Legume plants may facilitate *Zanthoxylum bungeanum* tolerance to extreme rainfall

Zilong Li¹,², Kaiwen Pan¹, Akash Tariq¹,², Feng Sun¹,², Sizhong Wang¹,², Lin Zhang³, Xiaoming Sun¹, Xiaogang Wu¹ & Dagang Song¹,²

A complete randomized design was implemented with two watering regimes (extreme rainfall and control) and three different plant combinations (*Zanthoxylum bungeanum, Z. bungeanum + Capsicum annuum, Z. bungeanum + Glycine max*) in order to assess the morphological and physio-biochemical responses of focal and neighbor plants. The results indicated that, extreme rainfall had significantly negative impacts on *Z. bungeanum* in three intercropping systems. However, intercropping with *G. max* improved the transpiration rate (*T*), and stomatal conductance (*g*), raised leaf relative water content (*LRWC*), increased chlorophyll a (*Chl a*) and carotenoid (*Car*) content, and enhanced the superoxide dismutase activity (*SOD*) of *Z. bungeanum*. After recovery, the *Z. bungeanum + G. max* mixed culture significantly increased soil NO₃⁻-N, improved the intercellular carbon dioxide concentration (*Cᵢ*), and higher root biomass of *Z. bungeanum*. Moreover, the recovery after extreme rainfall of the focal species *Z. bungeanum* with neighboring species *G. max* could have improved the tolerance of the focal species *Z. bungeanum* in response to extreme rainfall and its recovery after extreme rainfall.

Since the late 19th century, the global average surface temperature has increased by 0.85 (0.65–1.06)°C and is predicted to show a continuous warming of >1.5°C by the end of the century. Global warming is predicted to induce an increase in the frequency and magnitude of extreme rainfall over most regions of the world. Although the mean quantity of precipitation showed no significantly increasing or decreasing trend over 1960–2007 for southwestern China, extreme rainfall events have occurred frequently in recent years.

Plants are very vulnerable to extreme precipitation, which has severe impacts on different physiological processes such as photosynthesis, nutrient cycles, and plant productivity. Extreme rainfall can decrease photosynthesis, nutrient uptake, and plant productivity. Due to excess water, oxygen (O₂) supply to the root is reduced, and the oxygen demand of plant roots cannot be fulfilled, which reduces root respiration rates, decreases the water absorption and induces stomatal closure. However, the response of individual plants to expected extreme rainfall in relation to neighboring species remains a subject of debate. In a given ecosystem, the patterns in which plants recognize and respond to all aspects of their environment will influence their competitive ability, and thus have important consequences on the overall success of a species.

It is well known that community composition influences plant responses to climate change, with plant-plant interactions playing a key role. While they are still not well understood, competition and facilitation are important consequences on the overall success of a species. Recognizing and responding to all aspects of their environment will influence their competitive ability, and thus have important consequences on the overall success of a species.
As plants provide organic matter to decomposing microbes, the aboveground matter is closely linked with the belowground components. As such, the decomposer subsystem indirectly regulates plant growth and community composition by determining the supply of available soil nutrients. To alleviate the negative effect of climate change, the characteristics of subordinate species are more important for focal species than their plant diversity. Therefore, we assumed here that, under extreme rainfall, plant species composition might be crucial in promoting the absorption of nourishment and the physiological processes of focal species.

Z. bungeanum belongs to the family Rutaceae and is grown widely in India, North America, Australia and southwestern China. The fruits of Z. bungeanum are medicinal raw materials used for the treatments of toothache and rheumatism and are an important flavoring in Chinese food. It is drought resistant and grows quickly. Due to its important functions, research into Z. bungeanum has gained increased attention from scientists.

Most crops grow in soils with low nitrogen, which impacts plant nutrient uptake. Tree intercropping systems can enhance environmental and economic benefits, for example, increasing land use efficiency and crop yield and, conserving biodiversity and carbon storage. The use of nitrogen-fixing crops can be a strategy to enhance soil fertility. As the third largest crop grown worldwide, soybean is the main source of protein for both humans and animals. Soybeans have the very important feature of being able to establish symbiotic relations with N\textsubscript{2}-fixing soil bacteria. Moreover, soybean has been a well-studied crop with respect to environmental change. For this reason, soybean is the best choice to explain the response of a target plant to environmental change. Hot pepper (Capsicum annum L.) is a vital cash crop and is culinarily and medicinally important. It is also a non-nitrogen fixing plant that is sensitive to moisture. Furthermore, hot pepper requires low organic fertilizer inputs when compared to soybean.

However, Z. bungeanum is vulnerable to extreme rainfall, which results in declines in yield, quality of fruit and even death. A previous study has indicated that, under extreme rainfall conditions, the leaf nitrogen content of Z. bungeanum in a mixed legume culture was highest compared to other nonlegume mixed cultures. This is because a significant increase in the root biomass of the soybeans enhanced microbial resistance and the N mineralization rate and promoted the nitrogen absorption of the neighboring crop Z. bungeanum. However, there remains a lack of understanding about the physio-biochemical response of Z. bungeanum to extreme rainfall stress, when grown together in combination with leguminous and nonleguminous plants.

The purpose of this study was to 1) Explore the growth, physiological and biochemical responses of Z. bungeanum in different intercropping systems under extreme rainfall; 2) Evaluate whether legume species can alleviate the negative impact of extreme rainfall on Z. bungeanum. To achieve these goals, we studied the plant growth index, photosynthetic traits, pigment content, free radical and antioxidant enzymes in different systems subjected to extreme rainfall. The present study would benefit the management of sustainable agriculture through the development of a reasonable ecosystem with higher resistance to extreme rainfall.

Result

Soil properties. After the recovery treatment, under previous normal rainfall conditions, the soil NH\textsubscript{4}\textsuperscript{+}-N was higher in the Z-C mixed culture than in the Z monoculture and Z-G mixed culture (P < 0.05). Under the previous rainfall treatment, soil NO\textsubscript{3}\textsuperscript{-}-N was higher in the Z-G mixed culture than in the Z monoculture and Z-C mixed culture (P < 0.05) (Table 1). Planting systems, previous extreme rainfall, and the interactions of the planting system and previous extreme rainfall significantly affected both soil NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}\textsuperscript{-}-N (Table 1).

Nitrogen content of plant leaves. Irrespective of extreme rainfall, when compared with the Z-G mixed culture, the NH\textsubscript{4}\textsuperscript{+}-N content of the Z. bungeanum leaves decreased significantly in the Z monoculture and Z-C mixed culture. Extreme rainfall significantly decreased the NO\textsubscript{3}\textsuperscript{-}-N content of G. max. The two-way ANOVA showed that planting systems, extreme rainfall, and their interaction significantly affected the NH\textsubscript{4}\textsuperscript{+}-N content of the Z. bungeanum leaves (Table 2).

Focal plant growth and neighbor species biomass. Under normal water conditions, the LRWC of Z. bungeanum decreased significantly in the Z-C culture when compared with the monoculture and Z-G mixed culture (P < 0.05) (Table 3). Extreme rainfall reduced LRWC and the height of Z. bungeanum in all cultures, and it significantly decreased the aboveground weight of G. max and C. annum (P < 0.01). A two-way ANOVA showed that the planting system significantly affected the LRWC of Z. bungeanum (P < 0.001) and that the extreme rainfall significantly affected the LRWC and height of Z. bungeanum (P < 0.05) (Table 3).

Table 1. Soil physical and chemical properties after recovery. “Z-G” denotes Z. bungeanum intercropping with G. max, “Z-C” denotes Z. bungeanum intercropping with C. annum, and “Z” denotes the Z. bungeanum monoculture. Different uppercase letters indicate significant differences among all treatments. ANOVA was used to assess the effects of extreme rainfall on soil properties. *P < 0.05, **P < 0.01, ***P < 0.001, -non-significant (P > 0.05).
Recovery stages

| Treatments | Plant system | Z | Z-C | Z-G | System | Rainfall | System* Rainfall |
|------------|--------------|---|-----|-----|---------|----------|------------------|
| Control    |              |   |     |     |         |          |                  |
| Extreme rainfall |              |   |     |     |         |          |                  |
| NH₄⁺-N content of C. annuum leaves (mg g⁻¹) | 5.61 ± 0.68B | 3.24 ± 0.17C | 4.60 ± 0.49B | 3.97 ± 0.25C | 8.71 ± 0.38A | 5.44 ± 0.29B | *** | *** | * |
| NH₄⁺-N content of neighbor crops leaves (mg g⁻¹) | 2.60 ± 0.11A | 2.46 ± 0.20A | 2.65 ± 0.33A | 2.44 ± 0.14A | / | / | / |
| NO₃⁻-N content of C. annuum leaves (mg g⁻¹) | 13.03 ± 0.62A | 12.16 ± 0.67A | 12.78 ± 0.73A | 10.81 ± 0.98A | 13.08 ± 1.05A | 13.85 ± 1.01A | ns | ns | ns |
| NO₃⁻-N content of neighbor crops leaves (mg g⁻¹) | 20.14 ± 1.37A | 14.89 ± 0.96A | 17.36 ± 1.33A | 9.93 ± 0.83B | / | / | / |

**Table 2.** Leaf nitrogen content of *C. bungeanum* and neighbor crops. “Z-G” denotes *C. bungeanum* intercropping with *G. max*, “Z-C” denotes *C. bungeanum* intercropping with *C. annuum*, and “Z” denotes the *C. bungeanum* monoculture. Different uppercase letters indicate significant differences among all treatments. ANOVA was used to assess the effects of extreme rainfall on leaf nitrogen content. *P < 0.05, **P < 0.01, ***P < 0.001, “ns” non-significant (P > 0.05).**

**Table 3.** Effects on the growth parameters of *C. bungeanum* and neighbor crops after treatment and recovery. “Z-G” denotes *C. bungeanum* intercropping with *G. max*, “Z-C” denotes *C. bungeanum* intercropping with *C. annuum*, and “Z” denotes the *C. bungeanum* monoculture. “LRWC” denotes leaf relative water content. Different uppercase letters indicate significant differences among all treatments. ANOVA was used to assess the effects of extreme rainfall on plant growth. *P < 0.05, **P < 0.01, ***P < 0.001, “ns” non-significant (P > 0.05).**

After recovery treatments, the LRWC of *C. bungeanum* in the Z-G mixed culture was higher than that in the Z-C mixed culture and Z monoculture (P < 0.05). The root biomass of *G. max* previously treated with extreme rainfall was significantly larger than that of the control after recovery (P < 0.05). The previous extreme rainfall significantly reduced the yield of *C. annuum* and *G. max* (P < 0.01). A two-way ANOVA showed that after one month of the recovery treatment, the planting system did not have significant effects on the growth of *C. bungeanum* (Table 3).

**Photosynthetic parameters.** Under the normal rainfall treatment, the Ci of *C. bungeanum* in Z-C mixed culture decreased significantly in comparison with the Z-G mixed culture and Z monoculture. The extreme rainfall treatment significantly reduced the Pn of *C. bungeanum* in the Z monoculture, Z-C and Z-G mixed cultures by 12.83%, 25.71% and 22.96%, respectively (P < 0.05). The Tn of *C. bungeanum* in the Z-C mixed culture was significantly smaller than that in the Z-G mixed culture and Z monoculture under extreme rainfall (P < 0.05). The extreme rainfall treatment significantly decreased the Tn of *C. bungeanum* in the Z-C mixed culture by 30.27% (P < 0.05). The planting systems significantly affected the Pn, Gs, Ci and Tn; the extreme rainfall significantly affected the Pn and Tn (P < 0.001). There was a significant interaction effect between the planting system and extreme rainfall on Tn (Table 4). The order of the effects on the photosynthetic parameters of *C. bungeanum* is planting system > extreme rainfall > interaction of planting system and extreme rainfall.

After recovery with the previous normal rainfall treatment, the Pn of *C. bungeanum* of the Z-C and Z-G mixed cultures increased significantly (P < 0.05), while the Tn of *C. bungeanum* in the Z-C and Z-G mixed cultures decreased significantly in comparison with the Z monoculture (P < 0.05). After recovery with the previously extreme rainfall, the Ci of the Z-C and Z-G mixed cultures was significantly lower than that in the Z-G mixed culture (P < 0.05). Irrespective of whether the previous treatments were of normal or extreme rainfall, the Tn of *C. bungeanum* in the Z monoculture was significantly higher than that in the Z-C and Z-G mixed cultures (P < 0.05). The planting system significantly affected the Pn, Ci and Tn; the extreme rainfall significantly affected the
Table 4. Effects on the photosynthetic parameters of Z. bungeanum after treatment and recovery. “Z-G” denotes Z. bungeanum intercropping with G. max; “Z-C” denotes Z. bungeanum intercropping with C. annuum, and “Z” denotes Z. bungeanum monoculture. “P”, “G”, and “Tr” denote net photosynthetic, “G”, denotes stomatal conductance, “C”, denotes intercellular carbon dioxide concentration, “T”, denotes transpiration rate. Different uppercase letters indicate significant differences between the control (normal rainfall) treatments; different lowercase letters indicate significant differences between the extreme rainfall treatments. ANOVA was used to assess the effects of extreme rainfall on photosynthetic parameters. *P < 0.05, **P < 0.01, ***P < 0.001, “ns” non-significant (P > 0.05).

| Treatment stages | Plant system | Z | Z-C | Z-G |
|-------------------|--------------|---|-----|-----|
| Traits            | Control      | Extreme rainfall | Control      | Extreme rainfall | Control      | Extreme rainfall | System Rainfall |
| 30 days           | Pr (μmol m⁻² s⁻¹) | 15.36±0.03A       | 13.39±0.16B   | 14.39±1.15A     | 10.69±0.59C   | 13.94±0.87A   | 10.74±0.73C | **     | ***  | ns  |
|                   | Gs (mmol m⁻² s⁻¹) | 0.32±0.02A       | 0.29±0.02A    | 0.19±0.04A     | 0.18±0.03AB   | 0.32±0.06A   | 0.31±0.00A | **     | ***  | ns  |
|                   | Gi (μmol mol⁻¹) | 305.06±5.47A    | 279.54±12.69A | 271.15±3.57AB  | 269.35±0.38AB | 302.40±0.86A | 294.72±0.52A | **     | ns  | ns  |
|                   | Tr (g m⁻² h⁻¹) | 3.55±0.18B      | 3.56±0.01B    | 3.70±0.03B    | 2.58±0.14C    | 3.56±0.15B   | 4.05±0.04A | *      | ***  | *** |

Photosynthetic pigments. Under normal rainfall conditions for all cultures, no significant difference was found in the Car content of the Z. bungeanum leaves. Irrespective of normal or extreme rainfall treatments, the Chl a/Chl b of the Z.-bungeanum leaves of the Z.-mixed culture and Z-monoculture was significantly lower when compared with Z-G (P < 0.05). Under extreme rainfall, in comparison with the Z-monoculture, the content of Chl a, Chl b and Car, and Chl a/Chl b of Z.-bungeanum in the Z.-mixed culture significantly increased by 52.74%, 24.30%, 31.35% and 23.47%, respectively (P < 0.05). There were no significant differences in the Chl a, Chl b and Car contents, and the Chl a/Chl b of Z. bungeanum between the Z-C mixed culture and Z-monoculture. The planting system significantly affected the Chl a and Car contents and the Chl a/Chl b; the extreme rainfall significantly affected the content of Car, while the planting system and extreme rainfall interactively impacted the content of Chl a and Chl b (P < 0.01). In the extreme rainfall phase, the influence of extreme rainfall and that of the interaction of the planting system and extreme rainfall were more obvious that of the planting system alone (Table 5).

After recovery, the previously extreme rainfall treatment significantly increased the contents of Chl a, Chl b and Car of the Z. bungeanum leaves in the Z-monoculture (P < 0.05). There were no significant differences in the Chl a, Chl b, and Car contents of Z.-bungeanum in the Z-G between the previously extreme rainfall treatment and control. The Chl a and Chl b contents of Z.-bungeanum in the Z-C mixed culture with the previously extreme rainfall treatment increased significantly (P < 0.01), while the Chl a/Chl b significantly decreased. The planting systems significantly affected the Car content (P < 0.001), and the previously extreme rainfall significantly affected the Chl a, Chl b and Car contents, while the interaction of the planting system and extreme rainfall significantly affected the Chl b content and Chl a/Chl b (P < 0.01).

ROS production and lipid peroxidation. Under normal rainfall, the O$_{2}^·$ production rate of the Z. bungeanum leaves of the Z-G mixed culture decreased significantly (P < 0.05), while the H$_{2}$O$_{2}$ content increased significantly compared with the Z-C mixed culture and Z-monoculture (P < 0.05) (Fig. 1A,C). The extreme rainfall significantly increased the O$_{2}^·$ production rate of the Z. bungeanum leaves by 9.21%, 12.33% and 16.42% (P < 0.05); raised the H$_{2}$O$_{2}$ content by 89.69%, 20.94% and 4.73%; and increased the malondialdehyde (MDA) content by 11.49%, 4.09% and 4.00% in the Z-monoculture, Z-C and Z-G mixed cultures, respectively. The two-way ANOVA showed that the planting system and extreme rainfall both significantly affected the O$_{2}^·$ production rate and H$_{2}$O$_{2}$ content. The interaction effects of planting systems and extreme rainfall significantly affected H$_{2}$O$_{2}$ content (P < 0.001) (Fig. 1A,C).

After recovery with the previously normal rainfall treatment, the O$_{2}^·$ production rate and MDA content of the Z. bungeanum leaves of the Z-C mixed culture increased significantly while the H$_{2}$O$_{2}$ content decreased significantly compared with the Z-monoculture and Z-G mixed cultures (P < 0.05) (Fig. 1B, D). However, after recovery with the previously extreme rainfall treatment, the O$_{2}^·$ production rate of Z. bungeanum increased significantly while, compared with the Z-monoculture and Z-G mixed cultures, the H$_{2}$O$_{2}$ content decreased significantly in the Z-C mixed culture (P < 0.05). The planting system significantly affected the O$_{2}^·$ production rate and H$_{2}$O$_{2}$ content (P < 0.001) (Fig. 1B–D).

Antioxidant stress components. Irrespective of the normal or extreme rainfall treatments, no significant difference of SOD activity in Z. bungeanum leaves was found between the Z-monoculture, Z-C and Z-G mixed cultures (Fig. 2A). The extreme rainfall treatment significantly raised the SOD activity of the Z. bungeanum leaves

P$_{n}$, G$_{i}$, and T$_{r}$. There was a significant interaction effect between the planting system and extreme rainfall on $P_{n}$ and T$_{r}$ (Table 4). Either at the stage of extreme rainfall or at the recovery phase, the T$_{r}$ was the most affected parameter.
in the Z-G mixed culture by 33.21% ($P < 0.05$). Compared to the Z monoculture, and Z-C under normal rainfall and extreme rainfall treatments, the CAT activity of the *Z. bungeanum* leaves increased significantly in the Z-G mixed culture (Fig. 2C). Extreme rainfall significantly affected the SOD activity of the *Z. bungeanum* leaves ($P < 0.05$), the planting system significantly affected the CAT activity of the *Z. bungeanum* leaves ($P < 0.001$) (Fig. 2A–C).

After recovery under the previously extreme rainfall treatment, the SOD activity of the *Z. bungeanum* leaves of Z-C was significantly higher than that of Z-G (Fig. 2B). Under the previously normal rainfall condition, the Z monoculture had higher SOD activity compared to the Z-C and Z-G mixed cultures. Irrespective of previously normal or extreme rainfall treatments, the Z-C and Z-G mixed cultures had higher CAT activity than the Z monoculture (Fig. 2D). In the Z-G mixed culture, the previously extreme rainfall treatment significantly increased the CAT activity by 64.71%. The interaction effects of planting systems and extreme rainfall significantly affected the SOD and CAT activity of the *Z. bungeanum* leaves (Fig. B,D).

**Biochemical parameters.** Under normal rainfall, compared to the Z monoculture, the soluble sugar content of the *Z. bungeanum* leaves increased significantly and the proline content decreased significantly in the Z-C and Z-G mixed cultures ($P < 0.05$) (Fig. 3A,E). Compared with the Z and Z-G cultures, the soluble protein content of the *Z. bungeanum* leaves increased significantly in the Z-C culture (Fig. 3C). The extreme rainfall significantly decreased the soluble sugar content of *Z. bungeanum* in the Z-G mixed cultures. In the extreme rainfall treatment, the proline content of the *Z. bungeanum* leaves of the Z-C culture increased significantly ($P < 0.05$), while the soluble protein content of decreased significantly, compared to the control ($P < 0.01$). The interaction effects of planting systems and extreme rainfall significantly affected the proline content of the *Z. bungeanum* leaves ($P < 0.01$) (Fig. 3E).

After recovery with the previously normal rainfall treatment, the soluble sugar content of the *Z. bungeanum* leaves decreased significantly in the Z-C and Z-G mixed cultures ($P < 0.05$), and the soluble protein content increased significantly in the Z-G mixed culture, compared to the Z monoculture. The proline content increased significantly in the Z-C and Z-G mixed cultures than in the Z monoculture ($P < 0.05$). After recovery, compared to the control, the previously extreme rainfall treatment significantly increased the soluble sugar and proline content of the *Z. bungeanum* leaves in the Z-G mixed culture ($P < 0.01$) (Fig. 3B,F). The planting system significantly affected the soluble sugar, soluble protein and proline content of the *Z. bungeanum* leaves (Fig. 3B,D,F).

**Discussion**

Extreme rainfall has been increasing globally and seriously affecting plant growth and yield. The present study indicated that irrespective of the planting system, the LRWC and height of *Z. bungeanum* decreased under extreme rainfall compared to the control (Table 3). This was due to excessive water causing anoxia in the rhizosphere and hindering nutrient uptake by plants. Many previous studies have shown that the presence of a legume has a positive effect on the growth of neighboring species. Our results indicated that extreme rainfall did not significantly decrease the LRWC and height of *Z. bungeanum* in a legume mixed culture (G. max.), and it reduced the height of *Z. bungeanum* in the nonleguminous mixed culture (C. annuum) (Table 3). This could be attributed to the higher soil NH$_4$-$N$ and NO$_3$-$N$ in the legume mixed culture (as shown in our previous experiments). In the Z-G mixed culture, G. max could maintain adequate physiological functioning in wet soils. Its moisture resistance property is associated with the formation of aeration tissue. Moreover, under extreme rainfall, the NH$_4$-$N$ of *Z. bungeanum* leaves was higher in the Z-G mixed culture than in the Z monoculture and Z-C mixed cultures ($P < 0.05$) (Table 2). This indicates that under extreme rainfall,
Legumes facilitate focal species nutrient uptake via increasing N2 fixation48. After recovery, the LRWC of the focal species in three mixed cultures decreased compared to before the recovery. This may be due to the large diurnal temperature differences of the experiment site between different periods. Additionally, Z. bungeanum passes into a different growth phase, causing the lower LRWC.

Altered precipitation regimes influence plant eco-physiology by enhancing plant photosynthesis, transpiration and the leaf respiration rate49,50. Plant photosynthesis is a fundamental biological process and is greatly dependent on precipitation51. Previous studies have shown that increased precipitation could enhance plant photosynthesis and ecosystem carbon uptake52. An increase in precipitation can also indirectly affect focal species via its influence on the surrounding community53. The present study found that extreme rainfall significantly decreased the $P_n$ of the focal species in three mixed cultures. Previous studies have revealed that the changes in $P_n$ are mainly induced by the stimulation of stomatal conductance under increased precipitation54. Our results show that extreme rainfall significantly decreased $G_s$ and $T_r$, thereby resulting in the decreased $P_n$ of focal species in the Z-C mixed culture. However, the $T_r$ of focal species increased significantly in the Z-G mixed culture, which might be due to the formation of root aeration tissue of G. max44,45,55,56. After one month of rain recovery treatments, the $P_n$ of focal species decreased compared with before recovery. This result may be due to the growth stage changes in Z. bungeanum. Moreover, when compared to August, the photosynthetic radiation in September declined. The $P_n$ of the focal species in Z-C was lower compared with its control ($P < 0.05$). This clearly indicated that the

Figure 1. Effects of free radical and MDA content on focal species after extreme rainfall and recovery. The left column represents 30 days of rainfall, the right column represents recovery for 30 days; “Z-G” denotes Z. bungeanum intercropping with G. max, “Z-C” denotes Z. bungeanum intercropping with C. annuum, and “Z” denotes the Z. bungeanum monoculture. Vertical bars show ± SE of the mean (n ± SE). Different uppercase letters indicate significant differences between the control (normal rainfall) treatments; different lowercase letters indicate significant differences between the extreme rainfall treatments. “r” denotes extreme rainfall; “s” denotes planting system; “r × s” denotes the interaction of extreme rainfall and planting system; Proportion of explained variance by extreme rainfall and planting system effects and by their interactions (two-way ANOVA). Significant levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, “ns” no significant.
photosynthetic resilience of focal species in Z-C mixed culture was not strong enough. This shows that legumes can alleviate the decline of photosynthetic function in *Z. bungeanum*.

The content of chlorophyll pigments in leaves is an indicator of plant physiological status. Due to degradation of the chloroplast pigment in the *Z* monoculture and Z-C mixed cultures, extreme rainfall caused significant damage to the focal species. It has been previously demonstrated that, in the absence of any disturbance, pigment value also declines. Nitrogen is critical for the growth and development of crop plants. The literature data shows that, in mixed stands, depending on the legume species and cultivar, from forage legumes to companion grasses, the rates of nitrogen transfer range from 0 to 73%. Nitrogen fertilizer can increase chlorophyll and carotenoid content in the leaves of plants. Sun et al. found that the soil nitrogen content in the Z-G mixed culture was significantly higher than in the *Z* monoculture. They also found that the leaf NH$_4^+$-N and NO$_3^-$-N content of the focal species of the Z-G mixed culture was significantly higher than that of the *Z* monoculture and Z-C mixed culture. Moreover, the leaf NH$_4^+$-N and NO$_3^-$-N content of *G. max* significantly decreased. This proved that *G. max* could promote the nitrogen uptake of focal species. The present study shows that the Chl a, Chl b and Car content of the focal species in the Z-G mixed culture increased significantly under extreme rainfall. After recovery, the pigment content of the leaves of the focal species changed significantly in the *Z* monoculture and the Z-C mixed culture. This may be because, compared to the Z-G mixed culture, the pigment content of *Z. bungeanum* in the Z monoculture and Z-C mixed culture did not reach a steady state. This further proved that there was a significantly different impact on *Pn* in *Z. bungeanum* in the Z-C mixed culture (Table 4). However, in the Z-G mixed culture, there was no significant difference in the Chl a, Chl b, Car and Chl a/Chl b content of the focal species between the normal and extreme rainfall recovery treatments. These results indicate that the intercropping of leguminous crops could stabilize the pigmentation resilience of focal species.

Extreme rainfall causes a reduction in oxygen supply in the soil that further leads the over-production of ROS in plants. The dramatic increase in the ROS level triggers protein degradation, lipid peroxidation, and deoxyribonucleic acid (DNA) fragmentation and causes cell death. Our study found that, under extreme rainfall, when compared with their controls, the rates of O$_2^-$ production were significantly higher in all cultures. The H$_2$O$_2$ content was significantly higher only in the *Z* monoculture, and no significant differences were observed in the Z-C and Z-G mixed cultures. Therefore, under extreme rainfall, the MDA content of the focal species increased.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Effects of leaf SOD and CAT activity on focal species after extreme rainfall and recovery. The left column represents 30 days of rainfall, the right column represents recovery for 30 days; “Z-G” denotes *Z. bungeanum* intercropping with *G. max*, “Z-C” denotes *Z. bungeanum* intercropping with *C. annuum*, and “Z” denotes the *Z. bungeanum* monoculture. Vertical bars show ± SE of the mean (n ± SE). Different uppercase letters indicate significant differences between the control (normal rainfall) treatments; different lowercase letters indicate significant differences between the extreme rainfall treatments. “r” denotes extreme rainfall; “s” denotes planting system; “r × s” denotes the interaction of extreme rainfall and planting system; Proportion of explained variance by extreme rainfall and planting system effects and by their interactions (two-way ANOVA). Significant levels: *P < 0.05, **P < 0.01, ***P < 0.001, “ns” no significant.
significantly in the Z monoculture, while there was no significant difference in the MDA content of the focal species in the Z-C and Z-G mixed cultures. This demonstrated that increasing plant diversity could reduce the extreme rainfall damage to focal species.

Plants have developed antioxidant enzymes such as SOD and CAT required for the destruction of $O_2^-$ and $H_2O_2$. The SOD activity of $Z$. bungeanum was significantly enhanced in the Z-G mixed culture compared with its control. Moreover, the CAT activity of $Z$. bungeanum was the highest in the Z-G mixed culture in comparison with the Z-C mixed and Z monoculture cultures. This proved that species specificity plays a very important role in enhancing antioxidant enzyme activities to improve tolerance of focal species. The present findings are in line with a previous study that showed that facilitation should be more common when plants are subject to high abiotic conditions. After recovery, the MDA content of focal species recovered to control levels for all cultures. Moreover, in extreme rainfall recovery treatments, the CAT activity of $Z$. bungeanum was significantly higher in Z-G compared with its control. That means that the damage from extreme rainfall on $Z$. bungeanum in the Z monoculture, Z-C and Z-G mixed cultures could be recovered from in 30 days. The present finding gives an indication of the strong tolerance to extreme rainfall of focal species in a legume mixed culture.

**Figure 3.** Effects of leaf osmotic adjustment substances on focal species after extreme rainfall and recovery.

The left column represents 30 days of rainfall, the right column represents recovery for 30 days; “Z-G” denotes $Z$. bungeanum intercropping with $G$. max; “Z-C” denotes $Z$. bungeanum intercropping with $C$. annuum, and “Z” denotes the $Z$. bungeanum monoculture. Vertical bars show ± SE of the mean (n= SE). Different uppercase letters indicate significant differences between the control (normal rainfall) treatments; different lowercase letters indicate significant differences between the extreme rainfall treatments. “r” denotes extreme rainfall; “s” denotes planting system; “r × s” denotes the interaction of extreme rainfall and planting system; Proportion of explained variance by extreme rainfall and planting system effects and by their interactions (two-way ANOVA). Significant levels: *P < 0.05, **P < 0.01, ***P < 0.001, "ns" no significant.
Osmoregulation is an important adaptation strategy to external stress. Different patterns of carbohydrate accumulation in the leaves of flooded and control plants suggest that the decrease in water soluble carbohydrates in control plants is caused by the translocation from leaves to other plant organs. Our results show that, under extreme rainfall, the soluble sugar content of focal species in the Z-C mixed culture was higher than that in the Z monoculture and Z-C mixed culture. This may suggest either a higher rate of soluble sugar or a high molecular weight fructan. In addition, proline has several roles in osmotic adjustment, the elimination of ROS, and the maintenance of cell redox status under stress. Our study found that, under extreme rainfall the proline content of the focal species increased in the Z-C mixed cultures compared with their controls. This showed that proline accumulation in focal species might have a scavenging function and act as an osmolytes. In recovery treatments, the soluble sugar and proline contents of focal species leaves in the Z monoculture and Z-G mixed culture were significantly increased compared with the control. The accumulation of these osmolytes represents an important adaptive response during the recovery period.

Materials and methods

Study site. The experimental site was located in Mao county eastern Qinghai-Tibet Plateau (31°41′N, 103°53′E, elevation 1686 m). According to meteorological monitoring data from the Mao County Ecological Station of Chinese Academy of Science, the mean annual precipitation in the area is 920 mm, mean annual temperature is 8.9 °C and extreme minimum and maximum temperatures recorded are −11.6 °C, and 32.2 °C, respectively. The total precipitation in August is approximately 90 mm, and it is considered as the month with the most rainfall. According to a previous study, extreme rainfall is expected to increase in this area. The soils are classified as Udic Luvisols.

Experimental design. A batch of uniform, two-year-old seedlings of Z. bungeanum were planted in April 2013. Six experimental treatments were set up as a randomized design with three replicates, with 18 plots of 2.6 m × 2.6 m spaced at least 1 m apart from each other. The three planting systems were as follows: (1) Z. bungeanum + G. max (Z-G); (2) Z. bungeanum + C. annuum (Z-C); (3) Z. bungeanum monoculture (Z). G. max and C. annuum were planted in April 2015. One Z. bungeanum was grown in the center of each plot, while species of G. max and C. annuum were planted at the same density (0.27 m²/individual) in all plots. No additional fertilization was applied after the experiment commenced, and the weeds in each plot were completely removed by hand each week. Z. bungeanum and intercrops were grown under natural rainfall before simulating extreme precipitation. In August 2015, we exposed our plots, to the precipitation treatment at random, in which triplicate plots per system received either normal (control) or extreme rainfall. To avoid external rainfall effects, all plots were kept under rainout shelters during the experimental period (from 1st August to 30th September 2015) to control soil moisture. To minimize greenhouse effects, the rainout shelters for each plot were situated 2 m aboveground. Tap water was used to mimic extreme rainfall events, and a watering pot was used to compensate for rain. Rainfall regimes were designated, based on the average rainfall in the area during August of 3 mm/day (based on the average rainfall data during 1983–2013 from the Mao County Ecological Station of Chinese Academy of Science). This was designed as the control rain regime, while extreme rainfall was designated according to the abnormally high rainfall in August of 9.5 mm/day. Each planting system was first divided into two groups of different treatments: (1) Extreme rainfall (9.5 mm/day) and (2) Mean rainfall (Control, 3.0 mm/day). During the two-month-long experimental period, all the plots were watered in the morning (7–9 am) and evening (6–8 pm). After one month of extreme rainfall and control treatments, the systems were subsequently subjected to one month of recovery with rainfall of 3.0 mm/day. Around all plots, thick PVC panels were inserted to a depth of 0.5 m into the soil to prevent the lateral water movement between the plots and prevent interactions with roots from neighboring plots.

Plant leaf collection. At the end of each stage, the youngest fully expanded Z. bungeanum, G. max, and C. annuum leaves at the same developmental stage among plots were collected and placed in a liquid nitrogen container. The samples were taken back to the laboratory and stored at −80 °C to determine their physiological and biochemical parameters.

Soil properties analysis. Soil sampling was taken randomly at 50 cm distance from the Z. bungeanum (in 0–10, 10–20, and 20–30 cm depth) in each plot. The estimation of soil moisture content was performed gravimetrically by oven drying (105 °C for 24 h) 20 g of the soil sample. Soil NH4+-N and NO3--N were determined with the help of a flow injection auto analyzer (AA3, Bran + Luebbe, Germany).

Plant leaves nitrogen analysis. Approximately 0.1 g fresh leaves was ground and extracted in 1 mL of distilled water for 2.5 h. The NO3--N content of the solution was determined with 10% (w/v) salicylic acid in 96% sulfuric acid. The values were quantified after generating a standard curve. The NH4+-N content was determined with the colorimetric assay described by Krom.

Analysis of growth and biomass. Plant height (cm) was measured with the help of a measuring tape. The roots, shoots and leaves of neighboring plants were separated after digging the plants out from the soil, weighting, and subjecting them to oven drying at 70 °C for 24 h to measure dry weight. Determination of yield by weighing method.

Determination of leaf relative water content. Leaf relative water content (LRWC) was determined and calculated according to the equation:
The expanded leaves were collected from each pot as samples and weighed to obtain their fresh weight (FW). Then, the samples were immediately dipped into deionized water, in dark conditions at room temperature. After 12 h, the leaves were weighted to obtain turgor weight (TW) and then subjected to oven drying at 70 °C for 24 h to determine the dry weight (DW).

**Pigment and Photosynthetic parameter.** Chl a, Chl b, and Car were determined using 0.2 g of leaf sample and 5 mL of 100% acetone as a solvent. The samples were placed in dark conditions for 36 h at room temperature, and the extracting solution was determined spectrophotometrically at 662, 645, and 470 nm. The value of Chl a/Chl b was calculated by dividing Chl a by Chl b. The Pn, Gsi, and Tr were measured for fully expanded leaves at similar development stages with a portable open-flow gas exchange system (LI-6400, LI-COR Inc., USA) during the late morning (9:00–11:00 h). The air relative humidity, CO2 concentration and photon flux density were maintained at 70–80%, 400 μmol mol⁻¹ and 800 μmol m⁻² s⁻¹ respectively in all cases.

**Determination of biochemical parameters.** The leaves (0.2 DW) were extracted three times with 6 mL of 80% ethanol at 80 °C for 30 min. The resulting supernatant was analyzed for soluble sugar by the modified anthrone method. Proline was extracted with 2 mL of 10% acetic acid and 5 mL of 3% sulfosalicylic acid. The resulting supernatants were analyzed according to a previously described method. Soluble proteins were determined using the Bradford G-250 reagent.

**Determination of ROS and lipid peroxidation.** The production rate of O₂⁻ was measured by monitoring the nitrite formation from hydroxylamine in the presence of O₂⁻. Fresh leaves (0.2 g) were homogenized with 2 mL of 65 mM phosphate buffer (pH 7.8) and centrifuged at 5000 g for 10 min. The incubation mixture contained 0.9 mL of 65 Mm phosphate buffer (pH 7.8), 0.1 mL of 10 mM hydroxylammonium chloride and 1 mL of supernatant. After incubation at 25 °C for 20 min, 17 mM sulphanilamide and 7 mM α-naphthylamidine and 7 mM α-naphthylamidine were added to the incubation mixture, and kept at 25 °C for 20 min. The same volume of ethyl ether was added and centrifuged at 15000 g for 5 min. The absorbance wavelength of the aqueous solution was 530 nm. H2O2 was determined by monitoring the absorbance of the titanium-peroxide complex. Fresh leaf tissue (0.2 g) was homogenized with 5 mL of acetonitrile and centrifuged at 3000 g for 10 min. The reaction mixture contained 0.1 mL of titanium reagent (50 μL of 20% titanium tetrachloride in concentrated HCl), 0.2 mL of ammonia and 1 mL of supernatant, and was then centrifuged at 3000 g for 10 min. The resulting precipitate was washed five times with acetone and centrifuged at 10,000 g for 5 min. The precipitate was solubilized in 3 mL of 1 M sulfuric acid (H₂SO₄) and the absorbance was read at 410 nm. The MDA content was determined according to the thiobarbituric acid (TBA) method. Fresh leaf tissue (0.2 g) was homogenized with 2 mL of 50 mM phosphate buffer (pH 7.8) and centrifuged at 12,000 g for 20 min. One milliliter of supernatant was mixed with 3 mL of 20% trichloroacetic acid (TCA) solution containing 2% TBA. The reaction mixture was incubated in a water bath at 95 °C for 30 min, rapidly cooled in an ice bath and then centrifuged at 15,000 g for 10 min. The absorbance was read at 532 and 600 nm. The amount of MDA was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

**Determination of antioxidant enzyme activities.** The SOD activity was determined by the nitroblue tetrazolium (NBT) method. One unit of SOD activity was defined as the quantity of enzyme required for 50% inhibition of NBT reduction at 560 nm. The CAT was determined using the methods of Fu. CAT activity and soluble protein content of Z. bungeanum were measured for fully expanded leaves at similar development stages with a portable open-flow gas exchange system (LI-6400, LI-COR Inc., USA) during the late morning (9:00–11:00 h). The air relative humidity, CO2 concentration and photon flux density were maintained at 70–80%, 400 μmol mol⁻¹ and 800 μmol m⁻² s⁻¹ respectively in all cases.

**Statistical analysis.** Statistical analysis was performed using SPSS v.17.0 (SPSS Inc., Chicago, IL). One-way and two-way ANOVA were used to test the effects of planting systems and extreme rainfall on the soil properties and physio-biochemical parameters. Before the ANOVA, the data were checked for normality and the homogeneity of variances. Origin pro 8.5 was used for graphical presentation; error bars represent standard errors, and all data in the figures represent the mean ± SE.

**Conclusion**

Extreme rainfall significantly impacted the growth and metabolism of Z. bungeanum in the three intercropping systems, especially for the Z monoculture. In Z. bungeanum, a significant increase in ROS (O₂⁻ and H₂O₂) and MDA content was found, while there were no significantly changes between the antioxidative (SOD and CAT) activities and the accumulation of osmoprotectants (proline, soluble sugars and soluble proteins) confirming its weak resistance potential in response to extreme rainfall. However, under extreme rainfall, intercropping with G. max had a significantly positive effect on the antioxidative activities, pigmentation and photosynthesis of Z. bungeanum. Intercropping with C. annuum inhibited the growth, had a negative effect on Tn, and decreased the CAT activity and soluble protein content of Z. bungeanum. After recovery, the soil NO₃⁻–N significantly decreased in the Z-C mixed culture, resulting in a significant decrease in the Pₚ of Z. bungeanum. By increasing its root biomass, G. max enhanced soil NO₃⁻–N in the Z-G mixed culture and improved the Tn and CAT activity of Z. bungeanum. Legumes could enhance the tolerance of Z. bungeanum to extreme rainfall. The present findings provide baseline information about the effects of neighbor species (leguminous and nonleguminous) on focal species under extreme rainfall stress. However, more detailed studies are required to explore the interactive mechanisms of different agroforestry plant species, which could lead to a better understanding of the possible physiological responses in coping with future climate change.
References

1. IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T. F., Qin D. & Plattner, G.-K., et al.], Cambridge University Press, Cambridge, United Kingdom and New York, NY USA 4-20 (2013).
2. Donat, M. G., Lowry, A. L., Alexander, L. V., O’Gorman, P. A. & Maher, N. Addendum: More extreme precipitation in the world’s dry and wet regions. Nature Clim. Change 6, 508–513 (2016).
3. Qin, N., Chen, X., Fu, G., Zhai, J. & Xue, X. Precipitation and temperature trends for the Southwest China: 1960–2007. Hydrol. Process. 24, 3733–3744 (2010).
4. Qin, N. et al. Spatial and temporal variations of extreme precipitation and temperature events for the Southwest China in 1960–2009. Geos Environ. Disasters 2, 1–14 (2015).
5. Reyer, C. P. O. et al. A plane’s perspective of extremes: terrestrial plant responses to changing climatic variability. Global Change Biol. 19, 75–89 (2013).
6. Tawar, J. T. et al. Climate change induced rainfall patterns affect wheat productivity and agroecosystem functioning dependent on soil types. Ecol. Res. 31, 203–212 (2016).
7. Qiyue, Y., Wenzhi, Z., Bing, L. & Hu, L. Physiological responses of Haloxylon ammodendron to rainfall pulses in temperate desert regions, Northwestern China. Trees 28, 709–722 (2014).
8. Zhang, J. B. et al. Soil gross nitrogen transformations along the Northeast China Transect (NECT) and their response to simulated rainfall events. Sci. Rep. 6, 22830 (2016).
9. Jackson, M. & Drew, M. Effect of flooding on growth and metabolism of herbaceous plants. In: Flooding and Plant Growth. Academic Press, London, pp. 47–128 (1984).
10. Broz, A. K. et al. Plant neighbor identity influences plant biochemistry and physiology related to defense. BMC Plant Biol. 10, 115 (2010).
11. Gellesch, E. et al. Plant community composition is a crucial factor for heath performance under precipitation extremes. J. Veg. Sci. 26, 975–985 (2015).
12. Ballièr, E., Camarero, J. J., Ninot, J. M. & Gutiérrez, E. Seedling recruitment, survival and facilitation in alpine Pinus uncinata tree ecotones. Implications and potential responses to warming climate. Global Ecol. Biogeogr. 18, 460–472 (2009).
13. Brooker, R. W. Plant-plant interactions and environmental change. New Phytol. 171, 271–284 (2006).
14. Ekelin, A. & Harrison, S. Biotic context and soil properties modulate native plant responses to enhanced rainfall. Ann. Bot. 116, 963–973 (2015).
15. Tyilanakis, I. M., Didham, R. K., Bascompte, J. & Wardle, D. A. Global change and species interactions in terrestrial ecosystems. Ecol. Lett. 11, 1351–1363 (2008).
16. Grant, K., Keyling, J., Heilmeier, H., Beierkuhnlein, C. & Jentsch, A. Extreme weather events and plant–plant interactions: shifts between competition and facilitation among grassland species in the face of drought and heavy rainfall. Ecol. Res. 29, 991–1001 (2014).
17. Sun, E. et al. The response of the soil microbial food web to extreme rainfall under different plant systems. Sci. Rep. 6, 37662 (2016).
18. Kong, D. L. et al. Extreme rainfall events can alter inter-annual biomass responses to water and N enrichment. Biogeosciences 10, 8129–8138 (2013).
19. Sun, E. et al. Soil soybean supplementation increases the resilience of microbial and nematode communities in soil to extreme rainfall in an agroforestry system. The Science of the total environment 626, 776–784 (2018).
20. Dormann, C., Wal, R. & Woodin, S. Neighbour identity modifies effects of elevated temperature on plant performance in the High Arctic. Global Change Biol. 10, 1587–1598 (2004).
21. Klanderud, K. Climate change effects on species interactions in an alpine plant community. J. Ecol. 93, 127–137 (2005).
22. Wardle, D. A. et al. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633 (2004).
23. Wagner, D., Eisenhauer, N. & Cesár, S. Plant species richness does not attenuate responses of soil microbial and nematode communities to a flood event. Soil Biol. Biochem. 89, 135–149 (2015).
24. Mariotte, P., Le Bayon, R.-C., Eisenhauer, N., Guentet, C. & Buttler, A. Subordinate plant species moderate drought effects on earthworm communities in grasslands. Soil Biol. Biochem. 96, 119–127 (2016).
25. Taylor, A. R. & Walters, V. Responses of orbited mite communities to summer drought: The influence of litter type and quality. Soil Biol. Biochem. 37, 2117–2130 (2005).
26. Pan, K., Wang, J. & Song, L. Effects of leaf leachates of Zanthoxylum bungeanum on soil enzymes, chemical properties and its own seedlings growth. Allelopathy J. 22, 153–166 (2008).
27. Hong, L. et al. Inhibitory effect of Zanthoxylum bungeanum essential oil (ZBEO) on Escherichia coli and intestinal dysfunction. Food & function 8, 1569–1576 (2017).
28. Miller, H. G. Management of water and nutrient relations in European forests. Forest Ecol. Manag. 305, 425–436 (1990).
29. LeBauer, D. S. & Treseder, K. K. Nitrogen Limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379 (2008).
30. Dupraz, C. & Liager, F. Agroforesterie, des arbres et des cultures. Editions France-Agricole, Paris (2008).
31. Quinkenstein, A. et al. Ecological benefits of the alley cropping agroforestry system in sensitive regions of Europe. Environ. Sci. Policy 12, 1112–1121 (2009).
32. Alain, A. & Kandji, S. T. Carbon sequestration in tropical agroforestry systems. Agr. Ecosyst. Environ. 99, 15–27 (2003).
33. Herridge, D. F., Peoples, M. B. & Boddye, R. M. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311, 1–18 (2008).
34. Stacey, G., Vodkin, L., Parrott, W. A. & Shoemaker R. National science foundation-sponsored workshop report. Draft plan for soybean genomics. Plant Physiol. 135, 59–70 (2004).
35. Graham, P. & Vance, C. Legumes: importance and constraints to greater use. Plant Physiol. 131, 872–877 (2003).
36. Gil-Quiñones, E. et al. Local inhibition of nitrogen fixation and nodule metabolism in drought-stressed soybean. J. Exp. Bot. 64, 2171–2182 (2013).
37. Manavalan, L. P., Guttikonda, S. K., Tran, L. S. & Nguyen, H. T. Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol. 50, 1260–1276 (2009).
38. Qin, C. et al. Whole-genome sequencing of cultivated and wild peppers provides insights into Capsicum domestication and specialization. Proc. Natl Acad. Sci. USA 111, 5135–5140 (2014).
39. Andriuzzi, W. S., Pulleman, M. M., Schmidt, O., Faber, J. H. & Brussaard, L. Anecic earthworms (Lumbricus terestris) alleviate negative effects of extreme rainfall events on soil and plants in field mesocosms. Plant Soil 397, 103–113 (2015).
40. Sairam, R., Kumarth, D., Ezhilmathi, K., Deshmukh, P. & Srivastava, G. Physiology and biochemistry of waterlogging tolerance in plants. Biol. Plantarum 52, 401–412 (2008).
41. Tempertton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B. & Buchmann, N. Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia 151, 190–205 (2007).
42. Roscher, C. et al. Using plant functional traits to explain diversity-productivity relationships. PloS one 7, e36760 (2012).
43. Marquard, E. et al. Plant species richness and functional composition drive overyielding in a six-year grassland experiment. Ecology 90, 3290–3302 (2009).
44. Drew, M., Saglio, P. & Pradet, A. Larger adenylate energy charge and ATP/ADP ratios in anechymatous roots of zea mays in anaerobic media as a consequence of improved internal oxygen transport. *Planta* 165, 51–58 (1985).

45. Laan, P. Mj., B., Lythe, S., Armstrong, W. & Cowyn, B. Root morphology and anechymaformation as indicators of the flood-tolerance of *Rumex* species. *J. Ecol.* 77, 693–703 (1989).

46. Walther, E., Thomson, C. & Greenway, H. Root Development and Aerenchyma Formation in Two Wheat Cultivars and One Triticale Cultivars Grown in Stagnant Agar and Aerated Nutrient Solution. *Annu. Bot.* 81, 349–354 (1998).

47. Huang, J. et al. Effects of phosphorus addition on soil microbial biomass and community composition in a subalpine spruce plantation. *Eur. J. Soil Biol.* 72, 35–46 (2016).

48. Khan, M., Grant, K., Beierkahnlein, C., Krejling, J. & Jerisch, A. Climatic extremes lead to species-specific legume facilitation in an experimental temperate grassland. *Plant Soil* 379, 161–175 (2014).

49. Gerten, D., Schaphoff, S., Haberlandt, U., Lucht, W. & Stich, S. Terrestrial vegetation and water balance—hydrological evaluation of a dynamic global vegetation model. *J. Hydrol.* 286, 249–270 (2004).

50. Heimann, M. & Reichstein, M. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451, 289–292 (2008).

51. Barron-Gafford, G., Scott, R., Jenerette, G., Hamerlynck, E. & Huxman, T. Temperature and precipitation controls over leaf- and ecosystem-level CO2 flux along a woody plant encroachment gradient. *Global Change Biol.* 18, 1389–1400 (2012).

52. Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J. & Hunатег, B. A. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biol.* 17, 927–942 (2011).

53. Levine, J., Maceacma, A. & Cowan, C. Do competitors modulate rare plant response to precipitation change? *Ecology* 91, 130–140 (2010).

54. Niu, S. et al. Climatic warming changes plant photosynthesis and its temperature dependence in a temperate steppe of northern China. *Environ. Exp. Bot.* 63, 91–101 (2008).

55. Armastron, W. Aeration in higher plants. *Adv. Bot. Res.* 7, 225–332 (1979).

56. Zhuang, B. C. *Biological studies on wild soybean in China*. Science Press (1999).

57. Jaraearu, C. D., Toma, L. D., Slabu, C. & Marta, A. E. Effect of weather conditions on photosynthetic and flavonoid pigment contents in leaves of grapevine cultivars during growing season. *J. Food Agric. Environ.* 9, 793–798 (2011).

58. Mossidt, T. D. & Runn, S. E. Temporal patterns of rainfall stream epilithic algae in relation to flow-related disturbance. *Aquat. Bot.* 58, 181–193 (1997).

59. Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A. & Raizada, M. N. Erratum to: Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. *Agron. Sustain. Dev.* 36 (2016).

60. El-Tantawy, E. M. & Nawar, D. A. S. Nodulation, growth, photosynthetic pigment and yield of broad bean plants (*Vicia faba L.*) as affected by nitrogen source, Rhizobium inoculation and iron foliar application. *J. Appl. Sci. Res.* 9, 974–987 (2013).

61. Parvaiz, A. & Satyawati, S. Salt stress and phyto-biochemical responses of plants – a review. *Plant Soil Environ.* 54, 89–99 (2008).

62. Apel, K. & Hirt, H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399 (2004).

63. Jaleel, C. A. *Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints*. Acta Physiol. Plant 31, 427–436 (2009).

64. Davis, D. G. & Swanson, H. R. Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula L.*) *Environ. Exp. Bot.* 46, 95–108 (2001).

65. Bertness, M. D. & Callaway, R. Positive interactions in communities. *Trends Ecol. Evol.* 9, 191–193 (1994).

66. Ramanjulu, S. & Bartels, D. Drought- and desiccation-induced modulation of gene expression in plants. *Annu. Rev. Plant Biol.* 54, 373–399 (2003).

67. Jurczyk, B., Krępski, T., Kosmala, A. & Rapacz, M. Different mechanisms trigger an increase in freezing tolerance in Festuca pratensis exposed to flooding stress. *Environ. Exp. Bot.* 93, 45–54 (2013).

68. Dionne, J., Castonguay, Y., Nadeau, P. & Desjardins, Y. Freezing tolerance and carbohydrate changes during cold acclimation of green-type annual bluegrass (*Poa annua L.*) ecotypes. *Crop Sci.* 41, 443 (2001).

69. Yildiztagay, E., OZDAN KONAKÇI, C., KÜÇÜKÖDÜK, M. & Duran, Y. Modulation of osmotic adjustment and enzymatic antioxidant profiling in Apera intermedia exposed to salt stress. *Turk. J. Bot.* 38, 99–111 (2014).

70. Rare, P. D. & Van Staden, C. W. J. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot.* 50, 413–434 (1999).

71. Parida, A. & Das, A. B. Salt tolerance and salinity effects on plants: A review. *Exotus. Environ. Saf.* 60, 324–349 (2005).

72. Abdel wahab El-Emany, A. D. A., Nahla Dief, Wafaa Al-Taisan. Role of salt-tolerant enzymes in amelioration of water deficit and waterlogging stresses on Vigna sinensis plants. *J. Biol. Earth Sci.* 3, B144-B153 (2013).

73. Zhai, P., Zhang, X., Wan, H. & Pan, X. Trends in total precipitation and frequency of daily precipitation extremes over China. *J. Clim.* 18, 1096–1108 (2005).

74. IUSS Working Group WRB. *World reference base for soil resources 2006*, first update 2007. *World soilresources reports no.103. FAO*, Rome (2007).

75. Borken, W., Savage, K., Davidson, E. A. & Schimel, S. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biol.* 12, 177–193 (2006).

76. Ng, E. L. et al. Do organic inputs alter resistance and resilience of soil microbial community to drying? *Soil Biol. Biochem.* 81, 58–66 (2015).

77. Wang, S. et al. Combined effects of cropping types and simulated extreme precipitation on the community composition and diversity of soil macrofauna in the eastern Qinghai-Tibet Plateau. *J. Soil Sediment.* 1, 1–13 (2018).

78. Cataldo, D. A., Maroon, M., Schrader, L. E. & Youngs, V. L. Rapid colorimetric determination of nitrate in plant tissue by nitrification of salicylic acid. *Commun. Soil Sci. Plant Anal.* 6, 71–80 (1975).

79. Krom, M. D. Spectrophotometric determination of ammonium: a study of a modified Berthelot reaction using salicylate and dichlorosuccinate. *Analyst* 105, 305 (1980).

80. Galle, A., Haldimann, P. & Feller, U. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytol.* 174, 799–810 (2007).

81. Liu, C. et al. Carbon and nitrogen metabolism in leaves and roots of Dwarf Bamboo (*Fargesia denudata Yi*) subjected to drought for two consecutive years during sprouting period. *J. Plant Growth Regul.* 33, 243–255 (2014).

82. Quan, R., Shang, M., Zhang, H., Zhao, Y. & Zhang, J. Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize. *Plant Sci.* 166, 141–144 (2009).

83. LS, R. P., WP & ID, T. Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207 (1973).

84. Ke, D. S., Wang, A. G., Sun, G. C. & Dong, L. F. The effect of active oxygen on the activity of ACC synthase induced by exogenous IAA. *Acta Bot. Sin.* 44, 551–556 (2002).

85. Zhou, Q. Y., Huang, L. & Nogués, S. The relationship between CO2 assimilation, photosynthetic electron transport and water–water cycle in chilled-exposed cucumber leaves under low light and subsequent recovery. *Plant Cell Environ.* 27, 1503–1514 (2004).

86. Zhou, Y., Lam, H. M. & Zhang, J. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *J. Exp. Bot.* 58, 1207–1217 (2007).

87. Fu, J. & Huang, B. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45, 105–114 (2001).
Acknowledgements
We are grateful to the editor and two reviewers for their helpful advice and valuable comments that have greatly improved the quality of the manuscript. This study was supported by the Ministry of Sciences and Technology of China (Grant No. 2015BAD07B050304) and by the National Science Foundation of China (Grant Nos 31370632 and 31500517).

Author Contributions
K.P. designed the experiments. Z.L. performed the experiments, determined the parameters, and drafted the manuscript. K.P., A.T., F.S., S.Z., L.Z., X.M., X.G. and D.G. revised the manuscript. All authors reviewed the manuscript.

Additional Information
Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.