

# Effects of Water, Nitrogen, and Phosphorus Additions on Root Growth Strategies of *Stipa breviflora* under Drought Conditions

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## Abstract

Root growth strategies are important to predict plant – soil interactions under nutrient-limited soil environments. A dominant species *Stipa breviflora* is found to develop cluster fragmentation in the arid and semi-arid areas of China, where water, nitrogen and phosphorus are the most important limiting factors to plant growth. Here, we aimed to assess how water, N, P additions and their interactions, and cluster fragmentation affect root growth traits in a greenhouse experiment to reveal the adaptation of *S. breviflora* under drought conditions. A standard Taguchi L8(27) arrays design was conducted with four factors such as nitrogen (N), phosphorus (P) and water (W) and cluster fragmentation (C) and three interactions (N  $\times$  P, N  $\times$  W and P  $\times$  W). Each of four factors had two levels (N1 = 15 mg N / kg, N2 = 120 mg N / kg, P1 = 2 mg P<sub>2</sub>O<sub>5</sub> / kg, P2 = 24 mg P<sub>2</sub>O<sub>5</sub> / kg, W1 = 25 % ~ 30 % field moisture capacity of mixture (FMC), W2 = 75 % ~ 80 % FMC, C1 = individual with single ramet, C2= individual with three ramets). Water was the most important contributor to *S. breviflora* root system growth, while cluster fragmentation was the second contributor, followed by N and P in order. W2 and P2 both promoted root growth, whereas N2 significantly inhibited root growth. Moreover, under drought condition, N2 induced its roots developing an acquisition resource use strategy for escaping adverse environments compared to N1. While under sufficient P condition, W1 induced its roots developing an acquisition resource use strategy for acquiring more resource. And cluster fragmentation is beneficial to improving the adaptability of *S. breviflora* under low N condition.

## Introduction

The *Stipa* steppe is one of the most common steppe types continuously distributed in the Eurasian grassland (Kang et al. 2007; Lv et al. 2019b). Most *Stipa* species are xerophytes and the dominant species in the arid and semiarid grassland of Inner Mongolia (Hu et al. 2014; Hu et al. 2013; Qi et al. 2010), while *S. breviflora* populations are more widely distributed than other *Stipa* species (Zhang et al. 2012). This species has a wider range of ecological adaptations to diverse environments as compared to other *Stipa* species (Kang et al. 2007; Zhang et al. 2012). *S. breviflora* is not only one of the dominant species in the desert steppe of China (Kang et al. 2007), but also is a codominant species with *S. bungeana* and *S. krylovii* in typical steppe (Kang et al. 2007; Zhang et al. 2012).

During the long evolutionary process of plants adapting to heterogeneous environments, root systems of terrestrial plants have a functional importance in resource acquisition and storage (Doussan et al. 2003). In the arid or semiarid steppe ecosystems, water deficit is a severe environmental constraint to plant growth and distribution (Buxbaum and Vanderbilt 2007; Knapp et al. 2017). Reducing water loss by limiting transpiration rate and increasing water uptake with improved roots extension and proliferation are two main mechanisms for plants to adapt to drought environments (Farooq et al. 2009). A structured root system can allow plants to maintain a balance level of water status, which can ensure optimum shoot growth and development (Sánchez-Blanco et al. 2014). Since roots are the only organ to acquire water from soil, therefore, they are the main organs to respond, perceive and maintain the plant growth under drought stress (Wasaya et al. 2018). In general, the morphological variations of root system, its length, density, and proliferation

can directly reflect responses of plants to drought stress (de Vries et al. 2016; Wasaya et al. 2018; Zhou et al. 2018). Normally, plants with deeper root systems, longer root length, larger root surface area and more lateral roots can extract and absorb more water from soil (Fitz Gerald et al. 2006; Sánchez-Blanco et al. 2014; Wasaya et al. 2018). Under drought stress environment, plants would allocate more biomass to roots than shoots, enhancing water uptake and decreasing water loss synchronously (de Vries et al. 2016; Zhou et al. 2018). During this procedure of root biomass accumulation under drought condition, it is a result of storing more carbohydrates in root systems and ensure the plants regeneration after drought stress (Farooq et al. 2009). However, it is observed from previous studies that plants would choose to reduce C demand for reducing root elongating rate or the number of lateral roots (Bengough et al. 2011), and displayed a resource conservation strategy with low specific root length (SRL) and high root tissue density (RTD) under drought stress (Chapin III et al. 1993; de Vries et al. 2016; Pérez-Ramos et al. 2013). Simply, water is a key factor limiting root growth, while it is difficult to explain the wide distribution of *S. breviflora* in arid and semi-arid areas by studying root growth strategies solely under water stress. In fact, plant growth is always influenced by the synergy of water and soil nutrients under drought condition (Farooq et al. 2009; Wasaya et al. 2018; Xue et al. 2017). For example, water and nutrient absorption and availability may be limited in plants under drought condition (Stasovski and Peterson 1991; Xue et al. 2017). And an inappropriate water-nutrient application can degrade soil physicochemical properties and influencing plant growth (Farooq et al. 2009).

In semi-arid or arid steppe ecosystems, besides water, nitrogen (N) and phosphorus (P) are two main nutrient elements limiting plant growth (Gong et al. 2011; Vitousek et al. 2010; Yahdjian et al. 2011). In general, there would be strong interaction between water and nutrients for plant growth (Rouphael et al. 2012; Stasovski and Peterson 1991; Wasaya et al. 2018). However, most studies focused on assessing the effect of single stressful condition on plant root growth, it is rarely considered interactions of soil water and nutrients (Hill et al. 2006; Jin et al. 2005; Song et al. 2010; Walch-Liu et al. 2006). Soil water shortage limits the microbial mineralization for organic matter, and then negatively affect N, P availability, uptake and transport, which impact nutrient absorption by plant root growth (Rouphael et al. 2012; Stasovski and Peterson 1991; Wasaya et al. 2018). In this sense, under drought condition, the more severe the drought the lower the water and nutrient flow and the more limited is the availability of nutrients for absorption and transportation by the root system (Farooq et al. 2009; Rouphael et al. 2012). While other studies pointed that when there was sufficient water applied under drought condition, plants are capable of making the best use of soil nutrients, and N or P is easier to be limited factors during plant growth (Gong et al. 2011). However, Likewise, N addition also can improve water availability in root systems for plants, which can increase photosynthetic rate, and then promote plants growth (Zhang and Han 2008). Meanwhile, the root system architecture has been evolved to be responsive and extremely adaptive to the heterogenous soil habitats (Doussan et al. 2003; Wasaya et al. 2018), which greatly affects plants growth and development under drought condition (Farooq et al. 2009; Gong et al. 2011; Rouphael et al. 2012).

*S. breviflora* as a tussock species, its intact clusters are easily fragmented from stem base independently in nature or under environmental or human interferences, and then form multiple sub-clusters with the identical genetic information to enlarge their populations, this process is defined as cluster fragmentation (Lv et al. 2019a). The cluster fragmentation allows *S. breviflora* to make full use of patchy resources and thus enhance their tolerance to stressful environments (Wang et al. 2017). Whereas the resource acquisition and efficiency are different based on the size of fragmented sub-clusters. It is generally accepted that the size of sub-cluster is smaller, the fewer nutrients or water needed during the plant growth. Here, we want to confirm (1) whether soil water, N or P is the limiting factor for *S. breviflora* root growth; (2) how root traits of *S. breviflora* responds to soil water, N and P additions; and (3) the cluster fragmentation influences the root growth of *S. breviflora*. During the process of plants adapting to the environment, it is the key to quantify the root traits in response to the treatments of soil water, N, P addition and cluster fragmentation of *S. breviflora*, and revealed their primary adaptation strategies in arid or semiarid areas.

## Material and methods

## Experimental design

On 15 July 2018, plants of *S. breviflora* were dug from a temperate grassland area located at Horinger county, Inner Mongolia, China (111°50'E, 40deg29'N). Then, they were hand separated into two types of experimental subjects containing ramets with similar morphological size: individual with single ramet (C1) and individual with three ramets connected by rhizomes (C2). Twenty individuals of each experimental subject were transplanted to one square pot (60 cm length x 35 cm width x 18 cm height) filled with soil mix containing 20% field soil (from Horinger) and 80% vermiculite ((Mg, Fe, Al)<sub>3</sub>[(Si, Al)<sub>4</sub> O<sub>10</sub> (OH)<sub>2</sub>]. 4H<sub>2</sub>O) at the greenhouse of the College of Grassland, Resources, and Environment of the Inner Mongolia Agricultural University. There was a total of 12 square pots for C1 and 12 square pots for C2. The chemical properties of experimental soils were shown in Table 1. In the greenhouse, there was 14 h light/10 h dark photoperiod. The air temperature was 26 ± 2 and 20 ± 2 for day/night, respectively.

The experimental design was a standard Taguchi L<sub>8</sub>(2<sup>7</sup>) arrays including four factors such as nitrogen (N), phosphorus (P) and water (W) and cluster fragmentation (C) and three interactions (N x P, N x W and P x W). Each of four factors had two levels, and there was a total of 8 treatments (Table 2). This experiment used NO<sub>3</sub>NH<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub>\*2H<sub>2</sub>O as N and P fertilizer with the addition concentrations of 15 mg N / kg and 120 mg N / kg and 2 mg P<sub>2</sub>O<sub>5</sub> / kg and 24 mg P<sub>2</sub>O<sub>5</sub> (low level/high level) respectively. Water additions included 25 % ~ 30 % field moisture capacity of mixture (FMC) and 75 % ~ 80 % FMC, and cluster fragmentation consisted of individual with single ramet and individual with three ramets (Table 2). MINITAB 18.0 (Minitab, Inc., State College, PA, USA) software was employed in this data analysis step. The experiment was repeated 3 times with 5-day interval between them. Before treatments, individuals were allowed to grow for 21 days to establish roots. Each experimental period lasted 17 weeks. The nitrogen and phosphorus additions were repeated every 20 days, while water additions were repeated every 3 days.

## Measurements

Five plants were randomly selected from each treatment. To scan root traits of every plants, the roots were severed from the shoot at the root collar and then were gently washed with running tap water over a sieve with a mesh size of 0.2 mm. Each root sample was scanned in water with a flatbed scanner (Epson Perfection V700 Photo, Seiko Epson Corporation, Nagano, Japan). Subsequently, the images were analyzed using the software winRHIZO<sup>7</sup> (Regent Instruments Canada Inc., Quebec, QC, Canada). Root traits analyzed were total root length (calculated using a one pixel thinned image and multiplying the number of pixels by pixel size), root surface area (calculated by determining the root diameter and length), root volume (calculated using the root surface area and length), an root tips (Wang and Zhang 2009). After scanning, root samples were oven-dried at 65degC to constant weights and weighed to determine root dry biomass. Root tissue density (RTD) was determined by the ratio of root dry biomass to root volume. Specific root length (SRL) was estimated by the ratio of total root length to root dry biomass.

## Statistical analyses

The analysis of variance (ANOVA) was used to determine the effects of three factors (N, P, W), their interactions (N x P, N x W, and P x W) and C on seven root traits. Means were separated using Tukey's test at  $P$  [?] 0.05. Besides, the variance contribution rate (VCR) was calculated to determine the magnitude of the influence of the particular factor in terms of percentage on six root traits. The bigger the VCR value, the greater the influence of the particular factor to root traits (Song and Chen 2021). In our study, the VCR is the ratio of the sum of the squares (SS<sub>M</sub>) of the factor to the total sum of the square (SS<sub>T</sub>) of all the factors. Meanwhile, factor analysis was used to determine the dependence relationship between seven root traits of root morphological traits and root biomass. Factor analysis is a type of multivariate analysis that can be used to reduce a large number of correlated variables to a smaller number of main factors (Kaiser 1958). Principal factor analysis was used for factor extraction and varimax rotation was used to define factors (Kaiser 1958). Furthermore, one-way ANOVA was used to identify the effects of N, P, or W on six root traits (total root length, root surface areas, root volume, root tips, root dry biomass and specific root length) under cluster fragmentation. Means were separated using Tukey's test at  $P$  [?] 0.05.

## Results

### Effects of experimental factors on root traits

No interaction effects of NxP, NxW and PxW were observed for total root length (TRL), root surface area (RSA), root volume (RV), root tips (RT) and root biomass (RB) of *S. breviflora*. The interaction effects of NxW and PxW for specific root length (SRL) were significant ( $P[?] 0.05$ , Table 3).

Meanwhile, N addition significantly affected TRL (VCR = 22.23,  $P < 0.01$ ) and RT (VCR = 19.88,  $P < 0.001$ , Table 4), P addition greatly influenced RT (VCR = 22.51,  $P < 0.001$ ) and water addition had significant effects on RV (VCR = 45.83,  $P < 0.001$ ), RSA (VCR = 34.78,  $P < 0.05$ ) and RB (VCR = 21.19,  $P < 0.01$ ). In addition, cluster fragmentation was significant for TRL (VCR = 21.97,  $P < 0.01$ ), RSA (VCR = 20.79,  $P < 0.01$ ), RV (VCR = 17.97,  $P < 0.01$ ), RT (VCR = 26.54,  $P < 0.001$ ), RB (VCR = 22.81,  $P < 0.01$ ) and SRL (VCR = 16.51,  $P < 0.01$ ). Moreover, when considering the total VCR from each experimental factor to six root traits, water addition (Total VCR = 135.24) was the greatest contributing factor, cluster fragmentation (Total VCR = 126.59) was the second factor, followed by N addition (Total VCR = 69.54) and P addition (Total VCR = 52.22) (Table 4).

### Relationships among root traits

Based on the Kaiser's criterion (Kaiser 1958), data could be condensed in two factors, and the results indicated that the two – first eigenvalue represented 88% of all the variability (Figure 1). After varimax rotation, high root growth correlation for the first factor was observed among TRL, RSA, RV and RT, and they all had positive correlations with the first factor, or with each other respectively. For the second factor, high root growth correlation was observed among root biomass, SRL and RTD, whereas SRL had a negative correlation with the second factor (Figure 1). In brief, there were two response types for assessing root growth of *S. breviflora*, one was related to root biomass, and another was related to root morphological traits.

### Root morphological traits and root biomass respond to water addition

The root traits such as TRL, RSA, RV and RB were decreased by 16.5%, 31.6%, 44.3% and 41.4% respectively under 25% FMC water addition than these under 75% FMC water addition ( $P [?] 0.05$ , Figure 2 A-D).

### Effects of cluster fragmentation on root growth

The C2 (individual with three ramets) showed 1.26-fold, 1.34-fold, 1.44-fold, 1.42-fold and 1.84-fold higher TRL, RSA, RV and RT than that of C1 (individual with single ramet) ( $P [?] 0.05$ , Figure 3A-E).

### Root morphological traits and root biomass respond to nutrient addition

N2 addition (120 mg N/kg) had negative effects on root growth by decreasing TRL, RSA, RV and RT ( $P [?] 0.05$ , Figure 4A-C) as compared to N1 addition (15 mg N/kg). P2 addition (24 mg  $P_2O_5$ /kg) significantly promoted root growth via increasing RSA, RV and RT as compared to P1 addition (2 mg  $P_2O_5$ /kg) ( $P [?] 0.05$ , Figure 5A-C).

### Root morphological traits and root biomass respond to the interaction between water and nutrients

The N x W had significant effects on SRL (Table 3). Under the drought condition of 25 % FMC water addition, the SRL was significantly lower in the 15 mg N/kg addition than that in the 120 mg N/kg addition, while there were no variations of the SRL between two-level nitrogen additions under well – watered condition (75 % FMC water addition) ( $P < 0.001$ , Figure 6A). Besides, the P x W also had significant effects on SRL (Table 3), which showed that water addition had no effect on SRL under the condition of 2 mg  $P_2O_5$ /kg addition (P1), but under higher phosphorus condition with 24 mg  $P_2O_5$ /kg (P2), the SRL was significantly higher under drought condition (W1) than that under well-watered condition (W2) ( $P [?] 0.05$ , Figure 6B).

## Discussion

## Effects of drought on root growth

The wide distribution of *S. breviflora* in the desert steppe is closely related to its high level of drought tolerance (Lv et al. 2016; Ren et al. 2017). Root systems are the main organs to uptake water from soil and directly affect the water availability in plants, which are sensitive to drought stress by modifying their morphological traits or biomass as usual (Chen et al. 2013; Huang 2001; Zhang et al. 2018; Zhou et al. 2018). In this study, root biomass and morphological traits such as total root length, root surface area, root volume of *S. breviflora* at the 25 % FMC were significantly lower than at the 75 % FMC. These results are consistent with previous findings that drought caused a significant reduction on root biomass for grass species (Chen et al. 2013; de Vries et al. 2016; Zhang et al. 2018). In addition, Huang (2001) found that drought caused a significant decrease in the total root length of three tall fescue cultivars. A meta-analysis also showed that drought significantly decreased not only root biomass, but also total root length, root volume and root surface area (Zhou et al. 2018).

## Effects of nutrient additions on root growth

N and P are the two essential nutrient elements for plant growth, whereas they also are two main limited elements in grassland ecosystem (Gong et al. 2011; Vitousek et al. 2010). Generally, N or P deficiency can limit root branching or elongating (Gruber et al. 2013; Han et al. 2014; Lopez-Bucio et al. 2003; Walch-Liu et al. 2006). Variations of the number of root branching not only influenced total root biomass, total root length and total root surface area, but also the availability of soil nutrients for plants (Paez-Garcia et al. 2015). In general, N and P contents are closely associated with root architectures formation. In our research, we found that *S. breviflora*'s roots proliferated with larger root surface area, bigger root volume and more root tips at high-level P addition than that at low-level P addition respectively. This is in agreement with previous reports that low P supply decreased root length and root branching (Han et al. 2014). By contrast, in our study, high-level N addition restrained their root growth with significant lower values of the total root length, root surface area and root tips than those in the condition of low-level N addition for *S. breviflora*, which was inconsistent with most of former studies (Hodge et al. 1999; Walch-Liu et al. 2006). Hodge (2004) pointed that, whether plants' roots could proliferate in some nutrient – rich environment, not only depends on the content of nutrient in environments, but also depends on demand for the nutrient by themselves, and they may have a specific threshold of nutrients to induce root morphological traits or biomass changing. For example, though high – level N addition could induce root elongation and branching, sometimes the high internal nitrate/N may inhibit lateral roots growth, and influence the architecture or biomass of root system (Walch-Liu et al. 2006). Therefore, in our research, 120 mg N / kg may have exceeded the threshold value of N addition for promoting the root growth of *S. breviflora*, which cause its root growth restricted.

## Effects of interactions of water and nutrients on root growth

Mostly, soil moisture influences nutrients availability and their transportation or uptake in root systems (Rouphael et al. 2012), i.e., in some cases, sufficient nutrients in soil does not guarantee to improve the plant growth during drought conditions (Rouphael et al. 2012). Therefore, to some extent, root growth can be influenced by the interactions between soil moisture and nutrients. As results showed in this study, SRL was influenced by the interactions of N x W and P x W. During the procedures of plants adapting environments, it is noteworthy that there would be trade-off between resource acquisition and resource conservation use strategies for plants to adapting soil heterogeneity (Fort et al. 2016). RTD, SRL and biomass are always seen as indicators assessing this trade – off. Under poor nutrients environments, plants will develop an acquisition resource use strategy by producing thin roots (with high SRL, low RTD and low biomass), which can elongate root systems for acquiring more nutrients and water (de Vries et al. 2016). And more, in this study, RTD and biomass both had negative correlation with SRL for *S. breviflora*. While under drought condition, there were higher SRL after 120 mg N / kg addition than that at 15 mg N / kg addition, which indicated that plants invest more thin roots, not for acquiring more nutrients or water, it seems to escape high level N and drought condition, which both inhibited the *S. breviflora*'s root growth. By contrast, in general, under nutrient – rich environments, plants would develop a conservation resource use strategy with producing coarse roots (with low SRL, high RTD and high biomass), which have higher ability in transporting nutrients and water

(Hernandez et al. 2010) and can store more resource with longer root spans than thin roots (Craine et al. 2001; de Vries et al. 2016; Fort et al. 2016). In this study, under low – level N addition, the root system of *S. breviflora*'s would invest more coarser to store more organic matter for maximum longevity under drought environment, which may be related to 15mg/N addition being contribute to the root growth of *S. breviflora*. It indicated that high – level N addition caused *S. breviflora* to develop an acquisitive – resource – use strategy under drought condition, whereas developing a conservative – resource- use strategy under low – level N addition, one was for escaping and another for longevity. To some extent, higher SRL and lower biomass were found under 25 % FMC water addition (acquisition resource use strategy) than that under 75 % FMC water addition (conservation resource use strategy) under sufficient P condition, which indicated the former for acquiring more resource and later for storing more resource. This study was consistent with previous studies (Craine et al. 2001; de Vries et al. 2016; Fort et al. 2016).

### Effects of cluster fragmentation on root growth

At the beginning of this study, C2 had more than 2-fold root biomass than C1, but at the end of study, C2 showed 1.26-fold, 1.34-fold, 1.44-fold, 1.42-fold and 1.79-fold higher total root length, root surface area, root volume, root tips and root biomass than that of C1. This result might indicate that the root growth rate of *S. breviflora* was higher for the small sub-cluster than the large one. Moreover, the effects of nitrogen, phosphorus and water addition on root morphological traits and root biomass under the treatment of C1 and C2 were different. During the further analysis, within the treatment of C1 we found that the total root length, root surface area and root biomass were significantly lower under high-level N addition than that under low-level N addition, whereas there were no changes within the treatment of C2 ( $P \geq 0.05$ , Table S1). These results also can be explained by the ideas of Hodge (2004), the proliferation of root systems was determined by the content of extra and intra nutrients of plants together. For example, compared with the control, *Poa pratensis* had a shorter root length, lower root length density and smaller root biomass in a N-rich patch, where they can capture 13% of its total N content (Hodge et al. 1998), but in another study where N patch represented a larger proportion of its total N (18%), they had a higher root length density and higher root biomass (Hodge et al. 1999). In our study, compared with C2, C1 did not need more nitrogen to support their individual growth, which may be caused by high-N addition exceeding the specific thresholds of nitrogen to induce root growth for C1. So, N was a major limiting factor to the root growth of small sub-cluster.

### Conclusions

The root growth strategies of *S. breviflora* can be assessed by two kinds of traits, one was related to root biomass, and another was related to root morphological architecture. For *S. breviflora*, water was the most contributor to the growth of root system, and cluster fragmentation was the second contributor, followed by N and P in order. Under drought condition, high-level N addition induced roots of *S. breviflora* developing an acquisition resource use strategy for escaping adverse environments compared to low-level N addition; under sufficient P condition, low-level water addition induced their roots developing an acquisition resource use strategy for acquiring more resource compared to high-level water addition. *S. breviflora* can adjust root growth strategies of sub-clusters by cluster fragmentation, producing smaller clusters was beneficial for improving its adaptability in a low-N condition.

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### Conflict of interest

The authors declare no conflict of interest.

### Author contributions

**Ru-yue Fan:** Conceptualization(equal);data curation(lead);formal analysis(equal);

methodology(equal);visualization(lead);writing-original draft(lead);  
writing-review&editing(equal)

**Shi-jie Lv:** Formal analysis (equal); methodology (equal);writing-review&editing(equal)

**Qing-feng Li:** Conceptualization(equal);funding acquisition(Lead);methodology(equal);  
project administration(lead);supervision(lead);writing-review&editing(equal)

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## Data availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Table 1. Chemical properties of soil used in the experiment

Properties	
pH	8.29
Organic Carbon	3.30 g/kg
Total nitrogen	0.34 g/kg
Total Phosphorus	0.32 g/kg
Total Potassium	20.21 g/kg
Available N	29.25 mg/kg
Available P	1.85 mg/kg
Available K	73.70 mg/kg

Table 2. Standard Taguchi  $L_8(2^7)$  arrays experimental design

Treatments	Nitrogen addition (N)	Phosphorus addition (P)	N*P	Water addition (W)	N*W	P*W	Cluster f
1	N1	P1	N1P1	W1	N1W1	P1W1	C1
2	N1	P1	N1P1	W2	N1W2	P1W2	C2
3	N1	P2	N1P2	W1	N1W1	P2W1	C2
4	N1	P2	N1P2	W2	N1W2	P2W2	C1
5	N2	P1	N2P1	W1	N2W1	P1W1	C2
6	N2	P1	N2P1	W2	N2W2	P1W2	C1
7	N2	P2	N2P2	W1	N2W1	P2W1	C1
8	N2	P2	N2P2	W2	N2W2	P2W2	C2

Notes: N1 = 15 mg N/kg, N2 = 120 mg N/kg; P1 = 2 mg  $P_2O_5$ /kg, P2 = 24 mg  $P_2O_5$ /kg; W1 = 25% FMC (Field moisture capacity), W2 = 75% FMC; C1 = individual with single ramet; C2 = individual with three ramets.

Table 3. The analysis of variance of the effects of nitrogen addition (N), phosphorus addition (P), water addition (W) and their interactions (N×P, N×W and P×W), and cluster fragmentation (C) on seven root traits for *Stipa breviflora* .

Traits	N	P	N×P	W	N×W	P×W	C
Total root length (TRL)	**	NS	NS	*	NS	NS	**
Root surface area (RSA)	*	*	NS	***	NS	NS	**
Root volume (RV)	NS	*	NS	***	NS	NS	**
Root tips (RT)	**	**	NS	NS	NS	NS	***
Root biomass (RB)	NS	NS	NS	**	NS	NS	**
SRL	**	NS	NS	**	*	**	**
RTD	NS	NS	NS	NS	NS	NS	NS

**Note:** SRL: specific root length; RTD: root tissue density; Significant difference \*, P [?] 0.05; \*\*, P [?] 0.01; \*\*\*, P [?] 0.001; NS, not significant.

Table 4. Variance contribution rate (VCR) of the effects of nitrogen addition (N), phosphorus addition (P), water addition (W) and their interactions (N×P, N×W and P×W), and cluster fragmentation (C) on six root traits for *Stipa breviflora* .

Traits	VCR (%)	VCR (%)	VCR (%)	VCR (%)	VCR (%)	VCR (%)	VCR (%)
	N	P	N×P	W	N×W	P×W	C
Total root length (TRL)	22.23	6.46	0.12	13.68	1.75	0.83	21.97
Root surface area (RSA)	9.74	6.67	0.42	34.78	0.96	3.31	20.79
Root volume (RV)	3.80	6.38	1.74	45.83	0.57	5.08	17.97
Root tips (RT)	19.88	22.51	3.62	4.83	2.99	0.15	26.54
Root biomass (RB)	0.45	6.04	6.33	21.19	4.98	5.94	22.81
SRL	13.44	4.16	5.42	14.93	9.20	14.04	16.51
Total	69.54	52.22	17.65	135.24	20.45	29.35	126.59

**Note:** SRL: specific root length.

Figure 1. Factor analysis of seven root traits.

Figure 2. Effects of water addition on total root length (A), root surface area (B), root volume (C) and root biomass (D). Bars with the same letters were not significantly different ( $P > 0.05$ ) using Tukey's test. Error bars indicate standard error of means. W1 = 25 % FMC, W2 = 75 % FMC.

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Figure 3. Effects of cluster fragmentation on total root length (A), root surface area (B), root volume (C), root tips (D) and root biomass (E). Bars with the same letters were not significantly different ( $P > 0.05$ ) using Tukey's test. Error bars indicate standard error of means. C1 = individual with single ramet, C2 = individual with three ramets.

Figure 4. Effects of N addition on total root length (A), root surface area (B) and root tips (C). Bars with the same letters were not significantly different ( $P > 0.05$ ) using Tukey's test. Error bars indicate standard error of means. N1 = 15 mg N / kg, N2 = 120 mg N / kg.

Figure 5. Effects of P addition on root surface area (A), root volume (B) and root tips (C). Bars with the same letters were not significantly different ( $P > 0.05$ ) using Tukey's test. Error bars indicate standard error of means. P1 = 2 mg P<sub>2</sub>O<sub>5</sub> / kg, P2 = 24 mg P<sub>2</sub>O<sub>5</sub> / kg.

Figure 6. Effects of water addition × nitrogen addition (A) and water addition × phosphorus addition (B) on specific root length. Bars with the same letters were not significantly different ( $P > 0.05$ ) using Tukey's test. Error bars indicate standard error of means. W1 = 25 % FMC, W2 = 75 % FMC. P1 = 2 mg P<sub>2</sub>O<sub>5</sub> / kg, P2 = 24 mg P<sub>2</sub>O<sub>5</sub> / kg.