Qiao, Fusheng; Song, Xiaoyan; Wang, Changting; [et al.]. «Species Diversity and Stability of Dominant Species Dominate the Stability of Community Biomass in an Alpine Meadow Under Variable Precipitation». Ecosystems, Vol. 26, Issue 7 (November 2023), p. 1441-1455. DOI 10.1007/s10021-023-00842-4

Species diversity and stability of dominant species dominate the stability of community biomass in an alpine meadow under variable precipitation

Fusheng Qiao, Xiaoyan Song, Changting Wang, Yigang Hu, Xiangzhen Li, Gaofei Yin & Josep Peñuelas

Highlights:

(1) ANPP stability in the alpine meadow was relatively resistant to up to 50% change in annual precipitation.

(2) ANPP stability was mainly determined by a combination of asynchronous dynamics between coexisting species, population stability and grass stability.

(3) Asynchronous dynamics and population stability were driven by a combination of species alpha diversity and the stability of the dominant species.

Abstract:

The stability of aboveground net primary productivity (ANPP) is critical for ecosystem functions and services, and have been studied across a wide range of ecosystems. An intriguing and challenging question emerging from these studies is how precipitation fluctuations, especially extreme precipitation, affect the temporal stability of ANPP in alpine meadow. We investigated the changes in plant community composition and aboveground biomass in an alpine meadow over six consecutive years under five precipitation treatments (increase of 50%, natural precipitation, decreases of 30%, 50% and 90%) in order to reveal the response of ANPP stability to the precipitation change, especially extreme precipitation, and the relevant driving mechanisms. The alpha diversity of plant species did not differ significantly among the treatments. ANPP was resistant to changes in precipitation between 354 and 1336 mm (precipitation interval of 50% decrease in precipitation in the driest year and 50% increase in precipitation in the wettest year during the experiment), suggesting that normal interannual fluctuations in precipitation and recent changes in regional precipitation might not significantly influence ANPP stability. However, extreme precipitation treatment (90% decrease), significantly reduced ANPP, species asynchrony and ANPP stability. A path model indicated that ANPP stability was directly affected by population stability, species asynchrony and grass stability. While the effect of species diversity on ANPP stability depends on the extents to which it positively affects species asynchrony and negatively affects population stability. In addition, the dominant species stability did not directly affect ANPP stability, while indirectly affected ANPP stability by changing the asynchrony of species.

Keywords: Qinghai-Tibetan Plateau; precipitation changes; ANPP stability; species diversity; species asynchrony;

Introduction

Shifts in patterns of precipitation, such as amount, frequency, spatial and temporal heterogeneity, extreme precipitation or drought, influence the structure and function of terrestrial ecosystems by altering multiple ecological processes, e.g. formation of productivity, redistribution of water and biogeochemical cycling (IPCC, 2013). Changes in precipitation patterns are more difficult to predict than are warming and the deposition of atmospheric nitrogen (Beier et al., 2012), so the impact on ecosystems is more difficult to estimate (Wu Z T, 2011). Ecosystem stability refers to the ability of an ecosystem to maintain relatively stable interannual primary productivity in a changing environment and is critical for providing sustainable ecosystem functions and services (Tilman D, 2001; Isbell, 2015; Oliver, 2015). Precipitation may influence ecosystem stability either by altering biotic and abiotic factors such as amount of soil moisture (Knapp A K, 2008), temperature (Guo Q. 2015) and availability of nutrients (Niu S L. 2017) or by influencing the richness

(Isbell FL, 2009), evenness (Hallett, L, 2014) and asynchrony (Xu Z, 2015) of plant species. Comprehending how the stability of an ecosystem responds to different levels of changes in precipitation will therefore be of great importance in assessing the stability of ecosystem functions under future climate change.

Various global ecosystems are experiencing varying degrees of climate change, so many studies have been conducted on the response of the stability of ecosystem productivity and its potential mechanisms (Hooper D, 2005; Grman E, 2010; Loreau M, 2013; Hautier Y, 2014; 2015). Evidence is growing that the diversity of plant species is a major factor determining the stability of ecosystem productivity (Campbell, 2011; Gross K, 2014; Xu Z, 2015). The influence of species diversity on the stability of ecosystem productivity is mainly achieved by mechanisms such as a compensatory effect (Bai Y, 2004; Allan E, 2011), an overyielding effect (Tilman, 1999), a sampling effect (Loreau M, 2001) and a portfolio effect (Tilman, 2006). Compensatory effects suggest that the differentiation of ecological niches among community species allows different species to use resources in a temporally or spatially complementary manner, thus enabling the stable coexistence of multiple species (Cardinale, 2007). As a major manifestation of the temporal differentiation of ecological niches, species asynchrony represents the different responses of species with different functions to environmental fluctuations (Loreau & de Mazancourt, 2008). For example, a decrease in abundance of one species is more likely to be compensated by an increase in the abundance of other species (Loreau M, 2013). The phenomenon of overyielding refers to the tendency of the average biomass of a plant community to increase with species diversity (Tilman, 1999). The sampling effect assumes that the higher the species diversity, the more likely species that are resistant to environmental disturbances are included (Polley H W, 2003). The portfolio effect describes the phenomenon whereby the variance of a community is scaled by its mean, and in communities is usually expressed as the higher the species diversity, the more likely the variance of community biomass will be lower than the additive variance of the constituent species, ultimately stabilizing the community (Cottingham K L, 2001; Ruijven V, 2007). Hector (2010), however, reported that species diversity tended to reduce the stability of populations in terrestrial ecosystems and thus the stability of community biomass. In addition, the biomass-ratio hypothesis (Grime, 1998), which suggests that ecosystem function is mainly determined by the features of the dominant species and that ecosystem stability is mostly driven by the stability of the dominant species (Zelikova T J, 2014; Ma Z, 2017), has been confirmed by a growing number of studies (Sasaki T, 2011; Yang Z L, 2017; Polley, 2007). Changes in both species diversity and the stability of productivity of the dominant species may thus influence the stability of an ecosystem. The extent to which different changes in precipitation, especially extreme drought, influence the stability of ecosystems in alpine meadows and the mechanisms driving them, however, remain unclear.

As the 'third pole' of the Earth, the Tibetan Plateau is one of the most sensitive regions to global climate change (Zhang R H. 2015). The average annual temperature on the plateau has increased over the last few decades at a rate of 0.5 °C per decade (Yang, Y H. 2018), and precipitation has generally tended to increase, tending to be arid in some places (Yang G, 2014) and with increasing interannual fluctuations (Chen, 2013). Changes in precipitation can alter the amount of moisture in the soil, the effectiveness of nutrients, plant-community composition (Liu H, 2018), allocation of biomass (Zhang F, 2017), activity of soil enzymes (Chai J L, 2019) and litter quality (Schuster, 2016), leading to large fluctuations in ANPP. Most studies of ANPP stability on the Tibetan Plateau, however, have focused on its response to fertilization (Ma F, 2020), mowing (Wang H D, 2013) and warming (Ma Z, 2017), with few studies on the response of ANPP stability to changes in precipitation. The magnitude of the variability of rainfall and rainfall gradients in previous studies have also been relatively small, so including the strong fluctuations in precipitation in extreme years is difficult (Ma Z, 2017). We therefore monitored plant-community structure and ANPP for six consecutive years in a controlled field experiment with different rainfall treatments. We aimed to explore: 1) the range of rainfall variability over which ANPP and its stability in an alpine-meadow ecosystem could remain stable, 2) the key factors influencing ANPP and its stability in alpine meadows and 3) the mechanism maintaining ANPP stability in an alpine meadow under changes in precipitation. Ma et al. (2017) found that a 50% increase in rainfall significantly increased ANPP but did not significantly affect its stability, and Zhang et al. (2017) reported that a 25% increase and a 75% reduction in rainfall did not significantly affect ANPP. We therefore hypothesized that: 1) soil moisture, plant-community structure and ANPP would not change significantly within a particular range of rainfall, and the stability of ANPP would be maintained by an interspecific compensation effect and asynchronous dynamic adjustment, 2) available soil moisture, plant-community structure and ANPP would decrease significantly when rainfall reduction exceeded a threshold, and the compensatory effect between species would decrease and the competitive effect would increase, leading to a significant decrease in the stability of ANPP and 3) the diversity of plant species and the stability of dominant species would jointly determine the stability of ANPP.

Materials and methods

1.1 Study site

The study site was in an alpine meadow in Hongyuan County, Sichuan Province of the eastern Qinghai-Tibetan Plateau, China (32.83°N, 102.59°E). The altitude is approximately 3500 m. The local climate is temperate continental, with short springs and autumns, long and cold winters and mild and cool summers. The average annual temperature for 1963-2013 was 1.5 °C, with average maximum and minimum temperatures of 11.1 and -9.7 °C in July and January, respectively. The average annual precipitation is 747 mm, with 80% falling between May and September. The soil is >40 cm deep, the bulk density is 1.04 g/cm^3 at the experimental site and the soil type is a Mat Cry-gelic Cambisol, which is similar to an Inceptisol of the USDA classification (Zi H B, 2018). The vegetation type is typical of alpine meadows, with an average cover of >80%. The dominant plant species are the grasses *Kobresia humilis* and *Kobresia setchwanensis*, sedges *Elymus nutans* and *Koeleria macrantha*, legumes *Gueldenstaedtia diversifoltia* and *Caerulea* and forbs *Saussurea nigrescens*, *Anaphalis lactea* and *Anemone rivularis* (Yang D C, 2021).

1.2 Experimental design

An alpine meadow with relatively flat terrain and a relatively even distribution of aboveground vegetation was selected in 2015 as a test sample site in the study area and fenced off to prevent grazing. Five rainfall treatments, decreases of 90% (0.1P), 50% (0.5P) and 30% (0.7P), natural precipitation (1.0P) and an increase of 50% (1.5P), were set up based on the variation of precipitation in the region over the last 41 years (1970-2010) (Yang G, 2014) and the increase in precipitation on the Qinghai-Tibetan Plateau from 1960 to 2010 (Chen, 2013). Each treatment had six replicates in a randomized block design. Each trial plot was 2 × 2 m with at least 2 m between adjacent plots. A "V" shaped rainwater interceptor (L = 2.4 m, W = 14 cm) made of highly transparent organic glass (95% transmittance) was adopted to evenly cover 90, 50 and 30% of the area above the plot to achieve rainfalls of 0.1P, 0.5P and 0.7P, respectively. The interceptor was connected to a PVC pipe to direct the rainwater retained by the interceptor into a barrel. The rainwater collected in the 0.5P plots was evenly sprinkled by hand in the 1.5P plots after each rain. Rainwater collected in the other plots was discharged outside the plots. Each plot was surrounded by an aluminum sheet 45 cm in height and buried to a depth of 40 cm to prevent the lateral movement of water in the topsoil (Tang G, 2021).

1.3 Plant-community survey and measurement of soil moisture

One 1 × 1 m quadrat divided into four 0.5 × 0.5 m subsquares was laid in the center of each plot. One of the subsquares was set as a permanent observation sample. Surveys of the vegetation community were conducted in mid-August 2016-2021 to record the number of species, plant height and coverage and the richness, frequency and abundance of species were calculated. The other three subsquares were used for measuring biomass. The location of the plots used for determining biomass was changed every year to avoid harvesting the same subsquares in consecutive years. After the vegetation survey was completed, the plants were mowed flush to the soil surface by species, dried to a constant weight in a dryer at 65 °C and weighed as aboveground biomass for each species. The plants were classified into three groups based on their average relative abundance during the study period (2016-2021): dominant species (>5%), common species (1-5%) and rare species (<1%) (Mariotte, P. 2013). Relative species abundance is the ratio of aboveground biomass of each species to the aboveground biomass of the community (Song M H , 2019). Soil moisture was determined by weighing samples before and after drying (Xu Z, 2015).

1.4 Statistical analysis

The species diversity in each plot was estimated using species richness and the Simpson dominance index. Species richness was defined as the number of species within the permanent observation sample (Dong S K, 2017). The Simpson dominance index was calculated as:

(1)

where S is the number of species in a plot, bi is the biomass of species i and B is the biomass of a sample with S plants (Leps J. 2004).

The *ICV* index was used to indicate the temporal stabilities of the dominant species, common species, rare species, other functional groups (grasses, sedges, legumes and forbs) and the aboveground plant community. The relationship was described as:

(2)

where μ and σ are the temporal mean and temporal standard deviation of ANPP within the plot for 2016-2021, respectively. The larger the *ICV*, the higher the temporal stability (Tilman, 1999).

Population stability was calculated as:

(3)

where μ is the temporal mean of aboveground biomass in the sample, σ_i is the temporal standard deviation of aboveground biomass for each species in the sample and S is the number of species (Schnabel, 2021).

Direct comparisons of fluctuations in the community asynchrony of species are difficult when conducting studies of species diversity in natural communities where the number of species in different treatment communities is not equal, so we used changes in the synchrony of species at the community scale to indirectly represent species asynchrony (Loreau and de Mazancourt, 2008; Isbell, 2009). Calculations were carried out using:

(4)

where φ_y is the species asynchrony of each plot, φ_x is the species synchrony of each plot, σ_S^2 is the temporal variance of plant-community biomass, S is the number of species and σ_i is the temporal standard deviation of biomass of each species in a sample with S species. φ_x ranges between 0 and 1. The larger the value of φ_x, the higher the synchrony and the lower the asynchrony. $\varphi_x = 1$ indicates perfectly synchronous species fluctuations and the lowest community stability, and $\varphi_x = 0$ indicates perfectly asynchronous species fluctuations and the highest community stability.

We ascertained whether species diversity increased the stability of community biomass using mean-variance scaling and whether rainfall influenced this scaling. We calculated the mean-variance scaling relationship (Taylor's power law) for community-biomass stability (Taylor L, 1961) as:

(5)

where σ^2 is the variance of biomass for each species, c is a constant, m is the mean biomass of each species and Z is a scale factor. Diversity is expected to improve the stability of community biomass using the mean-variance scale when $1 < Z < 2$.

The effects of precipitation, duration (year) and their interaction on ANPP, species richness and dominance were examined using a two-way ANOVA with SPSS 20.0 (SPSS Inc., Chicago, USA). A one-way ANOVA and the least significant Difference (LSD) test were used to test the significance ($P < 0.05$) of soil moisture, features of the plant community (ANPP, species richness and dominance), stability of ANPP and species asynchrony for each treatment. Linear and nonlinear regressions were used to analyze the relationships between the stability of ANPP and species asynchrony, precipitation and other biotic and abiotic factors. We used a structural equation model (AMOS 23.0; AMOS Development Corporation, Chicago, USA) to analyze the potential pathways by which precipitation could affect the stability of ANPP.

Results

2.1 Precipitation and soil moisture

Annual precipitation in the region from 2016-2021 ranged from 576.3 (2021) to 890.4 mm (2020), with simulated annual changes in precipitation of 57.6-1335.6 mm in the precipitation treatments, which completely covered interannual changes in precipitation over the last 40 years (508-996.3 mm) (Fig. S1). Soil moisture in the 0-10 and 10-20 cm layers increased with rainfall (Table S1), with the concentration significantly lower in 0.1P than the other treatments ($P < 0.05$). Soil moisture was significantly lower at 0.5P than 1.5P ($P < 0.05$), but the concentration at both 0.5P and 1.5P did not differ significantly from 1.0P.

2.2 Characteristics of the plant community

ANPP was significantly lower at 0.1P (167.59 ± 3.93 g/m^2) than the other treatments over the six-year experiment period, averaging 27.72% (4.47-60.45%) lower than 1.0P ($P < 0.05$), and did not differ significantly between the other treatments (Fig. 1A). Species richness for each treatment was lowest in 2020 among the six years, but rainfall did not significantly affect the mean community species richness (Table 1, Fig. 1B). The overall species dominance tended to increase with rainfall, except at 0.5P (Fig. 1C). Mean species dominance was significantly higher at 1.5P than 0.1P and 0.7P ($P < 0.05$), but none of the treatments differed significantly from 1.0P.

S. nigrescens, *K. humilis* and *K. setchwanensis* were the dominant plant species at 1.0P. The 1.5P community had only one dominant species, *S. nigrescens*, whose biomass accounted for 42% of the community biomass. The biomass of *S. nigrescens* was lower and the biomass of *A. rivularis* was higher at 0.5P and 0.7P than 1.0P. The 0.1P community was dominated by six species, with a lower biomass of *S. nigrescens* and significantly higher biomasses of *Carex enervis*, *E. nutans* and *A. rivularis* ($P < 0.05$). The biomass of *S. nigrescens* varied among the treatments but did not differ significantly from 1.0P ($P > 0.05$)

(Fig. 1D).

Rainfall, year and their interaction significantly affected ANPP ($P < 0.05$). Species richness varied significantly with year ($P < 0.05$), and rainfall and year significantly affected species dominance ($P < 0.05$) (Table 1).

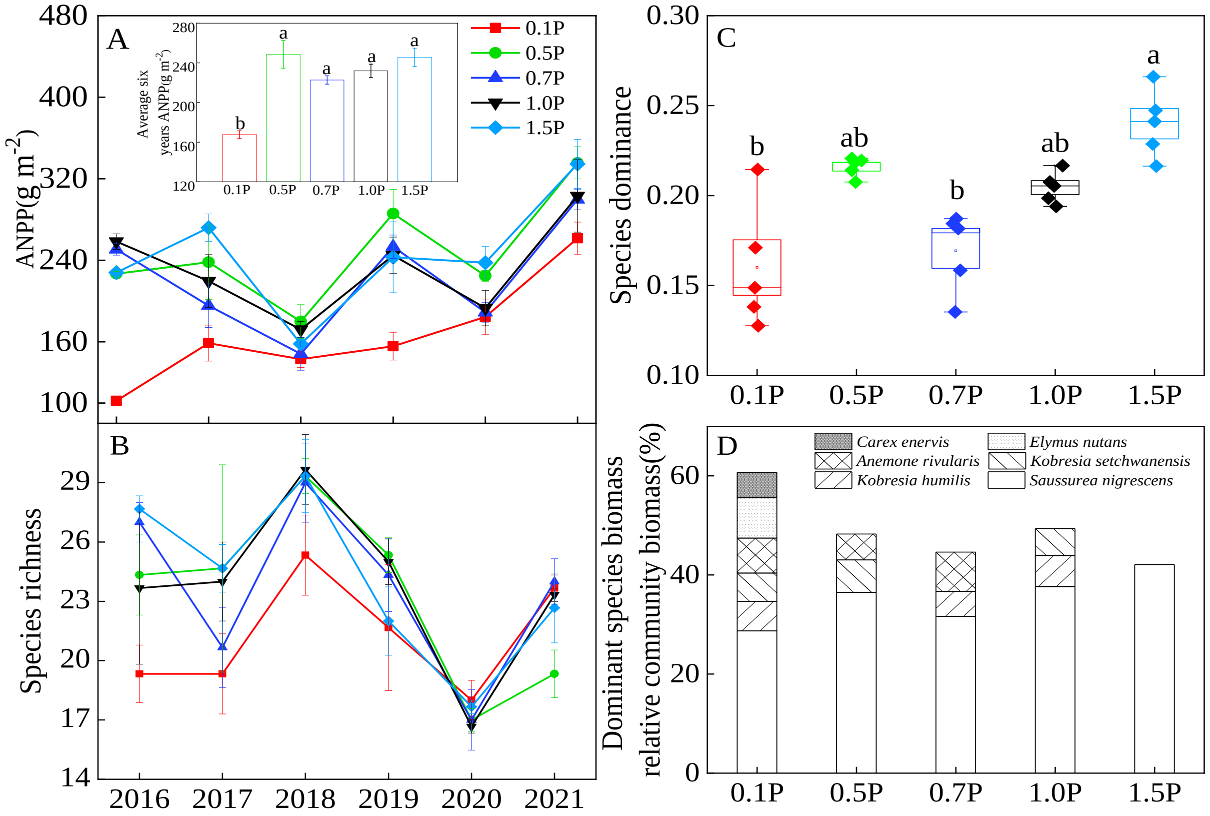

Figure 1 Responses of ANPP (A), species richness (B), species dominance (C) and dominant-species biomass (D) to changes in rainfall. 0.1P, 0.5P, 0.7P, 1.0P and 1.5P represent 90, 50 and 30% decreases in rainfall, natural precipitation and a 50% increase in rainfall, respectively.

Table 1 Effects of rainfall, duration (year) and their interaction on ANPP, species richness and species dominance.

	df	ANPP	Species richness	Species dominance
		F	*F*	*F*
Rainfall (R)	4	20.20***	2.49 ns	2.90*
Year (Y)	5	36.26***	30.12***	16.05***
R × Y	20	2.19**	1.35 ns	0.98 ns

*, **, *** and ns denote $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P > 0.05$, respectively.

2.3 Stability of aboveground biomass and species asynchrony

ANPP stability (Fig. 2A), species asynchrony (Fig. 2B), grass stability (Fig. 2G) and forb stability (Fig. 2J) were significantly lower at 0.1P than 1.0P ($P < 0.05$) by 32.39, 5.32, 34.52 and 43.74%, respectively. The stability of the population (Fig. 2C), sedges (Fig. 2H) and legumes (Fig. 2I) did not differ significantly between the treatments ($P > 0.05$). Increased or decreased rainfall, however, significantly ($P < 0.05$) reduced the mean stability of the dominant species (Fig. 2D). The stability of the common species was significantly lower at 0.5P than 0.1P (Fig. 2E) ($P < 0.05$). The stability of the rare species was significantly lower at 0.7P than 0.1P and 1.5P (Fig. 2F) ($P < 0.05$), and the stability of the forbs was significantly lower at 1.5P (Fig. 2J)

($P < 0.05$).

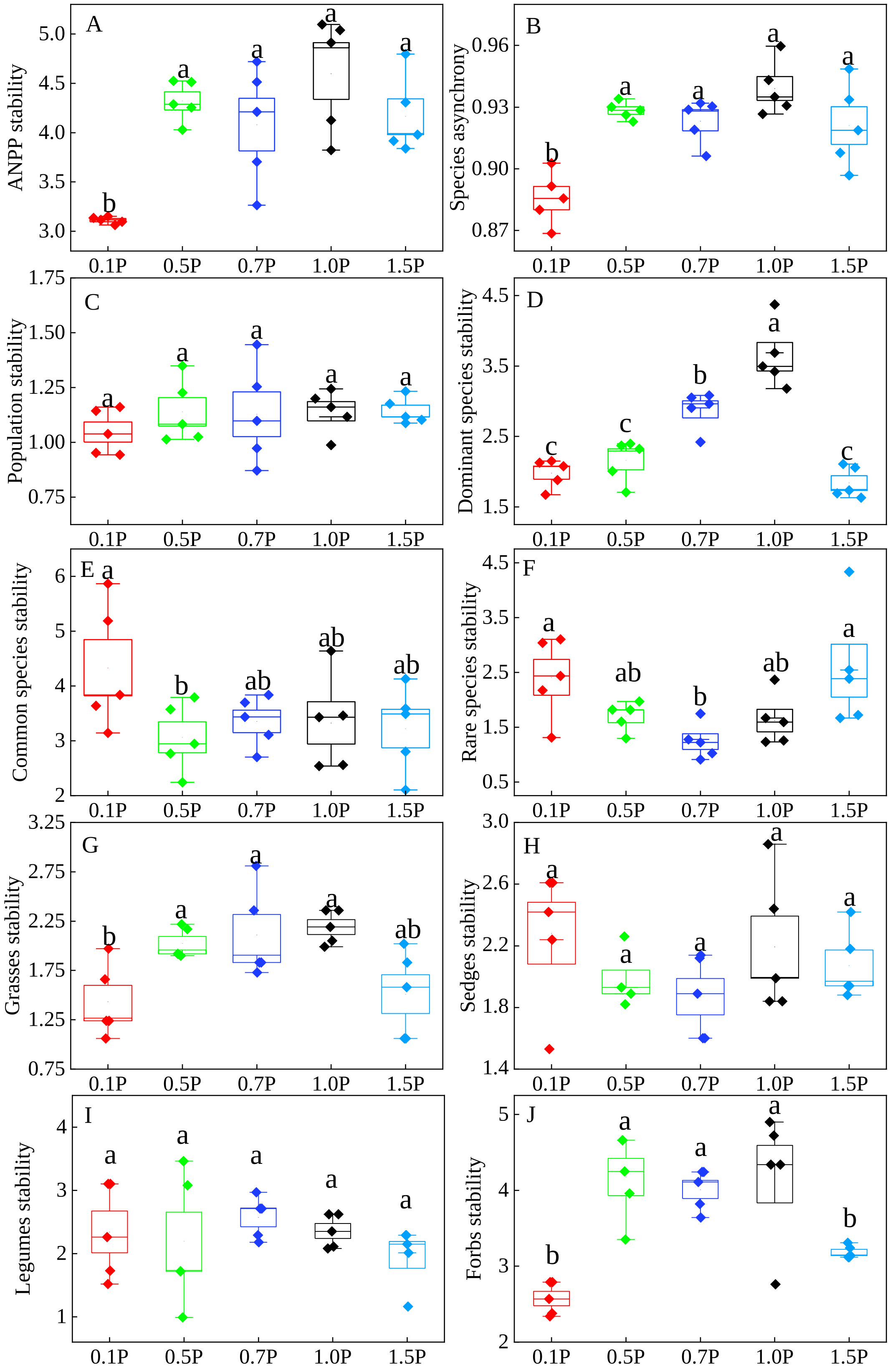

Figure 2 Stability of ANPP (A), species asynchrony (B) and stability of the population (C), dominant species (D), common species (E), rare species (F), grasses (G), sedges (H), legumes (I) and forbs (J) in the rainfall treatments. 0.1P, 0.5P, 0.7P, 1.0P and 1.5P represent 90, 50 and 30%

decreases in rainfall, natural precipitation and a 50% increase in rainfall, respectively.

2.4 Factors and pathways influencing the stability of ANPP

The variance in aboveground biomass for each plant species across the five rainfall treatments was strongly positively linearly ($R^2 \geq 94\%$) correlated with their means (Fig. 3), indicating that species richness contributed to ANPP stability via the mean-variance scale. The scaling coefficient (Z) varied from 1.57 (0.7P) to 1.66 (0.5P), with no significant differences among the treatments (Table S2).

Regression analysis indicated that ANPP stability and species asynchrony increased with precipitation, with 51 and 54% of the variation in ANPP stability and species asynchrony explained by precipitation, respectively (Fig. 4). ANPP stability was significantly positively correlated ($P < 0.05$) with soil moisture, species asynchrony and the stabilities of the population, dominant species, grasses and forbs. ANPP stability was not significantly correlated with species richness, dominance or the stabilities of the common species, rare species, sedges or legumes. Soil moisture, species asynchrony and the stabilities of the population, dominant species, grasses and forbs explained 22-59% of the variation in ANPP stability (Fig. 5). Species asynchrony increased linearly with species richness and dominance ($P < 0.05$) (Fig. S3).

Factor analysis was used to reduce the dimension of species richness and dominance, and the first principal component interpretation rate of species diversity was 62.41% after dimension reduction. Structural equation modelling (SEM) was used to explore the potential pathway whereby precipitation affected ANPP stability (Fig. 6A). The model explained 76% of the variation in ANPP stability. Rainfall indirectly affected ANPP stability by influencing species diversity, species asynchrony and the stabilities of the dominant species, population and grasses. Among these factors, species asynchrony had the largest total effect on ANPP stability, with standardized direct and indirect effects of 0.469 and 0.239, respectively (Fig. 6B).

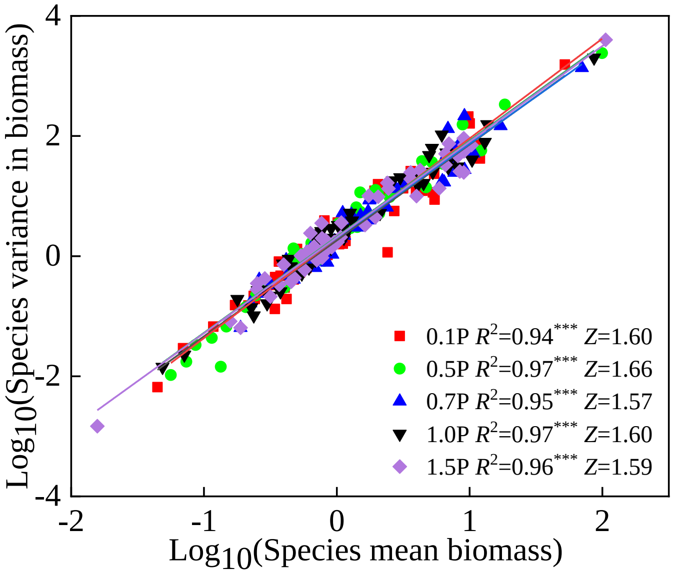

Figure 3 Relationships between the variance in ANPP and the mean of each species. *** denotes significance at $P < 0.001$.

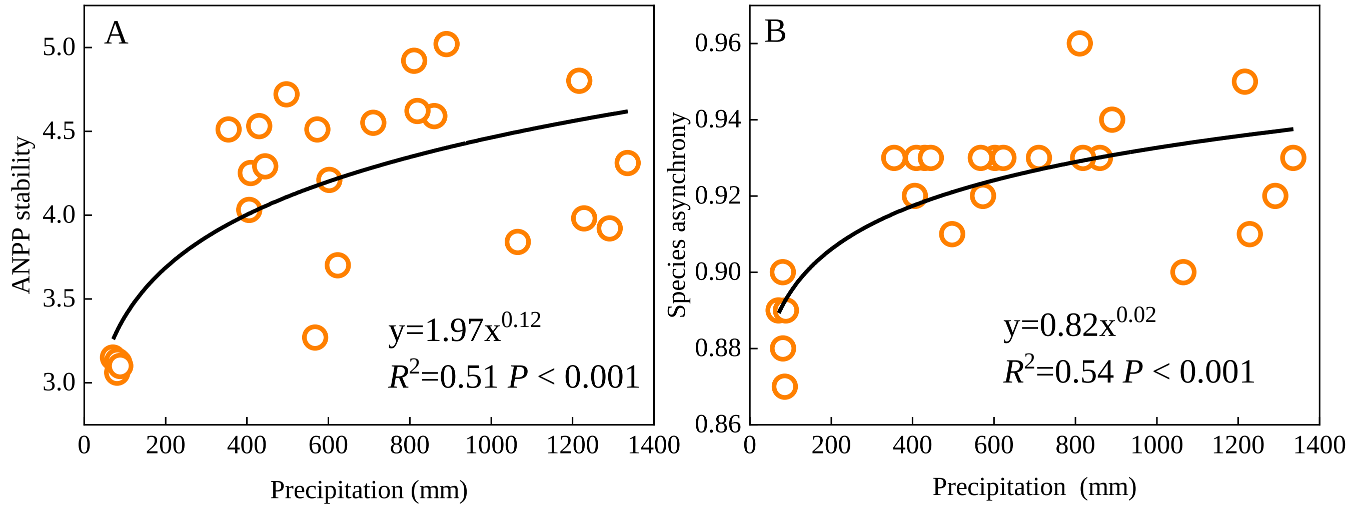

Figure 4 Relationships between ANPP stability (A), species asynchrony (B) and precipitation across the precipitation gradient.

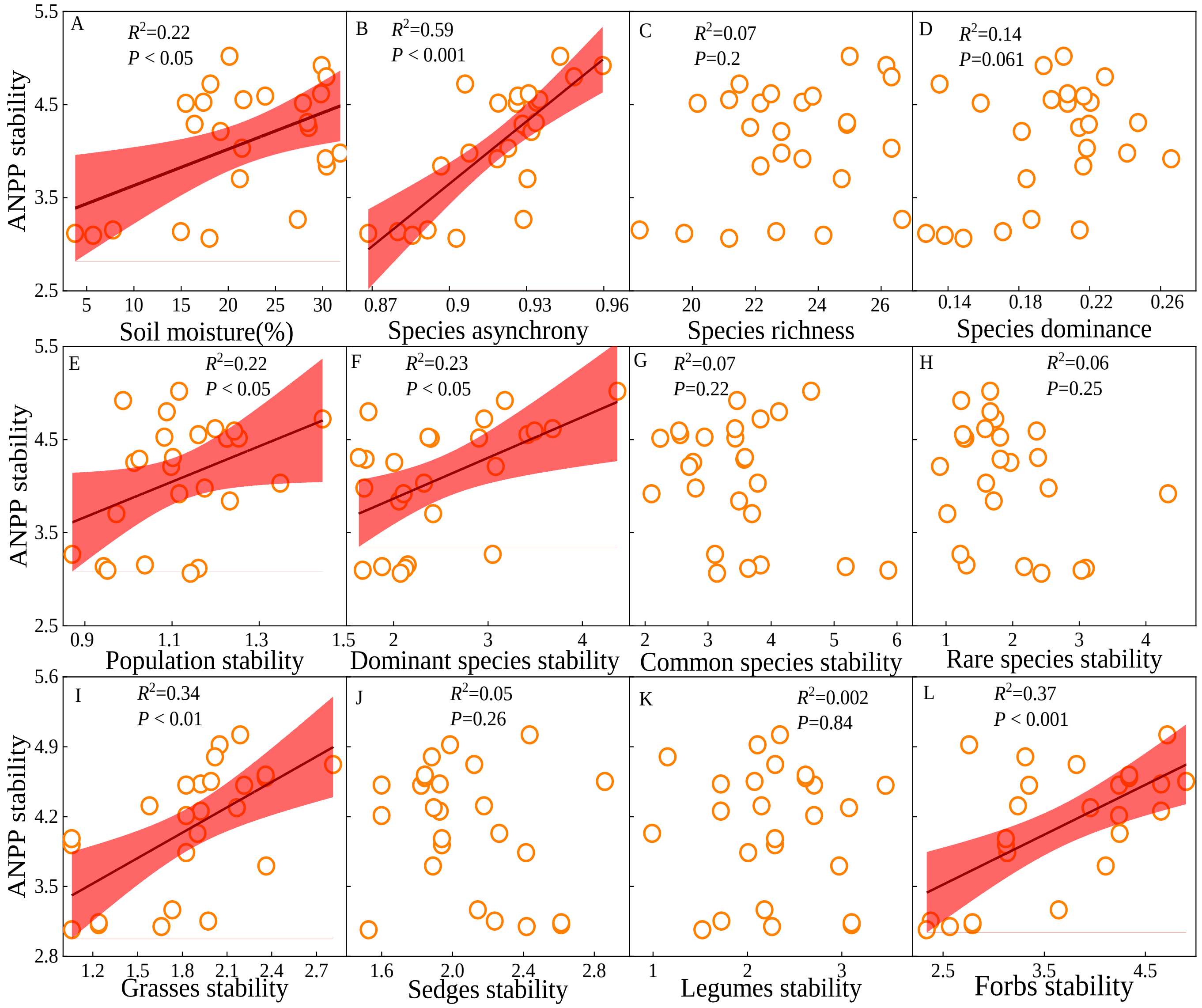

Figure 5 Relationships between ANPP stability and soil moisture, species richness, species dominance and the stabilities of the plant types. Pink shading represents 95% confidence intervals.

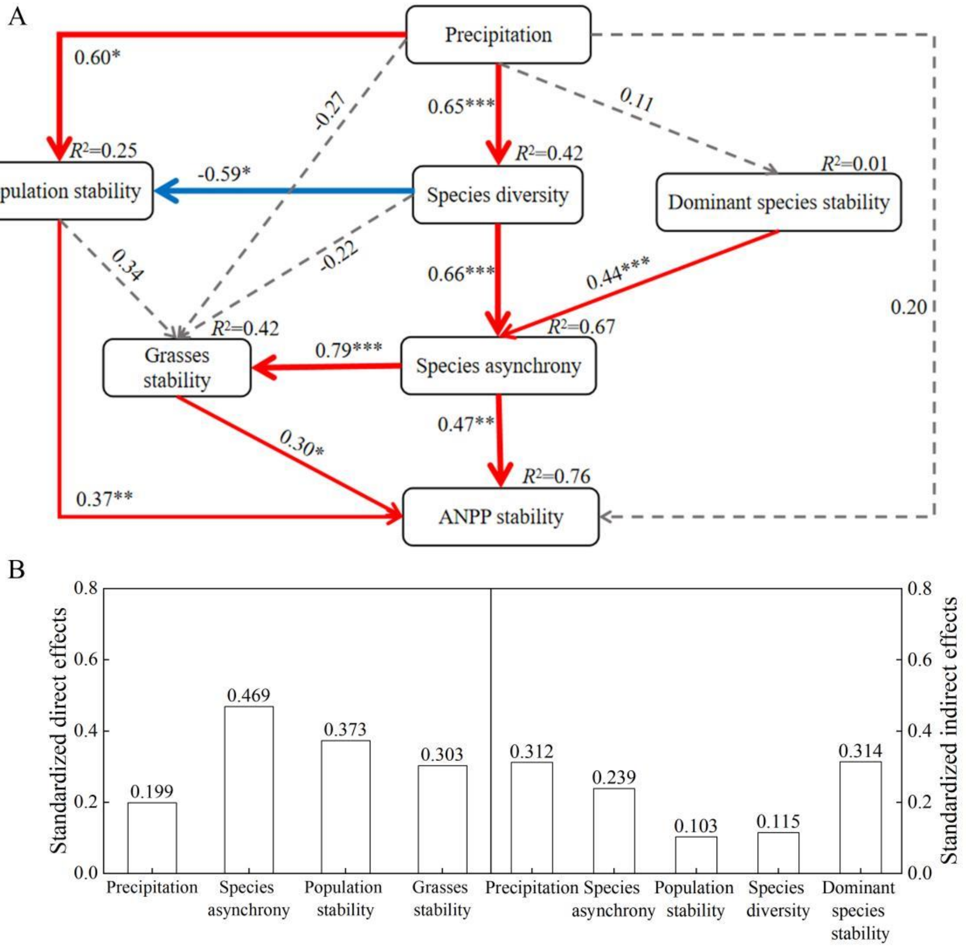

Figure 6 A Structural equation model showing the correlations between ANPP stability and other influencing factors. Line thickness indicates relative effect size. Solid red/blue lines indicate significant paths with positive/negative effects with standardized path coefficients on the lines, whereas dotted lines indicate nonsignificant paths. R^2 indicates the amount of variation explained. Goodness-of-fit statistics for the SEM are $\chi^2 = 2.965$, d.f. = 7, CHI/DF = 0.424, GFI = 0.967, AGFI = 0.868, RMSEA = 0.000 and $P = 0.888$. *, ** and *** represent significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. B represents the standard direct and indirect effects from the structural equation model for ANPP stability.

Discussion

3.1 Effect of precipitation on ANPP and its stability

The differences in ANPP and its stability in the alpine meadow under different rainfall treatments support our first and second hypotheses, i.e. the response of ANPP and its stability to changes in precipitation had a specific range, and ANPP and its stability significantly decreased when this range was exceeded. Aboveground biomass is an important indicator of the values of functions and services of grassland ecosystems (Yan Z Q, 2017), and its response to fluctuations in precipitation is very sensitive (Knapp A, 2001). We found that ANPP tended to decrease as rainfall decreased, even though ANPP did not differ significantly over the range of precipitation variation of 0.7P-1.5P. Possible reasons may be that (1) compensatory growth among the dominant species stabilized the primary productivity of the plant community (Fig. 1D), and (2) the plants may have adapted to drought stress by reducing stomatal conductance and the content or activity of photosynthetic enzymes, leading to a small reduction in biomass rather than an abrupt collapse of the ecosystem (Reddy, A, 2004). The significant reduction in ANPP at 0.1P, however, was mainly caused by a decrease in forb biomass (Fig. S2). Species in alpine-meadow ecosystems can be divided into four functional groups: grasses, sedges, legumes and forbs. More than 65% of the community biomass at our study site was derived from forbs. Forbs are more sensitive than grasses to changes in precipitation and are more susceptible to suppression by extreme drought, because they generally require more water to grow than do grasses (Knapp, 2001). A reduction in forb biomass thus led to a decrease in ANPP. In summary, the plants in this alpine meadow maintained the relative stability of ANPP by regulating their physiological activities or compensatory effects among functional groups within a specific range of variation in precipitation, but ANPP decreased significantly under severe drought stress, indicating that the response to ANPP to changes in precipitation had a specific threshold.

Similar to the variation in ANPP, ANPP stability did not vary significantly over the range of 0.5P-1.5P (288-1336 mm), indicating that normal interannual fluctuations in precipitation (508-996 mm) did not significantly affect the stability of this alpine-meadow ecosystem. Ma (2017) et al. found that ANPP stability in alpine meadows was independent of the effects of rainfall, which is partly consistent with our findings. The stability of ANPP was nevertheless significantly lower at 0.1P, mainly because the -50% and +50% rainfall treatments in the study by Ma (2017) et al. did not cover the 90% rainfall variability scenario of reduced rainfall. The temporal stability of ANPP is defined as the ratio of the temporal mean productivity of aboveground biomass of a community to its standard deviation (Lehman, 2000; Sasaki T, 2019). The reduction in ANPP stability at 0.1P in our study was therefore mainly due to a decrease in the temporal mean of biomass (Fig. S4) (Hautier, 2014). The temporal mean of ANPP represents the photosynthetic capacity of the plant community (Huang K, 2018), and the significant reduction in forbs at 0.1P likely decreased their photosynthetic capacity, leading to a significant reduction in ANPP and consequently to a reduction in ANPP stability. The study area has a semi-humid climate (mean annual precipitation of 747 mm), with interannual fluctuations in precipitation ranging from ±249 mm over the last 40 years. The range of variation of precipitation in the occasional years of extreme drought is also <50% of the annual precipitation. Some studies have found that precipitation on the Tibetan Plateau has recently tended to increase, and the average annual precipitation by 2100 may increase by 38 or 272 mm (Chen, 2013). ANPP in the region will therefore likely remain stable over most interannual periods under a scenario of future precipitation.

3.2 Effect of species diversity on ANPP stability

Field observations (Jiang L, 2009), experiments that directly manipulated diversity (Wright A J, 2015) and theoretical models (Cardinale B J, 2013) have all found that higher species diversity was associated with higher ANPP stability. Our study, however, found no direct correlation between species diversity and ANPP stability (Fig. 5C and D), although SEM indicated an indirect effect of species diversity via species asynchrony and population stability (Fig. 6).

Larger species pools are more likely to contain species that respond differently to environmental disturbances (Yachi, 1999), and different species may contribute uniquely to different functions to some extent at different times, so the relative importance of asynchronous responses to environmental disturbances increases with species diversity (Sasaki T, 2019). The asynchronous response of plant species to environmental fluctuations is an important potential mechanism by which species diversity affects the stability of community ANPP (Xu Z, 2015). Asynchrony among species is a major form of differentiation among temporal ecological niches (Isbell FL, 2009) and tends to promote ecosystem stability by compensatory growth (Bai Y, 2004; Song M, 2015). Our regression analyses and SEM indicated that species asynchrony positively influenced ANPP stability. The physiological and ecological characteristics of plants differ among functional groups, with grasses being taller and more upright and occupying a higher ecological niche than do short forbs and thus have a competitive advantage for light resources. Grasses and straight-rooted forbs extend their roots deeper into the soil and can absorb and use deeper soil moisture and nutrients, but the rhizomes of sedges are coiled and twisted, which is not conducive to underground extension, and their roots are mainly distributed in the subsurface soil layer where they can only use surface moisture and nutrients. This difference is consistent with classical ecological niche theory, whereby species in different ecological niches use different spatial resources under conditions of environmental stress (Hao A H, 2018), which also leads to decreases in the biomass of some species being compensated by increases in the biomass of other species, thus buffering temporal fluctuations in the biomass of the community as a whole. In addition to species asynchrony, population stability is another key factor affecting ANPP stability (Thibaut L M, 2013). SEM indicated that species diversity had a significant negative effect on population stability, with species diversity tending to reduce population stability in terrestrial ecosystems due to the competitive effects between species that affect ANPP at a higher level of species richness (Hector, A. 2010). From this perspective, interspecific competition supported by high species diversity reduces population stability but increases ANPP stability by increasing species asynchrony. The effect of species diversity on ANPP stability therefore depends on the extents to which it positively affects species asynchrony and negatively affects population stability. Reduced population stability in communities with higher species diversity may mask the positive effects of asynchronous dynamics (Sasaki T, 2011).

Species diversity can also affect the stability of ANPP by other mechanisms. One mechanism is the overyielding effect, although experiments controlling for species diversity have found that ANPP stability tended to increase with diversity (Cardinale B J, 2006), because experiments directly controlling for species diversity tend to produce an overyielding effect (relationship between positive diversity and productivity) that contributes to community stability (Gross K, 2014), but no consistent relationship between species diversity and productivity has been found in natural communities (Grace J B, 2007; Adler, P B. 2011). Most species that are lost or gained when the environment fluctuates are rare species, so they represent only a small fraction of the community biomass and contribute relatively little to ANPP stability (Ma Z, 2017; Ma F, 2020). Second, the mean-variance scale, as a pervasive mechanism of community stabilization, indicates that communities with higher diversity are more likely on average to reduce variation in their ecological characteristics (Doak DF, 1998), and the slope of the scale, Z, determines the strength of this mechanism (Tilman D, 1998). As Taylor's power law (Taylor L, 1961) summarizes, when $1< Z <2$, the mean-variance scale indicates that species diversity is expected to increase the stability of the community. Z for all rainfall treatments in our study ranged from 1 to 2 (Fig. 3), indicating that the positive effect of species diversity on the stability of ANPP was larger than its effect on the negative effect on population stability, which was also supported by the SEM results (Fig. 6). Species richness and dominance also did not vary significantly under each treatment compared to the control sample, but the stability of ANPP was significantly lower at 0.1P, indicating that species diversity and its statistical averages (mean-variance scale) contributed relatively little to the stability of ANPP. Species diversity was thus not a direct driving factor of ANPP stability in this alpine meadow but depended on the combined effect of species asynchrony and population stability to affect the stability of ANPP.

3.3 Effect of dominant-species stability on ANPP stability

Dominant species are the main contributors to ecosystem functions and services (Yang Z L, 2017). The biomass-ratio hypothesis suggests that ecosystem functions, including stability, are mostly controlled by the dynamics of the dominant species in the short term, and nondominant plants may regulate community stability by complementing the dominant species (Grime J P, 1998). The dominant species in our study accounted for only 2-12% of community species richness, but their biomass contributed 42-60% of the community biomass. An increasing number of studies have reported that community stability is often positively correlated with the stability of the dominant species (Wilsey B J, 2013; Song M, 2015), and our regression analysis confirmed this phenomenon (Fig. 2F), indicating that the contribution of different species to stability in alpine-meadow ecosystems depends strongly their ecological niches. Our study also found that the stability of the dominant species did not directly influence ANPP stability but indirectly influenced it by species asynchrony (Fig. 6). The dominance of species within the community changed accordingly with changes in rainfall (Fig. 1D), with some species that were drought-tolerant or insensitive to changes in moisture, such as *E. nutans*, *C. enervis* and *A. rivularis*, increasing in abundance and becoming dominant under the treatments of rainfall reduction, and *S. nigrescens* contributed >40% of the community biomass under the treatment with increased rainfall. Increases in the biomass of dominant species can compensate to some extent for the decrease in biomass of other species and can maintain the stability of community biomass, but increased biomass can also increase competition among species for available resources, which will lead to a reduction in their own stability and the stability of ANPP (Gross K, 2014). Our study also found that ANPP stability was not correlated with the stabilities of the common and rare species (Fig. 6G and

H). Rare species are the most diverse component of communities but are often at greater risk of extinction due to their small population sizes (Smith M D, 2003). Increased diversity due to increased numbers of rare species may therefore have little practical effect on ANPP stability in alpine-meadow ecosystems. Dominant species generally indirectly regulate the stability of ANPP by influencing the asynchrony of species in the community; the positive relationship between dominant-species stability and the stability of ANPP supports the biomass-ratio hypothesis and highlights the importance of dominant species in the productive function of alpine-meadow ecosystems on the Tibetan Plateau. Our results supported our third hypothesis, that species diversity and dominant-species stability would dominate the stability of ANPP in the alpine meadow under changes in rainfall.

Conclusion

We used six years of field experiments controlling rainfall to investigate the response and regulatory mechanism of the stability of a plant community to variation in rainfall in an alpine-meadow ecosystem. Our results indicated that ANPP stability was highly resilient to ±50% variation in rainfall, but extreme drought significantly reduced stability. These results suggest that plant communities in alpine-meadow ecosystems in semi-humid zones can maintain the relative stability of production functions under normal interannual fluctuations in precipitation and the small probability of abnormal future changes in precipitation, which is crucial for the development of local livestock farming and the sustainability of ecosystem services. Further analysis suggested that the stability of ANPP was mainly determined by a combination of asynchronous dynamics among coexisting species and population stability and that asynchronous dynamics among species and population stability were driven by a combination of species alpha diversity and the stability of the dominant species. These findings are important for guiding the conservation of arid meadow ecosystems and the practices of ecological restoration of degraded grassland on the Tibetan Plateau.

References

IPCC (2013). Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, UK. 1535. https://doi.org/10.1017/CBO9781107415324

Beier, Beierkuhnlein, Wohlgemuth, Penuelas & Emmett. (2012). Precipitation manipulation experiments - challenges and recommendations for the future. ECOL LETT, 15(8), 899-911. https://doi.org/10.1111/j.1461-0248.2012.01793.x

Wu, Z. T., Dijkstra P, Koch, G. W., & Josep Peñuelas. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology, 17(2), 927-942. https://doi.org/10.1111/j.1365-2486.2010.02302.x

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., & Lehman, C., (2001). Diversity and productivity in a long-term grassland experiment. Science, 294, 843–845. https://doi.org/10.1126/science.1060391

Isbell, F., Craven, D., Connolly, J., et al. (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature. https://doi.org/10.1038/nature15374

Oliver, T. H. et al. (2015). Declining resilience of ecosystem functions under biodiversity loss. Nat. Commun.6, 10122 https://doi.org/10.1038/ncomms10122

Knapp A K, Claus B, DD Briske, et al. (2008). Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems. Bioscience, 58(9), 811-821.https://doi.org/10.1641/B580908

Guo, Q., Hu, Z., Li, S., Yu, G., Sun, X., Zhang, L. & Zhao, W. (2015). Contrasting responses of gross primary productivity to precipitation events in a water-limited and a temperature-limited grassland ecosystem. Agricultural and Forest Meteorology, 214, 169–177. https://doi.org/10.1016/j.agrformet.2015.08.251

Niu, S., Fu, Z., Luo, Y., Stoy, P. C., Keenan, T. F., Poulter, B. & Yu, G. (2017). Interannual variability of ecosystem carbon exchange: From observation to prediction. Global Ecology and Biogeography, 26(11), 1225–1237. https://doi.org/10.1111/geb.12633

Isbell FL, Polley HW, Wilsey BJ. (2009). Biodiversity, productivity and the temporal stability of productivity: patterns and processes. Ecology Letters, 12, 443–451. https://doi.org/10.1111/j.1461-0248.2009.01299.x

Hallett, L. M., Hsu, J. S., Cleland, E. E., Collins, S. L., Dickson, T. L., Farrer, E. C. & Suding, K. N. (2014). Biotic mechanisms of community stability shift along a precipitation gradient. Ecology, 95(6), 1693–1700. https://doi.org/10.1890/13-0895.1

Xu, Z., Ren, H., Li, M., van Ruijven, J., Han, X., Wan, S. & Jiang, L. (2015). Environmental changes drive the temporal stability of semi-arid natural grasslands through altering species asynchrony. Journal of Ecology, 103, 1308–1316. https://doi.org/10.1111/1365-2745.12441

Hooper D. U. et al. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75, 3–35. https://doi.org/10.1890/04-0922

Grman E, Lau J A, Schoolmaster, D. R. & Gross, K. L. (2010). Mechanisms contributing to stability in ecosystem function depend on the environmental context.Ecol. Lett.13, 1400–1410. https://doi.org/10.1111/j.1461-0248.2010.01533.x

Loreau M, de Mazancourt C. (2013). Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecol Lett 16(Suppl 1), 106–15. https://doi.org/10.1111/ele.12073

Hautier, Y. et al. (2014). Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature508, 521–525. https://doi.org/10.1038/nature13014

Hautier, Y. et al. (2015). Anthropogenic environmental changes affect ecosystem stability via biodiversity. Science348, 336–340. https://doi.org/10.1126/science.aaa1788

Campbell V, Murphy G, Romanuk T N. (2011). Experimental design and the outcome and interpretation of diversity–stability relations. Oikos, 120(3), 399–408. https://doi.org/10.1111/j.1600-0706.2010.18768.x

Gross K, Cardinale B J, Fox J W, et al. (2014). Species Richness and the Temporal Stability of Biomass Production: A New Analysis of Recent Biodiversity Experiments. American Naturalist, 183(1), 1-12. https://doi.org/10.1086/673915

Bai Y, Han X, Wu J, et al. (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature, 431(7005), 181-184. https://doi.org/

Allan E, Weisser W, Weigelt A, et al. (2011). More diverse plant communities have higher functioning over time due to turnover in complementary dominant species. Proceedings of the National Academy of Sciences, 108(41). https://doi.org/10.1073/pnas.1104015108

Tilman D. (1999). The ecological consequences of changes in biodiversity: A search for general principles. Ecology, 80, 1455–1474. https://doi.org/10.2307/176540

Loreau M, Hector A. (2001). Partitioning selection and complementarity in biodiversity experiments. Nature, 412 (6842), 72-76. https://doi.org/10.1038/35083573

Tilman, David, Reich, et al. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature, https://doi.org/10.1038/nature04742

Cardinale, BJ, Wrigh, et al. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity[J]. PROC NAT ACAD SCI USA, 104(46)(-), 18123-18128. https://doi.org/10.1073/pnas.0709069104

Loreau M, de Mazancourt C. (2008). Species synchrony and its drivers: neutral and nonneutral community dynamics in fluctuating environments. The American Naturalist, 172, 48–66. https://doi.org/10.1086/589746

Polley H W, Wilsey B J, De Rner J D. (2003). Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures? Ecology Letters, 6(3), 248-256. https://doi.org/10.1046/j.1461-0248.2003.00422.x

Cottingham K L, Brown B L, Lennon J T. (2001). Biodiversity may regulate the temporal variability of ecological systems. Ecology Letters, 4(1). https://doi.org/10.1046/j.1461-0248.2001.00189.x

Ruijven V, Berendse. (2007). Contrasting effects of diversity on the temporal stability of plant populations. OIKOS, https://doi.org/10.1111/j.0030-1299.2007.16005.x

Hector, A., Hautier, Y. , Saner, P. , Wacker, L., & Dimitrakopoulos, P.. (2010). General stabilizing effects of plant diversity on grassland productivity at multiple sites through population asynchrony and overyielding. Ecology, 91. https://doi.org/10.1890/09-1162

Grime J P. (1998). Benefits of Plant Diversity to Ecosystems: Immediate, Filter and Founder Effects. Journal of Ecology, 86(6), 902-910. https://doi.org/10.1046/j.1365-2745.1998.00306.x

Zelikova T J, Blumenthal D M, Williams D G, et al. (2014). Long-term exposure to elevated CO_2 enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie. Proceedings of the National Academy of Sciences, 111(43). https://doi.org/10.1073/pnas.1414659111

Ma Z, Liu H, Mi Z, et al. (2017). Climate warming reduces the temporal stability of plant community biomass production. Nature Communications, https://doi.org/10.1038/ncomms15378

Sasaki T, Lauenroth W K. (2011). Dominant species, rather than diversity, regulates temporal stability of plant communities. Oecologia, 166(3), 761-768. https://doi.org/10.2307/41499879

Yang Z L, Qian, et al. (2017). Daytime warming lowers community temporal stability by reducing the abundance of dominant, stable species. Global Change Biology, . https://doi.org/10.1111/gcb.13391

Polley H W, Wilsey B J, Derner J D. (2007). Dominant species constrain effects of species diversity on temporal variability in biomass production of tallgrass prairie. Oikos, 116(12). https://doi.org/10.1111/j.2007.0030-1299.16080.x

Zhang R H, Su F G, Jiang Z H, et al. (2015). An overview of projected climate and environmental changes across the Tibetan Plateau in the 21st century (in Chinese). Chin Sci Bull, 60, 3036–3047. https://doi.org/10.1360/n972014-01296

Yang, Y. H. (2018). Ecological processes in alpine ecosystems under changing environment. Chinese Journal of Plant Ecology, 42, 1–5. https://doi.org/10.17521/cjpe.2018.0048

Yang G, Chen H, Wu N, et al. (2014). Effects of soil warming, rainfall reduction and water table level on CH_4 emissions from the Zoige peatland in China. Soil biology & biochemistry, 78, 83-89. https://doi.org/10.1016/j.soilbio.2014.07.013

Chen, H. et al. (2013). The impacts of climate change and human activities on biogeochemical cycles on the Qinghai - Tibetan Plateau. Glob. Change Biol.19, 2940–2955. https://doi.org/10.1111/gcb.12277

Liu H, Mi Z, Lin L, et al. (2018). Shifting plant species composition in response to climate change stabilizes grassland primary production. Proc Natl Acad Sci U S A, 115(16):4051-4056. https://doi.org/10.1073/pnas.1700299114

Zhang F, Quan Q, Song B, et al. (2017). Net primary productivity and its partitioning in response to precipitation gradient in an alpine meadow. Rep, 7(1), 15193. https://doi.org/10.1038/s41598-017-15580-6

Chai J L, Xu C L, Zhang D G, Xiao H, Pan T T & Yu X J. (2019). Effects of simulated trampling and rainfall on soil nutrients and enzyme activity in an alpine meadow. Acta Ecologica Sinica, 39(1), 333-344. https://doi.org/10.5846/stxb201801050029

Schuster MJ (2016). Increased rainfall variability and N addition accelerate litter decomposition in a restored prairie. Oecologia, 180, 645–655. https://doi.org/10.1007/s00442-015-3396-1

Ma F, Zhang F, Quan Q, et al. (2020). Common Species Stability and Species Asynchrony Rather than Richness Determine Ecosystem Stability Under Nitrogen Enrichment. Ecosystems, (Suppl 1). https://doi.org/10.1007/s10021-020-00543-2

Wang H D, Zhang L L, Zhu Z H. (2013). Effects of clipping and fertilizing on the relationships between species diversity and ecosystem functioning and mechanisms of community stability in alpine meadow. Chinese Journal of Plant Ecology, 37 (4), 279–295. https://doi.org/10.3724/sp.j.1258.2013.00028

Zi H B, Hu L, Wang C T, et al. (2018). Responses of soil bacterial community and enzyme activity to experimental warming of an alpine meadow. European Journal of Soil Science, 69(3), 429-438. https://doi.org/10.1111/ejss.12547

Yang, D. C., Hu, L., Song, X. Y. & Wang, C. T. (2021). Effects of changing precipitation on litter quality and decomposition of different plant functional groups in an alpine meadow. Chinese Journal of Plant Ecology, 45(12), 1314-1328. https://doi.org/10.17521/cjpe.2021.0211

Tang, G., Hu, L., Song, X. Y., Li, X. Z., Wang, C. T. (2022). Response of plant roots in different diameter classes to changing precipitation in an alpine meadow. Acta Ecologica Sinica, 42(15), **-** . https://doi.org/10.5846/stxb2021042610 96

Mariotte, P., Vandenberghe, C., Kardol, P., Hagedorn, F. & Buttler, A. (2013). Subordinate plant species enhance community resistance against drought in semi-natural grasslands. J. Ecol. 101, 763–773. https://doi.org/

Song, M. H., Zong, N., Jiang, J., et al. (2019). Nutrient-induced shifts of dominant species reduce ecosystem stability via increases in species synchrony and population variability. The Science of the Total Environment, 692 (Nov. 20), 441 -449. https://doi.org/10.1016/j.scitotenv.2019.07.266

Dong, S. K., Tang, L., Zhang, X. F., Liu, S. L., Liu, Q. R., Su, X. K., Zhang, Y., Wu, X. Y., Zhao, Z. Z., Li, Y., & Sha W. (2017). Relationship between plant species diversity and functional diversity in alpine grasslands. Acta Ecologica Sinica, 37(5), 1472-1483. https://doi.org/ 10.5846/stxb201509281981

Leps J. (2004). Variability in population and community biomass in a grassland community affected by environmental productivity and diversity. OIKOS 107, 64–71. https://doi.org/

Schnabel Florian, Liu Xiaojuan, et al. (2021). Species richness stabilizes productivity via asynchrony and drought-tolerance diversity in a large-scale tree biodiversity experiment. Science advances, 7(51): https://doi.org/10.1126/SCIAD V.ABK1643

Taylor L R. (1961). Aggregation, Variance and the Mean. Nature, 189(4766):732-735. https://doi.org/10.1038/189732a0

Yan, Z. Q., Qi, Y. C., P, Q., et al. (2017). Advances in the Effects of Simulated Precipitation and Nitrogen Deposition on Grassland Biomass. Acta Agrestia sinica, 25(6), 1165-1170. https://doi.org/10.11733/j.issn.1007-04 35.2007.06.001

Knapp, A. K. (2001). Variation Among Biomes in Temporal Dynamics of Above-ground Primary Production. Science, 291(5503), 481-484. https://doi.org/10.1126/science.291.5503.481

Reddy, A. R., Chaitanya, K. V. & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology 161, 1189–1202. https://doi.org/10.1016/j.jplph.2004.01.013

Knapp, A. K., Briggs, J. M., Koelliker, J. K. (2001). Frequency and Extent of Water Limitation to Primary Production in a Mesic Temperate Grassland. Ecosystems, 4(1), 19-28. https://doi.org/10.1007/s100210000057

Lehman, CL, Tilman, et al. (2000). Biodiversity, stability, and productivity in competitive communities. The American Naturalist, 156(5):534-552. https://doi.org/10.2307/3079056

Sasaki T, Lu X, Hirota M, et al. (2019). Species asynchrony and response diversity determine multifunctional stability of natural grasslands. Journal of Ecology, 107(4). https://doi.org/10.1111/1365-2745.13151

Huang K, Xia J, Wang Y, et al. (2018). Enhanced peak growth of global vegetation and its key mechanisms. Nature Ecology & Evolution, 2. https://doi.org/10.1038/s41559-018-0714-0

Jiang L, Pu Z. (2009). Different effects of species diversity on temporal stability in single-trophic and multitrophic communities. The American Naturalist 174(5), 651–659. https://doi.org/10.1086/605961

Wright A J, Ebeling A, Kroon H D, et al. (2015). Flooding disturbances increase resource availability and productivity but reduce stability in diverse plant communities. Nature Communications. https://doi.org/10.1038/ncomms7092

Cardinale B J, Gross K, Fritschie K, et al. (2013). Biodiversity simultaneously enhances the production and stability of community biomass, but the effects are independent. Ecology, 94(8). https://doi.org/10.1890/12-1334.1

Song M, Yu F. (2015). Reduced compensatory effects explain the nitrogen-mediated reduction in stability of an alpine meadow on the Tibetan Plateau. New Phytologist, 207(1), 70-77. https://doi.org/10.1111/nph.13329

Hao A H, Xue X, Peng F, You Q G, Liao J, Duan H C, Huang C H, Dong S Y. (2020). Different vegetation and soil degradation characteristics of a typical grassland in the Qinghai-Tibetan Plateau. Acta Ecologica Sinica, 40(3), 964-975. https://doi.org/10.5846/stxb201809162019

Yachi, Shigeo, Loreau, et al. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proceedings of the National Academy of Sciences of the United States of America. https://doi.org/10.1073/pnas.96.4.1463

Thibaut L M, Connolly S R, He F. (2013). Understanding diversity–stability relationships: towards a unified model of portfolio effects. Ecology Letters, 16(2). https://doi.org/10.1111/ele.12019

Cardinale B J, Srivastava D S, Duffy J E, et al. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature, 443(7114):989-992.

Grace J B, Anderson T M, MD Smith, et al. (2007). Does species diversity limit productivity in natural grassland communities? Ecology Letters, 10(8).

Adler, P. B., Seabloom, E.W., Borer, E.T., Hillebrand, H., Hautier, Y., Hector, A. et al. (2011) Productivity is a poor predictor of plant species richness. Science, 333, 1750–1753. https://doi.org/10.2307/23060307

Doak DF, Bigger D, Harding EK, Marvier MA, O’Malley RE & Thomson D. (1998). The statistical inevitability of stability - diversity relationships in community ecology. Am Naturalist, 151, 264–276. https://doi.org/10.1086/286117

Tilman D, Lehman C L, Bristow C E. (1998). Diversity-stability relationships: statistical inevitability or ecological consequence? American Naturalist, 151(3), 277-282. https://doi.org/10.1086/286118

Wilsey Brian J, Daneshgar Pedram P, Hofmockel Kirsten & Polley H Wayne. (2013). Invaded grassland communities have altered stability-maintenance mechanisms but equal stability compared to native communities. Ecology Letters, 17(1), 92-100. https://doi.org/10.1111/ele.12213

Smith M D, Knapp A K. (2003). Dominant species maintain ecosystem function with non-random species loss. Ecology Letters, 6(6), 509-517. https://doi.org/10.1046/j.1461-0248.2003.00454.x