube
182    (Eid & Shaltout 2014). FEP tubes with 8 ml of nitric acid (65%) (Merck GR, Germany) and 2 ml
183    of $H_2O_2$ (30%) were incubated overnight. The next morning, the tubes were shaking gently and
184    placed in a graphite furnace for digestion. Gradient heating was used for the acid digestion (first
185    step: heated to 80°C for 10 min; second step: 100°C for 30 min; and third step: raised to 110°C
186    and maintained until the white smoke was exhausted; last, the obtained solution was
187    concentrated to approximately 1 to 2 ml). FEP tubes were taken out and cooled at normal
188    atmospheric temperature, and then Milli-Q water was added to a constant volume. Finally, the
189    sample solutions filtered by 0.45 μm microporous membranes were stored in the refrigerator for
190    detection (Malea & Kevrekidis 2013). Plant trace element concentrations were shown in mg.kg⁻¹
191    by dry weight.
192    **Method for measuring water stress**
193    To verify the effectiveness of isolated rainfall, the leaf water potential values (Ψd) of *P.*
194    *massoniana* in different treatment groups were determined within one year after the beginning of
195    isolated rainfall. The six target individuals (three from the uphill plot and three from the downhill
196    plot) were chosen from each treatment, then south branches with health leaves from the upper

197  crown of which were collected at 6:00 am on a clear day every month. They were placed
198  immediately in a cold closet, and then taken to the laboratory to determine the leaf water
199  potential by a WP4 dew-point potential meter (Decagon Device, Pullman, WA, USA) (Klein et
200  al. 2010).
201      The soil moisture contents at 20 cm and 80 cm from each treatment were determined
202  automatically at intervals of 15 min using Time Domain Reflectometry (Trime-T3 Moisture
203  Meter, IMKO, GER) (Sardans et al. 2013).
204  **Statistical Analyses**
205      SPSS 19.0 (SPSS Inc. Chicago, USA) for Windows statistical software was used for all
206  results. Samples from each treatment were replicated for six times. Statistical analyses were
207  applied to obtain a distinctive view of the influence of treatment and time on the data variability.
208  We used a one-way ANOVA to detect the effects of different seasons or treatments on the
209  concentrations of trace elements. Repeated measures analysis of variance (ANOVA) were used
210  to study the effects of season and treatment on the amount of trace elements. The correlation
211  analysis was used to compare the relationships among different trace elements. Principal
212  component analysis (PCA) was used to provide scores for observations (samples per treatment
213  and season) and variables (trace element concentrations), which could be interpreted together.
214  Significance and high significance levels for all tests were set at $p < 0.05$ and $p < 0.01$.

# Results

## The relationship between soil moisture content and leaf water potential during a 3-yr period

218      Figure 3 shows the dynamic changes in soil moisture content between the control and
219  drought treatments. From the beginning of the experiment (2013), the general trends of soil
220  moisture content in 20 cm and 80 cm soil layers of the control group both showed a significant
221  seasonal variation trend ($p < 0.01$) (Figure 3). The valleys at 20 cm depth appeared in the control
222  group on days 185 (27 October 2013, autumn) and 542 (25 October 2014, autumn), and the
223  valleys at 80 cm depth appeared on days 185 (27 October 2013, autumn) and 638 (30 December
224  2014, winter). The 1032 days of 100% rainfall exclusion caused a mean reduction in the soil
225  moisture contents at 20 cm (mean 10.69%, CV: 4.69%) and 80 cm (mean 17.31%, CV: 10.44%)
226  compared to the soil moisture at 20 cm (mean 22.56%, CV: 18.4%) and 80 cm (mean 30.85%,
227  CV: 18.4%) in the control group ($p < 0.01$), decreasing by 11.87% and 13.54%, respectively
228  (Figure 3). The observed effects of experimental drought from 2013 to 2016 resulted in mild
229  drought conditions in the drought group (Hsiao & Acevedo 1974; Zhang et al. 2015), which was
230  likely because of the movement of soil capillary water caused by the moderate slope in the
231  sample plot.
232      Leaf water potential is the most sensitive indicator of water deficit and drought resistance in
233  plant organs. In the control group, the general trend of $\Psi d$ showed obvious seasonal variation.
234  However, 100% rainfall exclusion resulted in $\Psi d$ weakening continuously in the drought group
235  from April 2013 to March 2014. The mean value in the control group ($\Psi d$: -2.80 MPa, CV:
236  10.00%) was significantly higher than that in the drought group ($\Psi d$: -3.73 MPa, CV: 7.78%)

237   ($p$<0.05) (Figure 4). The decline in leaf water potential was an important indicator of plant water
238   shortage, forming a large gradient between plant water potential and soil water potential, which
239   was beneficial for plants to absorb water from soil and showed that *P. massoniana* in the drought
240   group initiated the physiological mechanism that aims to use the lower leaf water potential to
241   enhance the water absorption capacity of plant cells to improve their drought resistance.

**Characteristics of trace element concentrations in plant leaves and branches**

243   In our 3-yr experiment, the average concentrations of six trace elements in leaves of *P.*
244   *massoniana* were in 100 to 500 mg.kg$^{-1}$ (Fe), 10 to 100 mg.kg$^{-1}$ (Zn), and less than 10 mg.kg$^{-1}$
245   (Cu, Ni, Cr, Cd) (Table 1). The average concentrations of elements from high to low are Fe>
246   Zn> Cu> Cr> Ni> Cd. In branches, the average concentrations of six trace elements were all
247   significantly higher than those in leaves ($p$<0.01), but except for the Cu concentration, which
248   was different from the leaves at 10 to 100 mg.kg$^{-1}$, and the ranges and size sorting of other
249   elements were the same. Compared with world terrestrial vascular plants, the average
250   concentrations of Fe, Ni and Cr in leaves and branches of *P. massoniana* were all significantly
251   higher than those of global terrestrial vascular plants ($p$<0.01), where Fe and Ni were nearly 2
252   times higher than the maximum, and Cr was 8 to 13 times higher than the maximum value. The
253   average concentrations of Zn and Cu in branches were slightly higher than that of world
254   terrestrial vascular plants, and the remaining elements fell in the normal range. The results
255   showed that plants in this area were at a potential risk of being poisoned by heavy metals.

**Temporal variation in trace element concentrations**

257   An analysis of the seasonal dynamics of each trace element concentration behavior in the
258   control group during these 3 years allowed us to distinguish three groups (Figure 5): (1) nutrient-
259   like elements such as Cu, Zn, Ni, and Cd had the highest trace element concentrations at the
260   beginning of the growing season (spring), and then decreased in concentration from spring, to
261   summer to autumn; notably, changes were more pluralistic from autumn to winter to spring; (2)
262   the indifferent element Fe exhibited an increase in concentration from spring to summer and then
263   a decrease in concentration to autumn, with the highest concentration occurring in summer; and
264   (3) the accumulating element Cr showed an increase in concentration from spring-summer to
265   autumn, but the situations in winters were different. However, this classification was slightly
266   different from that revealed by statistical analysis.

267   Figure 6A presents the results of the unconstrained PCA. PC1 × PC2 revealed two groups
268   of variables. One group comprised Cu, Zn, Ni, Cd and Cr. The elements of this group exhibited
269   continuous change, that is, continuing to rise or decline during the growing season (Figure 5).
270   The other group comprised Fe. This element first increased and then decreased. On PC1 × PC2,
271   time scatter plots represented the position of each season category (Figure 6B). It appeared that
272   summers from these 3-yr study periods were significantly distinguished from other seasons
273   (Figure 6B). This difference could be linked to plant growth and photosynthetic processes during
274   this active period. At the same time, spring, autumn and winter during these three years were
275   concentrated together in Figure 6B. Therefore, we hypothesized that trace element

276    concentrations in leaves of *P. massoniana* in the control group collected at different seasons
277    during the 3-yr study period could be analyzed in terms of seasonal variations.
278        As for branches, the Cu, Zn, Ni, Fe, Cr, and Cd concentrations of the control group changed
279    more diversely. Although they had a certain seasonal dynamic, the annual peak appeared in
280    different seasons during the study period (Figure 7). An analysis of the branches revealed that all
281    trace elements did not have a completely consistent trend with leaves of the control group
282    (Figure 8). With branch trace element concentrations as variables in the control group, we could
283    not obtain useful information, and the PCA distinguished was messy (Figure 8).
284    **Effect of drought on trace elements**
285        The concentrations of trace elements in leaves and branches of the drought group showed
286    increased concentrations of trace elements compared with those in the control group, such as Cu
287    (0.51%), Zn (9.13%), Ni (1.23%), Fe (67.8%), and Cr (19.85%) in leaves; Cu (6.99%), Zn
288    (14.11%), Fe (2.16%), and Cr (23.11%) in branches and decreased Cd (-14.8%) in leaves and Ni
289    (-3.00%) and Cd (-14.5%) in branches. However, drought general resulted in no significant
290    concentration differences under water stress conditions, except in the case of leaf Fe ($p<0.05$),
291    leaf Cr ($p<0.05$) and branch Cr ($p<0.05$). The leaf Fe concentration exhibited a clear upward
292    trend from day 332 to day 1032, while the leaf Cr concentration increased except on days 115
293    and 332, and the branch Cr concentration decreased except on days 332 and 941 (Figure 5,7).
294    Figure 9 presents the results of the unconstrained PCA and shows the dates of the trace element
295    concentrations in the leaves collected from different treatments in the 3-yr study period. The PC1
296    × PC2 treatment scatter-plot represents the position of each sample time in the control and
297    drought groups. It appeared that most sample times in the drought group could be significantly
298    distinguished from sample times in the control group. Similar processes seemed to occur in the
299    branch tissues, and it was difficult to distinguish the sample times in the different treatments, but
300    the 8 groups of different treatments were clustered together in the same sampling time (Figure
301    10). As a result, trace element concentrations in leaves of *P. massoniana* could be analyzed in
302    terms of treatment variation.

## Discussion

**Seasonal dynamics of trace element concentrations in *P. massoniana* in the control group**

305        The seasonal patterns of leaf nutrient-like elements such as Cu, Zn, Ni, and Cd
306    concentrations were similar and in agreement with the data for other plants from various
307    environments (Hagemeyer JA 1992; Viers et al. 2012). Leaf Cu, Zn, Ni and Cd concentrations in
308    the control group all showed the highest in the early growth season, indicating a strong metabolic
309    processes (high photosynthetic activity,  protein synthesis energy requirements) (Arneth et al.
310    1996). Notably, from winter to spring, Cu, Zn, Ni and Cd increased. However, during these
311    periods, the soil temperature was low and the roots could not absorb nutrients from soil
312    (Prokushkin et al. 2010). Therefore, these elements probably came from the pool of elements that
313    plant preserved in the early stage of their growth. These elements could be mobilized to the
314    phloem before old leaf senescence and redistributed through xylem the following year to
315    younger tissues that needs to grow (Viers et al. 2012). Then, the decrease in leaf Cu, Zn, Ni and

316  Cd concentrations of the control group in the middle and late growing seasons may be explained
317  by two processes: (1) nutrients accumulated by mature leaves were absorbed into active growing
318  areas (e.g., fine roots and shoots) or reproductive organs; and (2) nutrients were dilution by
319  carbon accumulation. The indifferent element Fe exhibited an increase in concentration from
320  spring to summer and then a decrease in concentration to autumn. The highest concentration
321  appeared in the most vigorous summer with the strongest photosynthesis. It is believed that many
322  enzymes involved in chlorophyll biosynthesis and respiratory metabolism are Fe compounds;
323  thus, the stronger photosynthesis was, the higher the Fe concentration. The accumulating element
324  Cr showed an increase in concentration from spring-summer to autumn, but the situation in
325  winter was different.
326  **Effects of drought on trace element concentrations in _P. massoniana_ in the drought group**
327       The 3-yr of 100% rainfall exclusion, which provoked an average reduction of 11.87% (20
328  cm) and 13.54% (80 cm) in the relative moisture of the soil, had some effects on trace element
329  concentrations in the drought group. In general, water stress increased most trace elements in
330  leaves and branches, but by statistical analysis, there were no significant concentration
331  differences under water stress conditions except for increased leaf Fe and leaf and branch Cr
332  concentrations significantly ($p<0.05$). Most studies found that the increase in trace elements in
333  the stress environment were related to the general enhancement of resource capture in soil
334  microbe activity (Sardans et al. 2006), and photosynthesis capacity (Llorens 2004). Then
335  enhancement of the photosynthetic capacity and the demand for resources of young leaves made
336  the soluble organic matter migrate before senescence by the , which was the reason for the
337  retention of trace elements in the aboveground biomass and mainly in leaves under stress
338  conditions (Sardans et al. 2007). In addition, the increase in trace element concentrations in the
339  stress environment was related to the plant showing high plant uptake, retranslocation and
340  production capacities. Therefore, we believed that in our study site, _P. massoniana_ individual as
341  a pioneer specie of the red soil erosion area in Changting County, were able to survive easily in
342  adverse situations due to their strong regulatory capacity. For example, _P. massoniana_, as a
343  resprouting species, has a superficial root system, and under the water stress conditions, it could
344  change the root structure to develop a deep root system to obtain water from the deepest layers
345  where the impacts of drought and pollution were reduced (Sardans et al. 2007). Even it seemed
346  to have a dural root system (Filella & Peñuelas 2003), where the deeper roots supplied moisture
347  to the superficial roots, thereby allowing the plant to remain active and able to exploit water
348  resources in different soil layers. In our study, the significant enhancement of the leaf Fe
349  concentration and leaf and branch Cr concentrations in the drought group seemed to be related to
350  an increase in the internal mobilization of resources due to the increases in photosynthetic and
351  metabolic capacities (Llorens 2004). Fe is involved in the production of chlorophyll pigment
352  molecules. It is a component of many enzymes associated with energy transfer, nitrogen
353  reduction and fixation and lignin formation (Ahanger et al. 2016). A greater leaf Fe
354  concentration could be associated with a higher quantity of chlorophyll, which would accelerate
355  photosynthesis in _P. massoniana_ (Sánchez-Rodríguez et al. 2010). However, less is known about

356 why drought could increase the Cr concentration in leaves and branches, which even enhances
357 potential toxicity.

## Conclusions

359 The distribution of trace elements in plants could effectively reflect ecological adaptation
360 strategies. The results showed that the concentrations of Fe, Cu, Zn, Cd, Ni, and Cr in leaves
361 and branches had an obvious seasonal variation tendency in the control group, which could be
362 divided into three types according to seasonal pattern: the first type was leaf nutrient-like
363 elements such as Cu, Zn, Ni, and Cd, which decreased continuously with the growing season;
364 the second type was the indifferent element Fe, which exhibited an increase in concentration
365 from spring to summer and then a decrease from summer to autumn, and the highest
366 concentration appeared in the most vigorous summer; the third type was the accumulating
367 element Cr, which showed an increase in concentration from spring-summer to autumn. The
368 concentrations of trace elements in the control group were significantly higher than those in
369 leaves. The concentrations of trace elements in the leaves and branches of *P. massoniana*
370 showed no significant differences with mild drought stress, except for the leaf Fe and Cr
371 concentrations and the branch Cr concentration, which were significantly increased ($p<0.05$).
372 The increases in Fe and Cr concentrations were the result of self-selection by *P. massoniana*
373 under mild drought stress.

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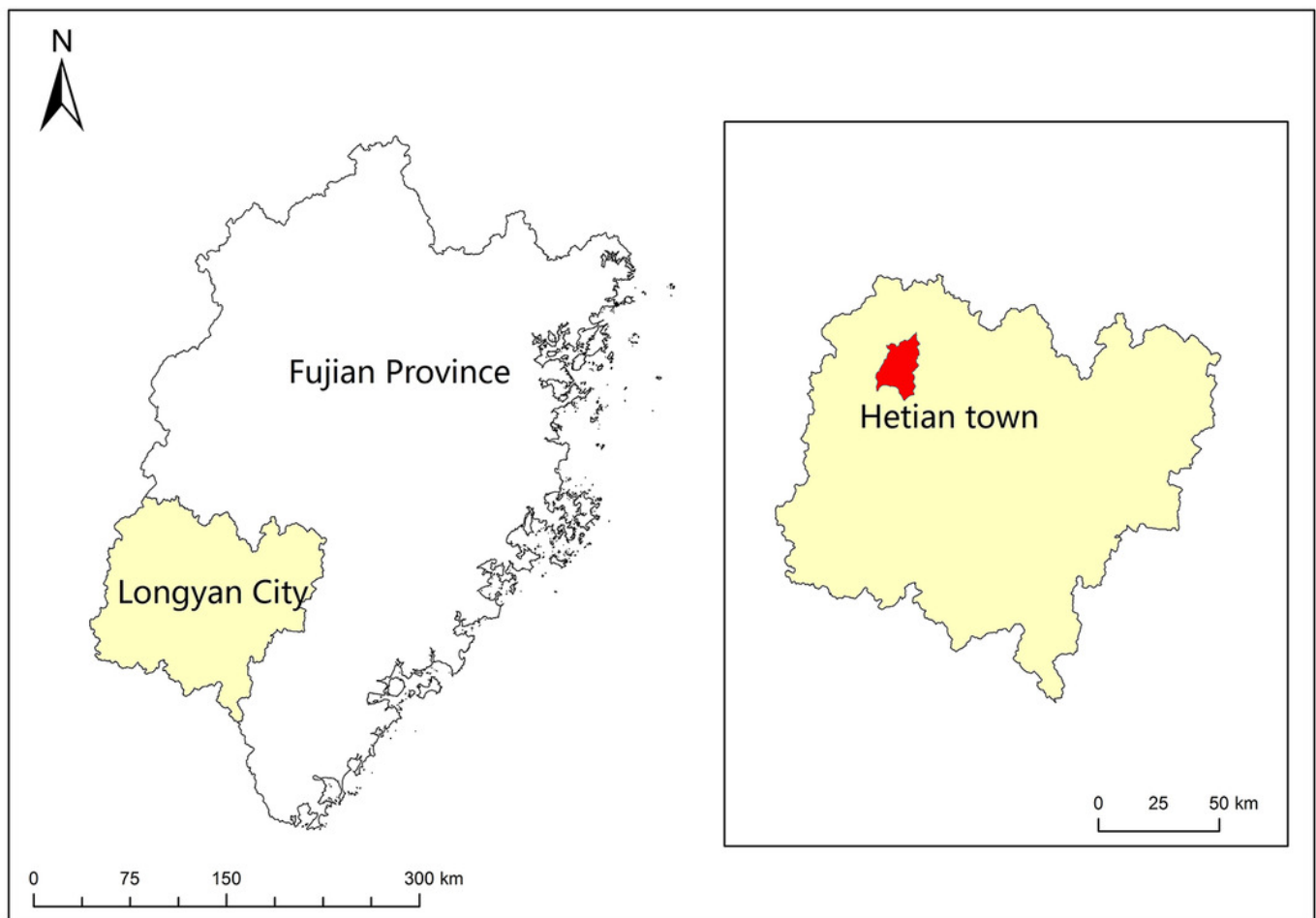
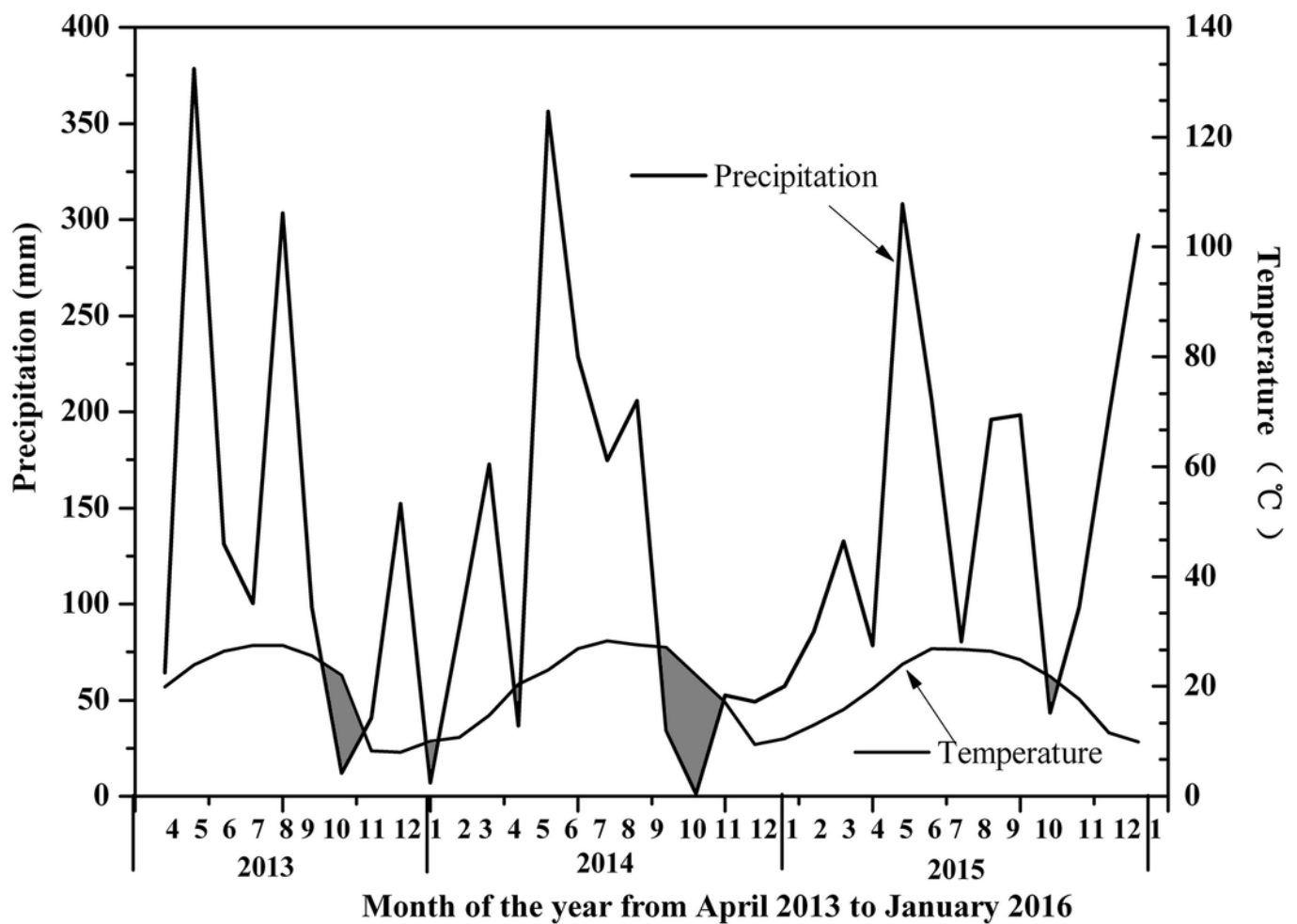
509

# Figure 1

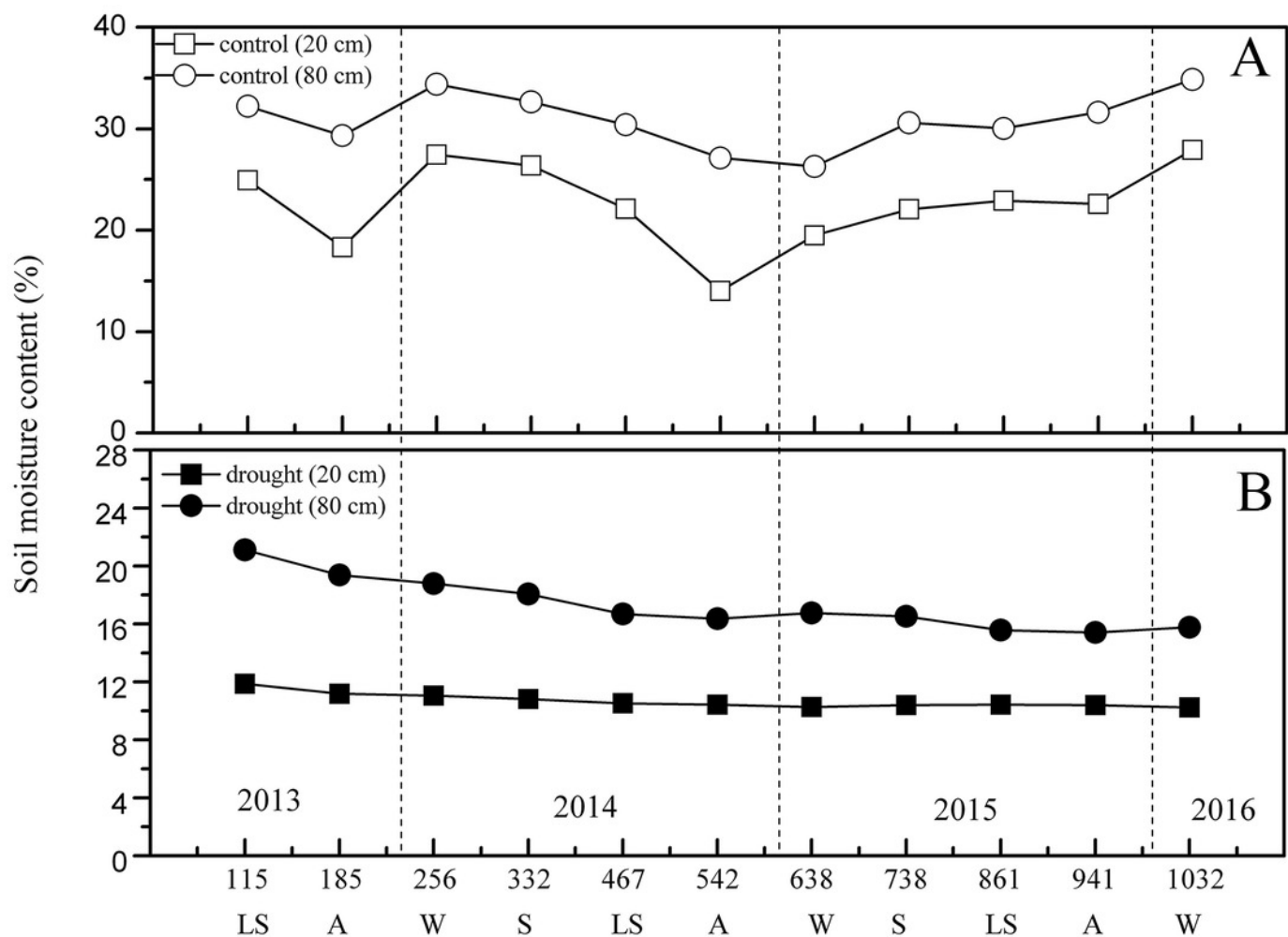
Map of the study area.

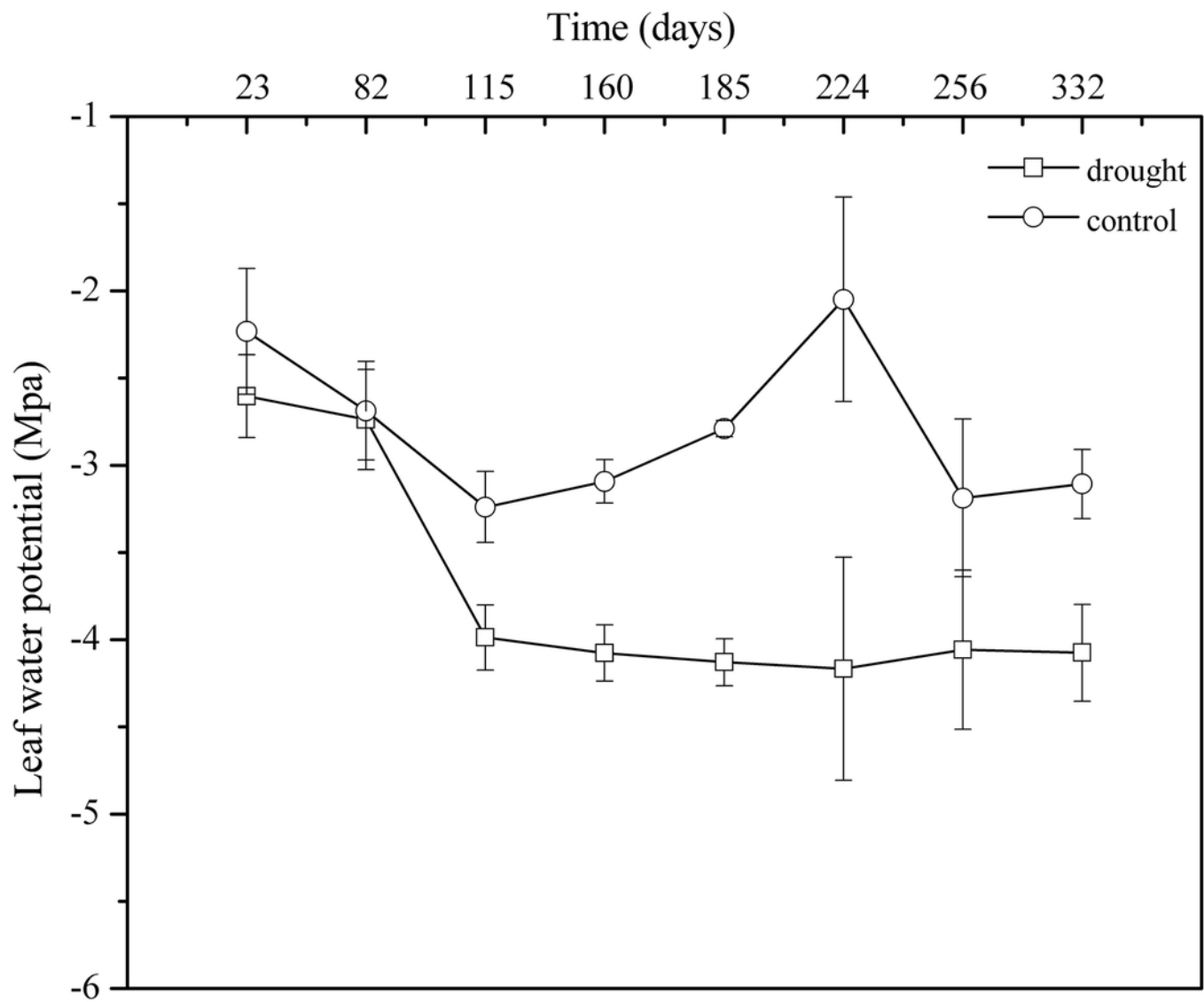
![Map of the study area showing Fujian Province with Longyan City highlighted, and an inset of Longyan City with Hetian town highlighted in red]

# Figure 2

Bagnouls-Gaussen bioclimatic diagram.

# Figure 3

Seasonal variation of soil moisture content under different treatment in the study period.

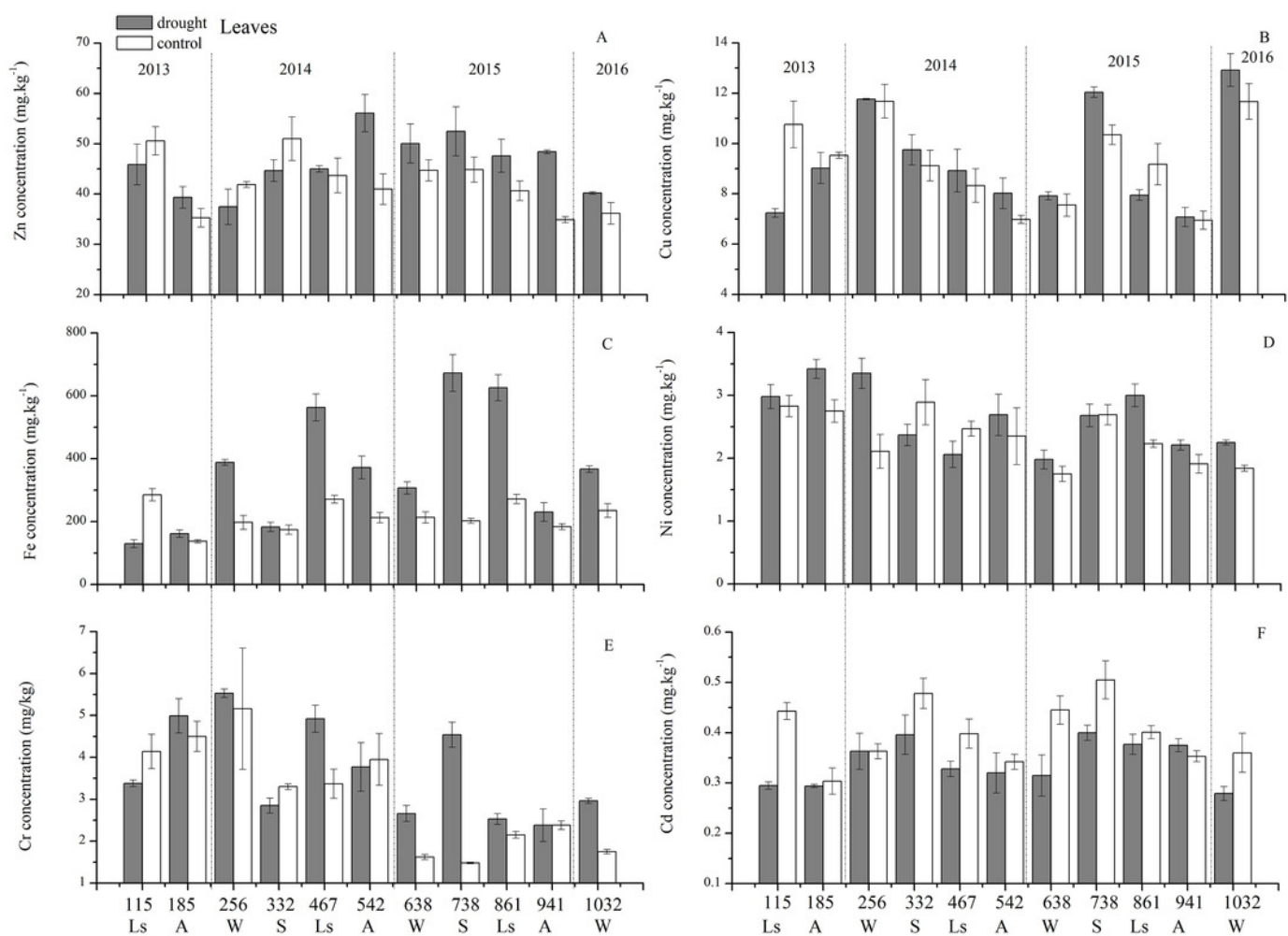
(A) In the control group. (B) In the drought group.

S: spring; LS: late summer; A: autumn; W: winter.

# Figure 4

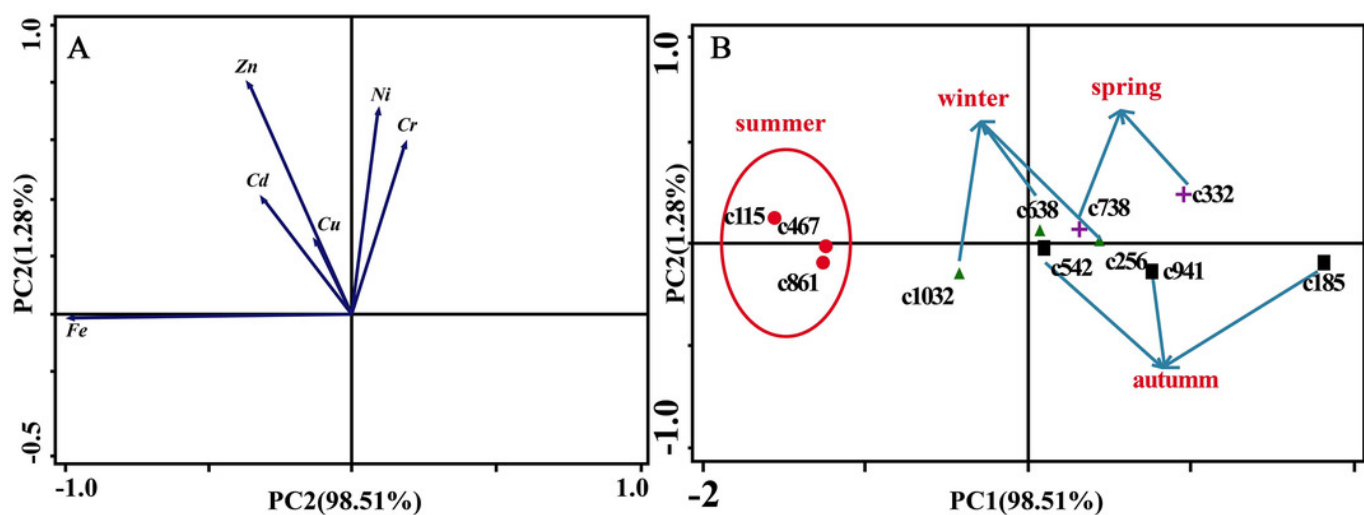
Monthly patterns of dawn leaf water potentials from April 2013 to March 2014.

# Figure 5

Leaf trace element concentrations of Zn, Cu, Fe, Ni, Cr, Cd under different treatments (mean±SD).

(A) Zn. (B) Cu. (C) Fe. (D) Ni. (E) Cr. (F) Cd.

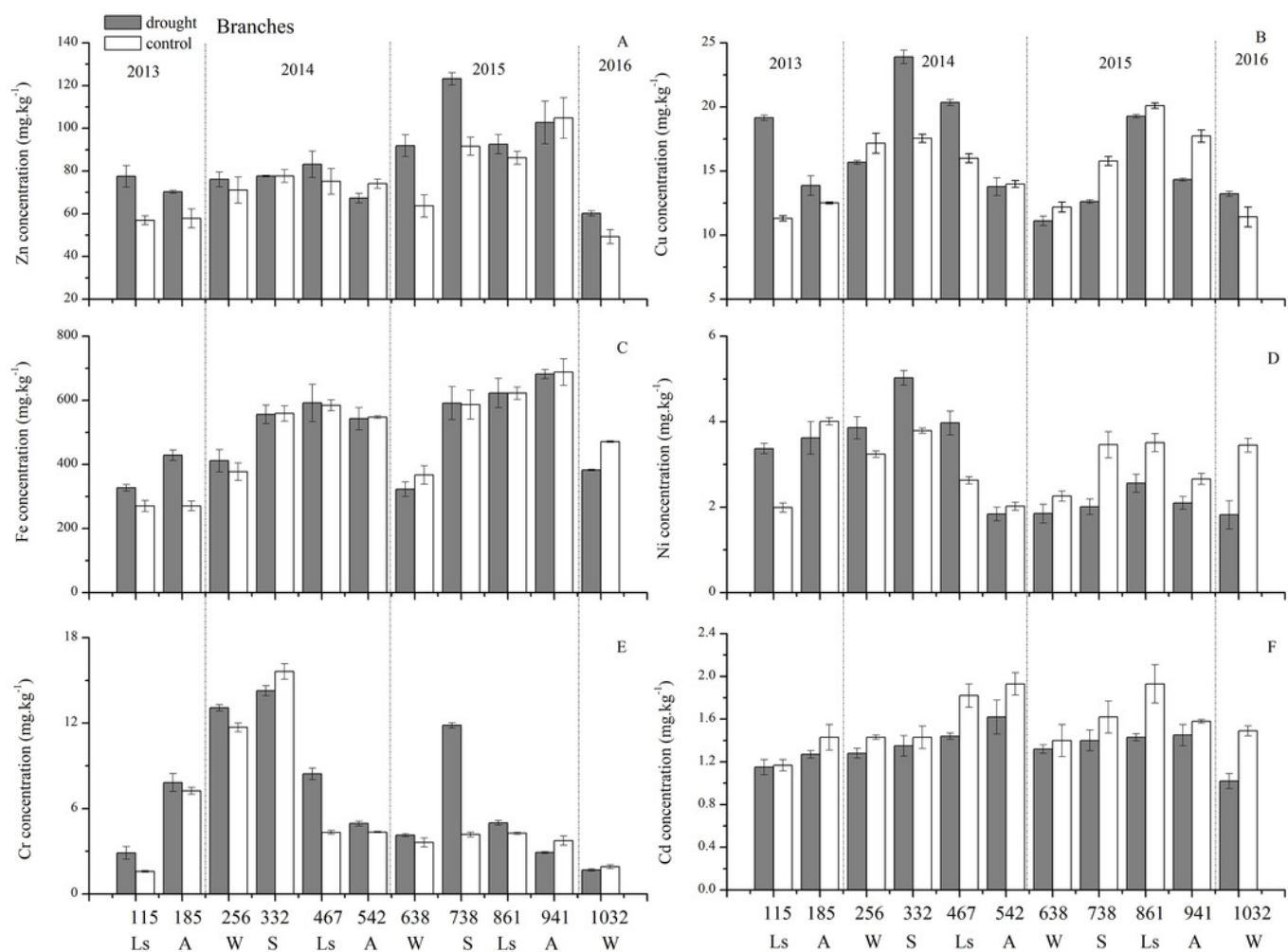
S: spring; Ls: late summer; A: autumn; W: winter.

# Figure 6

Principal component analyses of seasonal samples of *P. massoniana* leaves based on trace element concentrations in the control group.

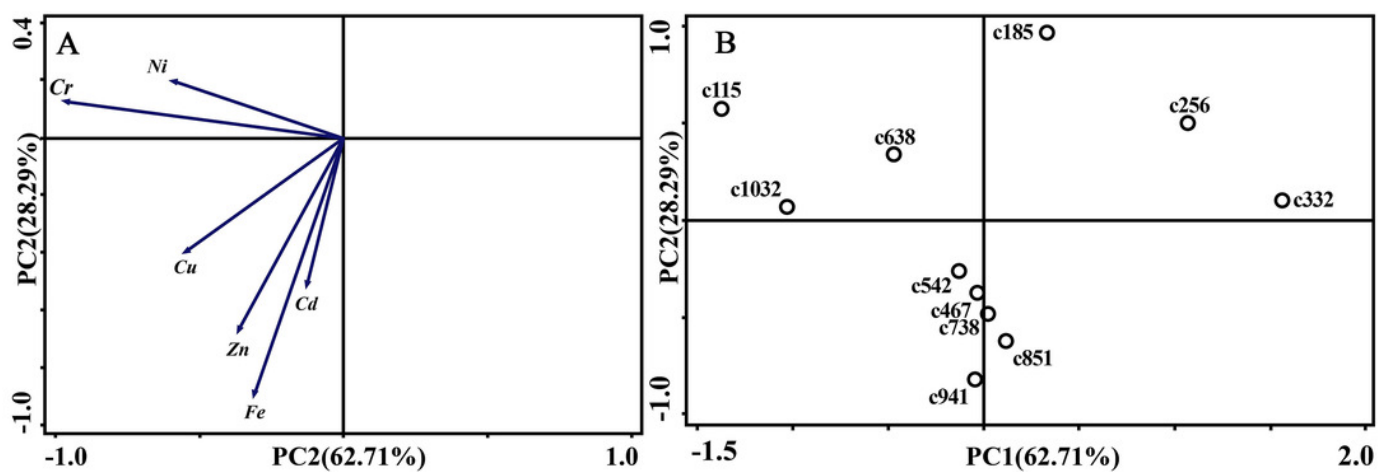
(A) Correlation of variables. (B) Time scatter-plot of observations. C1032=day 1032 in the control group.

# Figure 7

Branch trace element concentrations of Zn, Cu, Fe, Ni, Cr, Cd under different treatments (mean±SD).

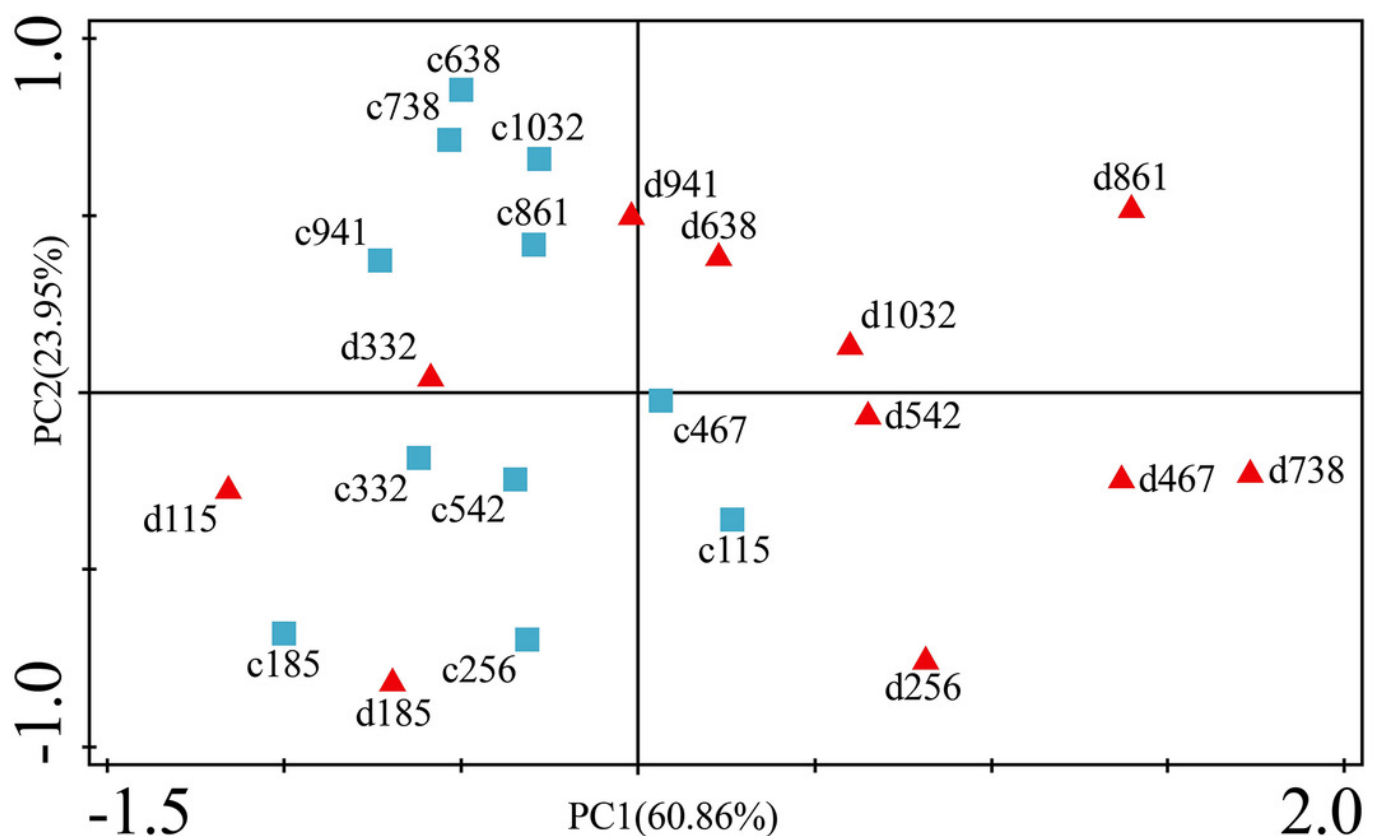
(A) Zn. (B) Cu. (C) Fe. (D) Ni. (E) Cr. (F) Cd.

S: spring; Ls: late summer; A: autumn; W: winter.

# Figure 8

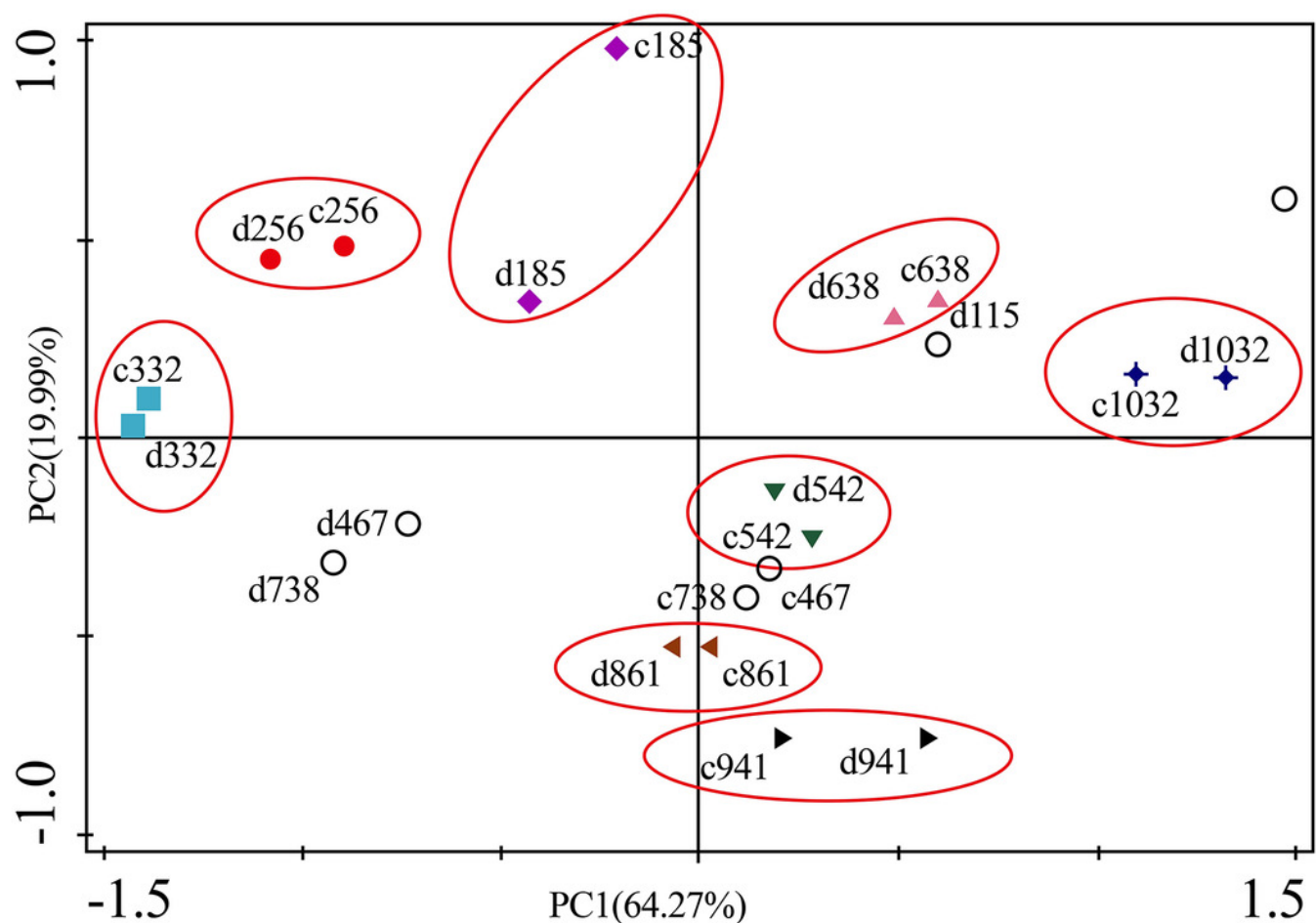
Principal component analyses of seasonal samples of *P. massoniana* branches based on trace element concentrations in the control group.

(A) Correlation of variables. (B) Time scatter-plot of observations. C1032=day 1032 in the control group.

# Figure 9

Principal component analyses of *P. massoniana* leaves based on trace element concentrations in different treatments.

PC1×PC2 time scatter-plot of observations. c1032=day 1032 in the control group. d1032=day 1032 in the drought group.

# Figure 10

Principal component analyses of *P. massoniana* branches based on trace element concentrations in different treatments.

PC1×PC2 time scatter-plot of observations. c1032=day 1032 in the control group. d1032=day 1032 in the drought group.

The mean trace element concentrations of *P. massoniana* across the study period in the control and drought group mg.kg$^{-1}$

1    Table 1 The mean trace element concentrations of *P. massoniana* across the study period in the control and

2    drought group mg.kg$^{-1}$

| Element | In the control group | | In the drought group | | Range of terrestrial vascular plant |
|---|---|---|---|---|---|
| | Leaf | Branch | Leaf | Branch | |
| Fe | 216.72±14.73 | 485.93±21.94 | 363.79±26.09 | 496.41±28.97 | 70~180 |
| Zn | 42.25±2.30 | 73.50±4.47 | 46.11±2.63 | 83.87±3.77 | 34~68 |
| Cu | 9.28±0.53 | 15.07±0.38 | 9.33±0.41 | 16.12±0.32 | 6~14 |
| Ni | 2.35±0.19 | 3.00±0.13 | 2.64±0.17 | 2.91±0.22 | 0~1.4 |
| Cd | 0.40±0.02 | 1.57±0.10 | 0.34±0.02 | 1.34±0.07 | 0.03~10 |
| Cr | 3.07±0.32 | 5.69±0.22 | 3.68±0.25 | 7.00±0.26 | 0.1~0.5 |