Timing outweighs magnitude of rainfall in shaping population dynamics of a small mammal species in steppe grassland

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Climate change–induced shifts in species phenology differ widely across trophic levels, which may lead to consumer–resource mismatches with cascading population and ecosystem consequences. Here, we examined the effects of different rainfall patterns (i.e., timing and amount) on the phenological asynchrony of population of a generalist herbivore and their food sources in semiarid steppe grassland in Inner Mongolia. We conducted a 10-y (2010 to 2019) rainfall manipulation experiment in 12 0.48-ha field enclosures and found that moderate rainfall increases during the early rather than late growing season advanced the timing of peak reproduction and drove marked increases in population size through increasing the biomass of preferred plant species. By contrast, greatly increased rainfall produced no further increases in vole population growth due to the potential negative effect of the flooding of burrows. The increases in vole population size were more coupled with increased reproduction of overwintered voles and increased body mass of young-of-year than with better survival. Our results provide experimental evidence for the fitness consequences of phenological mismatches at the population level and highlight the importance of rainfall timing on the population dynamics of small herbivores in the steppe grassland environment.

climate variability | consumer–resource dynamics | phenology mismatch | rainfall pattern | steppe grassland

The Earth is facing a great challenge from accelerated climate change. The global surface air temperature has increased by about 1° during the past century and is projected to exceed 1.5 to 2 °C by the end of the 21st century (1). Climate change has caused profound impacts on the Earth's ecosystems, such as local extinctions (2), range shifts (3), and population fluctuations (4, 5) of many species. Many organisms have advanced the timing of phenological events in response to climate warming, such as earlier leaf-out in plants, earlier emergence of insects, or accelerated egg hatching dates for birds (6). For consumers, phenological events are timed to match peak food resources for breeding; however, the direction of consumer's phenological response to climate change may differ from the response of species occupying lower trophic levels, leading to asynchrony between resources and consumers (7, 8). With respect to climate change, numerous studies have focused on the impact of temperature and its role in driving phenological asynchrony (9–11) since this is especially critical for species population dynamics and ecosystem functioning. However, relatively little is known about how rainfall mediates asynchrony between resources and consumers and its potential demographic consequences, especially in arid environments.

Shifts in rainfall patterns have been greatly affected by climate warming (12) and play a key role in regulating vertebrate population dynamics (13), the species composition of communities, and ecosystem functions and services (14). Both the timing and the amount of rainfall are recognized as distinct but major components that synergistically influence the timing of vegetation phenology, e.g., the timing of plant germination and seed ripening (15, 16). However, it remains unclear whether changes in the timing or the amount of rainfall play the more dominant role in the processes of phenological asynchrony between interacting species despite their distinct effects on aboveground annual net primary productivity (17). It is therefore important to disentangle the independent effects of rainfall timing and amount if we are to predict responses of species' populations and ecosystems to global climate change.

Among small herbivores, rainfall is well recognized to induce a bottom-up increase in abundance via increasing food availability, as observed in *Phyllotis darwini* and *Octodon degus* in South America (18, 19), *Pseudomys hermannsburgensis* and *Mus domesticus* in Australia (20, 21), *Spermophilus dauricus* (22) and *Cricetulus barabensis* (23) in East Asia, *Dipodomys*

Significance

Disentangling the effects of rainfall timing and magnitude on animal and plant populations is essential to reveal the biological consequence of diverse climate change scenarios around the world. We conducted a 10-y, large-scale, manipulative experiment to examine the bottom-up effects of changes in rainfall regime on the population dynamics of Brandt's voles in the steppe grassland of Inner Mongolia, China. We found that a moderate rainfall increase during the early growing season could produce marked increases in vole population size by increasing the biomass of preferred plant species, whereas large increases in rainfall produced no additional increase in vole population growth. Our study highlights the importance of rainfall magnitude and timing on the nonlinear population dynamics of herbivores.

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merriami in North America (24), and *Mastomys natalensis* in Africa (25). However, these observations are all based on the correlation between rodent abundance and precipitation; the mechanism underlying the bottom-up effects of precipitation on rodents through plant productivity is often assumed but has been rarely investigated by manipulative experiments. While valuable in their own right, most previous studies have been unable to elucidate fully the role of rainfall as a potential proximate cue in regulating phenology. In natural environments, many biotic factors (e.g., predation and interspecific competition) and abiotic factors (e.g., flooding of burrows) may interact to influence how phenological processes can affect population dynamics. To understand the effects of rainfall on the role of phenological asynchrony in the population dynamics of target species, including the effects of rainfall amount and timing, it is therefore necessary to exclude or control for confounding factors. Conducting more tightly controlled manipulative experiments is a requirement when assessing the fitness consequences of phenological asynchrony (6, 7), although it is very challenging for small rodents owing to the need for large field enclosures that prevent immigration/emigration of individuals and impacts by predators.

We conducted a 10-y, large-scale, manipulative experiment to examine the bottom-up effects of changes in rainfall regime (including timing and amount; *SI Appendix*, Fig. S1) on the phenological asynchrony between plants and herbivores, demographic parameters, and population dynamics of Brandt's voles *Lasiopodomys brandtii*. In our study region in Inner Mongolia, an increase in annual rainfall, especially during the early growing season, can markedly enhance annual net primary productivity (26), with more rain increasing the biomass of rye grass *Leymus chinensis* (27, 28), a major and favored food source for Brandt's voles (29). Additional rainfall in the early growing season can provide a match between the peak food resources and peak food requirements of young voles. Therefore, we hypothesized that rainfall would change the population density of voles by mediating the timing and peak amount of preferred foods and that rainfall timing (in the early growing season) would be of vital importance in triggering population increases, or outbreaks, of voles in arid steppe grassland.

Results

Impacts on Food Resource and Population Density. We captured and marked a total of 18,452 Brandt's voles from 2010 to 2019. The population density of voles fluctuated dramatically between years (ranging from 59 to 667 voles/ha). Rainfall increases produced various effects on the food resources and population densities of Brandt's voles depending upon the timing and amount of increased rainfall (Fig. 1*A*). In Phase 1 (2010 to 2015), the experimentally increased amount of rainfall was 50 and 100 mm (averaging 18 and 36% increase compared to the natural rainfall amount; termed the R50 and R100 treatments, respectively; *SI Appendix*, Table S1) and was evenly distributed throughout the growing season (*SI Appendix*, Table S1). We found that allocating 50 and 100 mm evenly through the growing season to enclosures had no significant effect on either the biomass of preferred plant species or the population abundance of voles (Figs. 1*A* and 2*A* and *SI Appendix*, Table S2).

In Phase 2 (2016 to 2018), we increased the amount of added rainfall to 130 and 260 mm (averaging 56 and 112% increase compared to the natural rainfall amount; termed the ER130 and ER260 treatments, respectively; *SI Appendix*, Table S1) and this time allocated more rainfall (61.5% of increased rain) to the early growing season (May to June; *SI Appendix*, Fig. S1). As compared to the control group, the ER130 treatment displayed significant increases in both the biomass of preferred plant species (all $P < 0.05$; Fig. 2*A*) and population density for all 3 y (all $P < 0.05$; Fig. 1*A* and *SI Appendix*, Table S2), whereas the ER260 treatment exhibited a substantial increase in the biomass of preferred plant species and vole density only in 2017 (both $P < 0.05$).

In Phase 3 (2019), we fixed the total amount of added rainfall to 130 mm and allocated most water (61.5%) to the early growing season (ER130 treatment) or to the late growing season (LR130 treatment). This rainfall increase in ER130 treatment and LR130 treatment significantly increased both the biomass of the vole's preferred plant species by 570 and 280%, respectively, and vole population density by 62.2 and 37.7%, respectively, compared with the control group (*SI Appendix*, Table S2). Furthermore, the biomass of preferred plant species and population density of voles in the ER130 treatment were significantly higher than those in the LR130 treatment (both $P < 0.05$; Figs. 1*A* and 2*A*).

Impacts on Reproduction and Body Growth. The response of reproduction and body growth of voles to increased rainfall depended on the rainfall treatment schedule (Fig. 1 *B* and *C*). In Phase 1, there was no difference in either the total number of recruits or body mass of young-of-year (YOY) between the control, R50, and R100 treatment groups. In Phase 2, the total number of recruits in 2016, 2017, and 2018 increased significantly by 71.8, 87, and 167%, respectively, in the ER130 treatment compared with the control group (all $P < 0.05$), while numbers in the ER260 treatment increased significantly only in 2017 ($P = 0.004$). Voles also showed greater body mass in the ER130 group compared with the control group in October over all 3 y (all $P < 0.05$), while those in the ER260 treatment showed increased body mass only in 2018 ($P = 0.02$). In Phase 3, the total number of voles recruited and their mean body mass were significantly greater in both the ER130 and LR130 groups compared with the control group (Fig. 1 *B* and *C*; all $P < 0.05$). There was no difference in the total number of voles recruited or body mass between the ER130 and LR130 treatment groups (both $P > 0.05$).

Overall, mass recruitment of *L. brandtii* occurred between the beginning of June and the end of August, with a pronounced peak at the beginning of July. In Phase 1, adding 50 or 100-mm rainfall into enclosures had no effect on the seasonal patterns of recruitment of *L. brandtii* (Fig. 2 *B* and *E*). In Phase 2 and 3, the constrained principal coordinates analysis revealed that rainfall manipulation had a significant impact on the recruitment patterns of Brandt's voles (Adonis permutation test; Phase 2: $F = 1.92$, $P = 0.023$; Phase 3: $F = 2.64$, $P = 0.015$), with an apparent discrimination of the seasonal distribution of recruitment between the control and ER130 groups (Fig. 2 *C* and *D*). More rainfall in the early plant growing season advanced the timing of reproduction of Brandt's vole, which was manifested by more than twofold increases in the percentage of recruits in June (Fig. 2 *F* and *G*).

Impacts on Recapture Probability and Survival Probability. For the overwintered cohort (i.e., founder population), the recapture probability was mostly affected by the timing of trapping sessions (*SI Appendix*, Fig. S2). The weak positive effect of rainfall increase on the recapture probability was detected only in 2010, 2012, and 2016. For YOY, the recapture probability was affected by the timing of trapping sessions (in 2013, 2017, and 2018) and the interaction between rainfall increase and the timing of trapping sessions (in 2010, 2011, 2014, 2016, and 2019; *SI Appendix*, Fig. S2). We found the independent effect of rainfall increase on the recapture probability of YOY only in 2012 and in 2015.

As with survival rate, Cormack-Jolly-Seber (CJS) models showed that rainfall increases positively affected the survival of

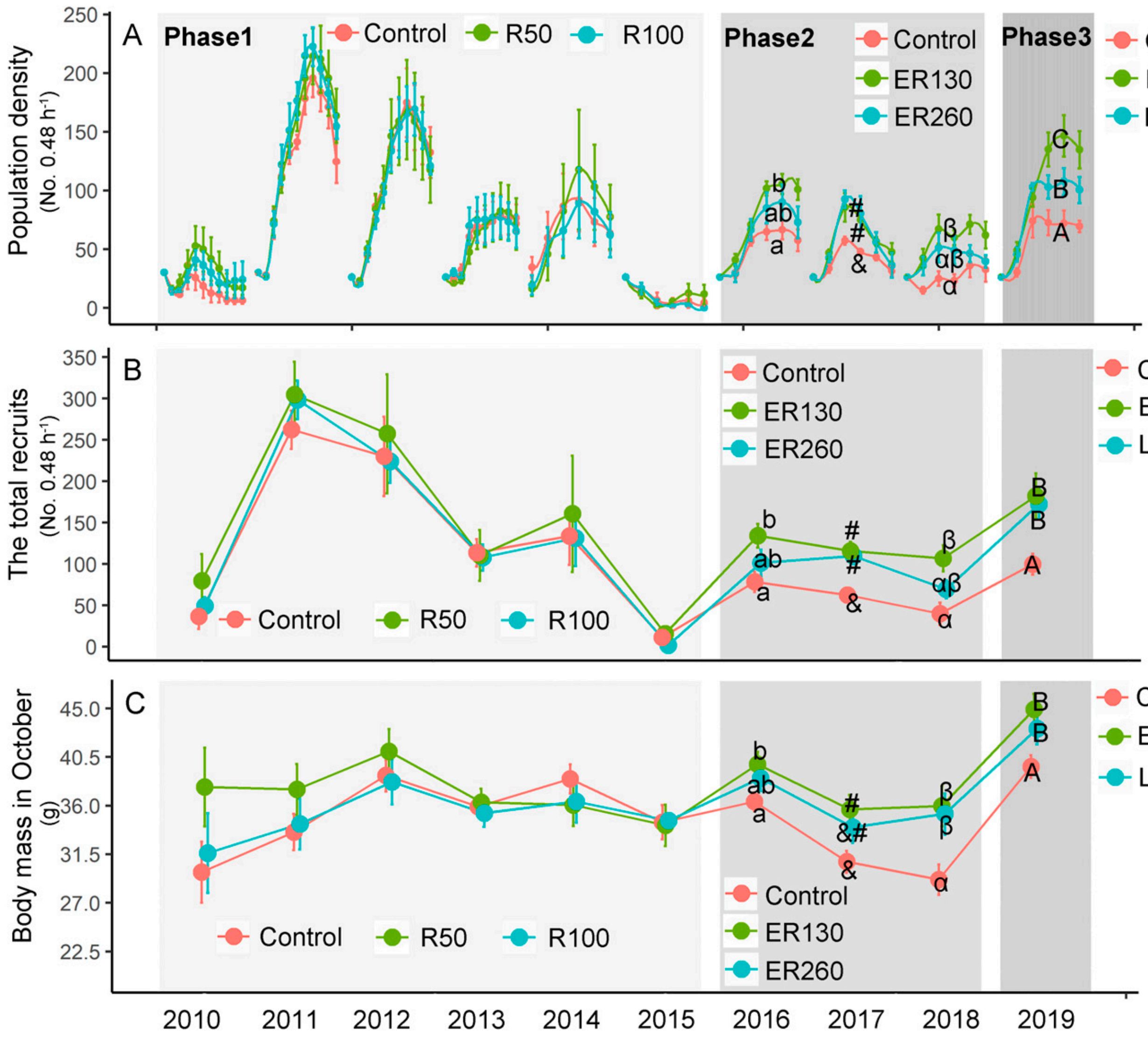

Fig. 1. Changes (means ± SE) in population density (*A*), numbers of recruits (*B*), and body mass (*C*) of yearling Brandt's voles between different rainfall increase scenarios from 2010 to 2019. For the results of pairwise comparisons, different types of symbol were used to report the statistical significance in different years (lowercase letters in 2016, special characters in 2017, greek letters in 2018, and uppercase letters in 2019). Different symbols denote significant difference between treatment groups ($P < 0.05$) in the same year. R50/R100: 50 mm/100 mm rainfall were added evenly throughout enclosures during the growing season; ER130/ER260: 130 mm/260 mm rainfall were added during the growing season but with more rainfall allocated to the early growing season (May to June); LR130: 130 mm rainfall were added during the growing season but with more rainfall allocated to the late growing season (July to September).

overwintered individual in 2010 and in 2019 (Fig. 3). In 2010, the survival rates in rainfall increase treatments (0.73 and 0.75 for the R50 and R100 treatments, respectively) were all higher than that in control group ($\varphi = 0.64$). In 2019, overwintered voles in ER130 had higher survival rates than voles in the control group in the late growing season. For most years, the survival rate of overwintered individuals was season-dependent, showing lower values in the autumn than in the spring (Fig. 3). In contrast, there was no significant independent effect of rainfall increase on yearling survival through the whole study using CJS models (Fig. 3 and *SI Appendix*, Table S5). The survival probability of YOY was mainly affected by the timing of trapping sessions, showing various response patterns in different years (Fig. 3).

Discussion

The timing of rainfall addition, instead of the magnitude of rainfall addition, played a more important role in facilitating the rate of increase of the Brandt's vole population. More rainfall addition in the early breeding season significantly increased reproduction and population density of voles by increasing the abundance of the voles' preferred food plant species. A doubling of rainfall addition did not produce any additional positive effect on the vole population. Many populations that increase due to rainfall treatment display synchronous increases in the numbers of recruits and body mass but little change in survival parameters. Here, we provide experimental evidence to confirm the bottom-up-regulation by rainfall on vole population that acts via increasing plant primary productivity and provide insights into some population dynamics consequences of interspecific phenological asynchrony under global climate change.

Climate change is shaping not only the annual amount of rainfall but also its seasonal distribution worldwide (12). Changes in the rainfall pattern are expected to induce a dramatic shift in animal fitness through bottom-up-regulation, especially in arid and semiarid regions where water availability is the major limiting factor for ecosystem structure and function (30).

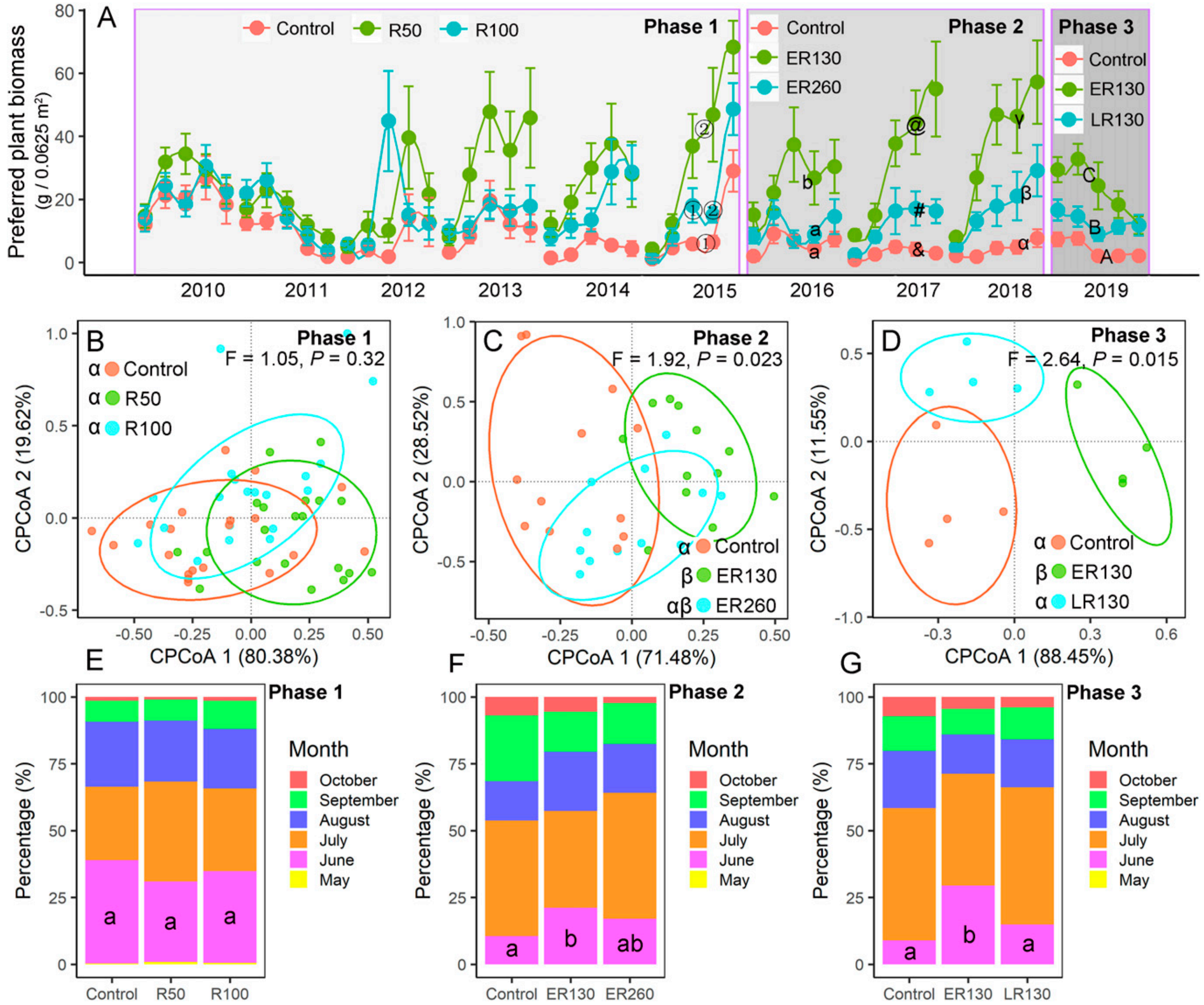

Fig. 2. Changes in food resources and seasonal patterns of recruits for Brandt's vole. (*A*) Changes in the preferred plant biomass between different rainfall increase scenarios from 2010 to 2019. (*B–D*) Constrained PCoA plot based on Bray-Curtis dissimilarity matrices of seasonal reproduction structure (i.e., the temporal distribution of recruits in the whole breeding season) during three phases of rainfall manipulation. (*E–G*) The composition of recruit percentages from May to October during three phases of rainfall manipulation. Different letters (a and b) assigned to purple bars indicate significant differences in the percentage of recruits in June between treatment groups ($P < 0.05$). For abbreviations, see Fig. 1.

The bottom-up effects of climate have been suggested to explain the population dynamics of small mammals (31, 32). Many studies show the significant associations between rodent abundance and precipitation in various global ecosystems using field monitoring of populations (18, 20, 24, 25), but the causal mechanism of climate change on small rodents—that is, through its effect on food plant species—has been rarely tested using manipulative experiments except for one by Carrier and Krebs (33). Association analysis based on monitoring data in the field is often affected by confounding factors such predators, competitors, or human disturbance (34), making it necessary to examine the causal effect of climate on animals by excluding these confounding factors. In this study, we excluded the impacts of predators and interspecific competitors by the use of experimental enclosures and conducted a three-phase rainfall manipulation experiment to examine the effects of rainfall alone. Our results indicate that the timing of rainfall addition plays a greater role than the rainfall magnitude in facilitating vole populations. In steppe grassland in Inner Mongolia, plants begin to sprout in the middle of April. Rain in May and June is then key for further plant growth, which could affect the first round of reproduction in voles and other subsequent demographic processes (35). In our study site, long-term meteorological data show that there is indeed an increasing trend for rainfall in May and June (*SI Appendix*, Fig. S3*A*), which augments the practical significance of our research in predicting rodent population responses to climate change.

Rainfall could exert bottom-up increases in plant productivity, affecting food resources and further influencing the population density of small mammals (30, 36). In our experiment, the plant community response to rainfall manipulations depended on both the timing and the magnitude of rainfall addition, which in turn determined the pattern of response of vole population. The high evaporation rate in semiarid regions could reduce the water-use efficiency of plant and weaken the positive effect of rainfall additions on food resources for voles, especially when the magnitude of rainfall addition is relatively low (37). Carrier and Krebs conducted a 5-y rainfall addition

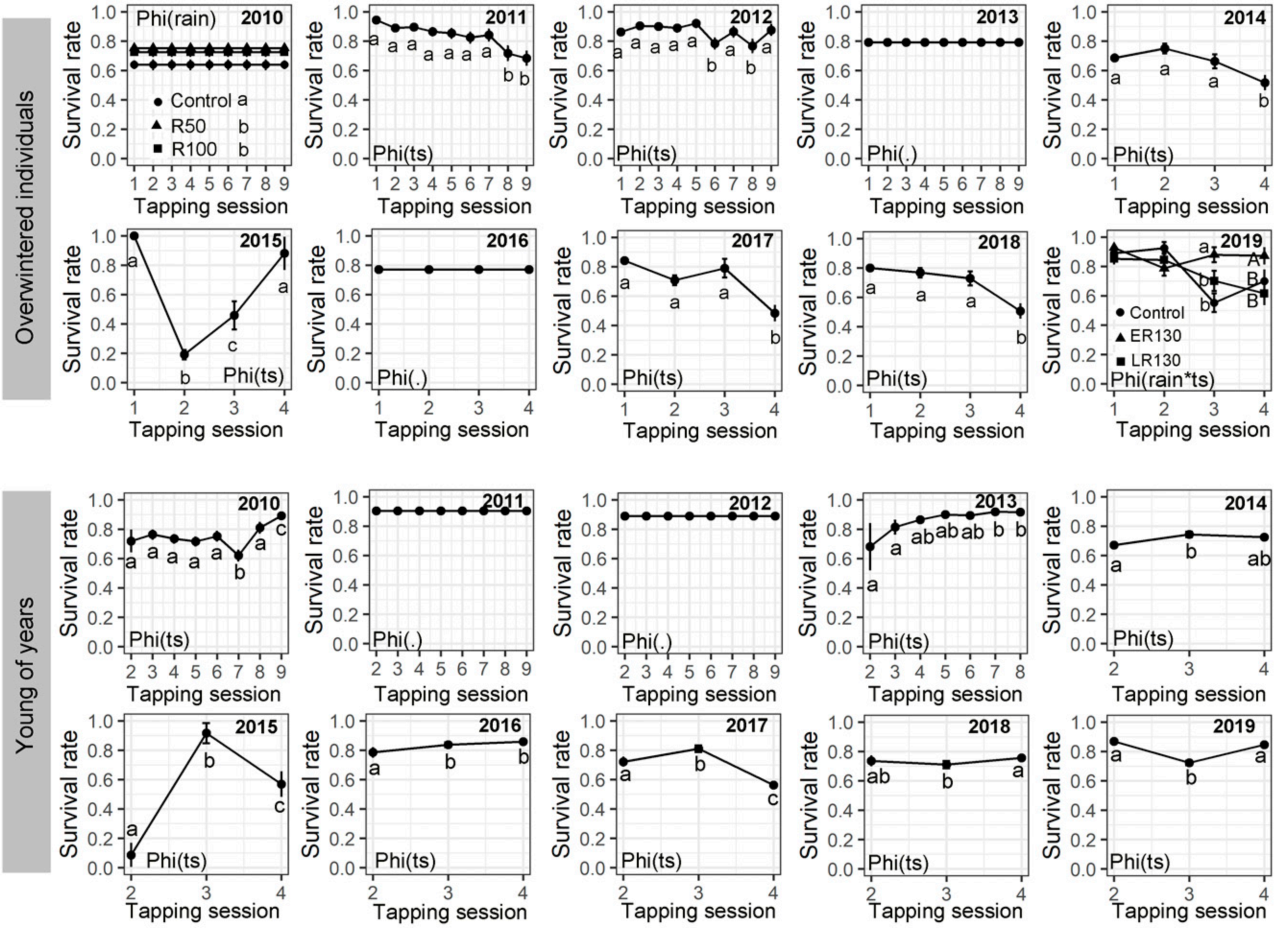

Fig. 3. Estimates of survival probability of Brandt's voles (both overwintered individuals and YOY) during the plant growing season (May to October) under different rainfall addition scenarios from 2010 to 2019. Different letters denote significant differences between treatment groups ($P < 0.05$). Phi indicates survival probability. Phi(rain), Phi(ts), and Phi(rain*ts) indicate that the survival probability was determined by different rainfall treatments, trapping sessions, and the interaction between rainfall treatments and trapping sessions, respectively. Phi(.) indicates that the recapture probability was constant.

experiment in in the Yukon Territory without considering the rainfall addition pattern (timing and magnitude) or excluding confounding factors such as predators and interspecific competitors. They found little effect of rainfall addition on food resources for the boreal red-backed vole *Clethrionomys rutilus*, indicating a neutral effect of rainfall on this species (33). Likewise, in our Experiment Phase 1, the biomass of the plant community (both preferred and less preferred plants) was not affected by the low-magnitude rainfall addition, which could explain why Brandt's vole populations showed no response to small rainfall additions in Phase 1. However, in our Experiment Phases 2 and 3, after resetting the timing and magnitude of rainfall addition, the biomass of preferred plants was significantly increased, which was accompanied by an increase in vole density. Some studies have showed that rainfall addition could induce a sharp increase in the biomass of *L. chinensis* (main food in summer for Brandt's voles) (27, 29). Due to the high content of fructose and oligosaccharides in *L. chinensis*, increasing the intake of *L. chinensis* would alter the composition of gut microbiota of Brandt's vole, likely increasing the production of short-chain fatty acids and facilitating the body growth and population density (29). In addition, rainfall addition can promote the sprouting and growth of vegetation, especially of grasses (Gramineae), which often produce a plant secondary metabolite (i.e., 6-methoxybenzoxazolinone [6-MBOA]) that triggers reproduction in rodents such as the montane vole (*Microtus montanus*) (38) and African multimammate rat (*M. natalensis*) (39). For Brandt's vole, 6-MBOA has been detected in the seedlings of its main food plant species (i.e., *L. chinensis*), and intraperitoneal injection of 6-MBOA has also been proved to effectively influence the outset of reproduction by increasing the contents of luteinizing hormone and testosterone in the serum and the relative mRNA expression levels of reproduction-related genes (e.g., *StAR* and *CYP11a1*) in the testes of Brandt's vole under a short photoperiod (40). Such a regulation pathway may also explain the positive bottom-up effect of rainfall addition on Brandt's vole populations in the early growing season.

Several studies have shown that rainfall may have a complex nonlinear effect on rodent populations (30, 41, 42). In this study, we found that vole population responses to the magnitude of rainfall addition may saturate under very wet conditions. For example, in Experiment Phase 2, although the amount of rainfall doubled, there was no significant difference in vole population density between the ER130 and ER260 treatment groups. This result may be explained by the nonlinear response of net primary productivity to changes in annual rainfall (43). For example, in Phase 2, we found a significant increase in the biomass of all plant species in the ER130 group as compared with the control group, while there was no significant increase in total plant biomass in the ER260 group as compared to the ER130 group. Extreme rainfall may create surface runoff, which fills burrow systems with water, causing many voles to drown and leading to population collapse (30, 44). The population density was lowest in 2015 in our study, which was most likely caused by two successive intense thunderstorms that deposited over 30 mm of rainfall in less than 2 h on June 22 and 24 mm of rainfall in less an hour on July 9 in the breeding season. The burrow system of Brandt's voles is usually built shallowly to a depth of about 30 cm below the ground surface. After the intense thunderstorms, we found many drowned young voles in the enclosures (*SI Appendix*, Fig. S1*B*), confirming the harmful effect of extreme rainfall events on vole populations as described before (30, 45). The variation in annual natural rainfall was large at our study site (e.g., 512 mm annual rainfall in 2012 but only 169 mm in 2017), which influenced the effect size of experimental rainfall additions on the vole populations. For example, in Experiment Phase 2, adding an extra 260 mm rainfall during the growing season (ER260) significantly increased the vole population in 2017 when natural rainfall during the growing season was low (i.e., 130.7 mm). In contrast, natural rainfall was relatively high in 2016 and 2018 (215 and 230 mm, respectively), and we found no such facilitating effect of experimental rainfall addition on vole populations even though we completed the same experimental treatments as in 2017. The beneficial effect of rainfall in indirectly increasing food resources may be offset by the harmful influence of rainfall through direct flooding of burrows, which may lead to a nonlinear effect of rainfall additions on vole populations.

Population dynamics reflect functional variations in population growth rates, which are determined by various demographic rates (survival, growth, and reproduction). The relative contributions of different demographic rates to changes in population growth rates vary between populations (46). Due to limited food resources in the natural environment, individuals are under selective pressure to allocate resources in an optimal way to growth, reproduction, and survival in order to maximize their fitness (47). At the population level, the tradeoffs between survival versus growth and reproduction are vital determinants of population fluctuations and species coexistence, which are constrained by physiological limitations and ecological modes of life (47). According to the pace-of-life syndrome (POLS) hypothesis, individuals with a fast pace of life (fast growth rate, early reproduction, and short lifespans) are expected to invest more in reproduction than in survival, while the reverse can be expected for the slow-pace-of-life individuals (48). Small rodents typically exhibit fast lifestyle and have relatively short lifespans. They may adopt a high-fertility/low-survival strategy to maximize their fitness, especially when there are short pulses of productivity after rainfall events. In our study, Brandt's vole responded consistently to the rainfall-induced increase in food resource, which was characterized by increased fertility and growth rate but less change in survival, supporting the POLS hypothesis.

To check whether the positive effects of large rainfall supplements in May and June (>120 mm) on vole population abundance that we observed under enclosure conditions are consistent with historical population outbreaks of voles under open field conditions, we reviewed meteorological records and literature from 1970 to 2019 and found that there were 7 y when the amount of rainfall in May and June exceeded 120 mm during this time period. Five of the seven years with wet seasons in May and June were reported to have population outbreaks of Brandt's vole (>300 individuals per hectare; *SI Appendix*, Fig. S3*A*), consistent with our experimental results. However, the predictive power score of rainfall in May and June was very low (i.e., 0.08), which suggests that using only one such indicator (>120 mm rainfall during the growing season of May and June) to predict population outbreaks of Brandt's voles may not be sufficiently accurate. The experimental errors were assessed by calculating the SE and coefficient of variation (CV) in population density within each treatment for each year in our study. Although the SE was larger in Experiment Phase 1 (*SI Appendix*, Fig. S6*A*) as compared to Phases 2 and 3, the CV of the three phases was similar (*SI Appendix*, Fig. S6*B*). The higher population abundance in Phase 1 than in Phases 2 and 3 was likely caused by a founder population effect: low-intensity plant suppression by vole herbivory or climate variation across years. Our previous studies have indicated that vole grazing can reduce the biomass of palatable vegetation (49). The yearly variation in vole populations in this study may be explained by various factors (natural rainfall, temperature in spring, and founder population effects), which will be analyzed separately in future studies.

Natural rodent populations are jointly influenced by endogenous (density dependence) and exogenous factors (climate, food, and predation) (13, 18, 50), with predation as an important force in regulating the population dynamics of small rodents (51–55). In this study, predator effects were excluded, with the main focus being to test the bottom-up effects of rainfall addition on vole populations, which may not fully reflect the true effect of rainfall in the field. Under natural conditions, the effects of rainfall may interact with predators through promoting vegetation height and biomass that influence hunting success. Previous studies have indicated that the response of plant biomass to rainfall is often dome-shaped in arid ecosystems (44, 56). Brandt's voles prefer vegetation of intermediate height and density in steppe grassland due to the tradeoff between food demand and predator avoidance (41). Tall or dense standing vegetation is detrimental to voles because of more difficulty in spotting predators (57), while sparse vegetation could cause population declines due to food shortage (58). In our study region, the abundance of Brandt's voles (41) and Daurian pika (*Ochotona dauurica*) (43) is highest in years with moderate cover of vegetation associated with moderate rainfall. In this study, we also found a dome-shaped association of precipitation in May and June with maximum vole population in the enclosures (*SI Appendix*, Fig. S3*B*). This may explain why high rainfall increases in our study did not produce extra effects on vole abundance. In the future, it will be necessary to test the interactive effects of rainfall and vegetation cover on predation in vole populations.

During the study period from 2010 to 2013, the initial densities were all set at 30 individuals (female: male = 1:1) per enclosure. Due to the difficulties in capturing the living voles in the field, we lowered the initial densities to 26 individuals per enclosure (female: male = 1:1) from 2014. This change in initial density may not influence the robust of our main results. As we can see, there was no significant effect of rainfall increase either before 2014 or after 2014 during Phase 1. In contrast, positive effects of rainfall increase were both found during Phase 2 and Phase 3, suggesting the importance of mode of rainfall increase in influencing the population growth of Brandt's voles. In addition, we analyzed the carryover effect caused by vegetation. If successive rainfall supplementation had a positive carryover effect on vegetation, there should be an increasing trend of vegetation and then an increasing trend of population density or recruits or body mass. Otherwise, the carryover effect of vegetation on population density may be small or limited. Based on plant data in Fig. 2, with the increase of experimental years, we found there was a significant increasing trend of preferred plants for ER130 and ER260 in Phase 2; there was no such significant increasing trend of preferred plants in other groups or Phase 1, indicating a likely positive carryover effect of vegetation. Based on population data in Fig. 1, with the increase of experimental years, there was significant decreasing trend of vole density for Phase II but either no trend for vole density, recruits, and body mass in Phase I or no trend for recruits and body mass in Phase II, suggesting no positive carryover effect of vegetation on vole population growth. The interyear variation in population abundance, body mass, and recruits was likely caused by background weather conditions (e.g., temperature in spring).

In summary, we present evidence that the rainfall timing is more important than magnitude in facilitating Brandt's vole populations in steppe grasslands. In the context of global climate change, especially with increased rainfall variation, studies on how animal populations respond are becoming more urgent and important for community biodiversity conservation and ecosystem stability. However, our understanding of such patterns now is restricted to Brandt's vole. More experimental studies with other species are further required to reveal whether timing of rainfall indeed determines demographic processes, population fluctuations, and species distributions more generally.

Materials and Methods

Study Area and Experimental Enclosures. This study was conducted at the Research Station of Animal Ecology (44°11′N, 116°27′E) in Inner Mongolia, China. This region is a steppe grassland that experiences a continental arid temperate climate, with a mean annual temperature of 3.4 °C and mean annual precipitation of 259.2 mm (1980 to 2009 climate data from China Meteorological Administration). The small mammal community in this region consists mainly of Brandt's vole (*L. brandtii*), Daurian ground squirrels (*S. dauricus*), striped hamsters (*C. barabensis*), Mongolian gerbil (*Meriones unguiculatus*), and house mouse (*Mus musculus*). Brandt's voles are the dominant rodent species, and the lifespan of Brandt's voles in the field is less than 12 mo. They usually breed from late April to August. Population density is highest in August, and most voles die during the winter with the harsh environment; thus, the population density is lowest by the next April. Overwintered voles can breed 2 to 3 times with litter size ranging from 7 to 10, contributing largely to recruitment of voles and population growth in the current year. They are highly reproductive in good years with plenty of food. Most overwinter voles died by August. Newborn voles have the potential to breed at 1 mo old, but few of them are able to breed in the current year due to the strong social suppression by overwintered individuals (59, 60).

In 2008, we constructed a set of 24 large outdoor enclosures (60 × 80 m each), each surrounded by a galvanized sheet metal wall extending 1 m underground and 1.4 m aboveground. Wire mesh netting (mesh size: 1 cm) extending 50 cm above the sheet metal wall was welded onto the metal wall, and nylon netting (mesh size: 10 cm) was placed on the top of the enclosures (for details, see ref. 60). The design of the enclosures was intended to prevent voles from emigrating or immigrating, as well as to exclude all mammalian and avian predators. To manipulate rainfall, a spraying irrigation system was built in each rainfall increase enclosure using steel pipe and consisting of 30 two-meter-high rotating sprinklers arranged in a 5 × 6 array (*SI Appendix*, Fig. S4*A*). Each sprinkler was pressure-regulated, allowing even application of water over each enclosure at a rate of ∼5 mm h^{-1}. Water was delivered from a well into a storage tank (6 × 3 × 1.5 m) before transfer to the irrigation system with a pump.

Rainfall Manipulation. Twelve enclosures were used for these manipulations that were randomly assigned to control and two levels of rainfall treatment. One enclosure stands for one experimental replicate. Each treatment has four replicates in our study. There were three distinct phases for the rainfall manipulation experiment (*SI Appendix*, Fig. S5). Phase 1 was conducted from 2010 to 2015 and was designed to test the effect of low and moderate increases in the amount of rainfall (averaging 18% and 36% increases as compared to the natural rainfall amount; *SI Appendix*, Table S1) on vole populations. In China, the 400 mm isohyet for the mean annual rainfall is considered the boundary between semihumid (>400 mm) and semiarid regions (200∼400 mm) and also corresponds to the boundary between grassland and forest or farmland. Given that the mean annual rainfall in our study site was 259.2 from 1980 to 2009, the enclosures receiving the two levels of rainfall increase were set to receive in total 50 or 100 mm of water (termed the R50 and R100 treatment groups, respectively) during the growing season (May to September), thus ensuring that the total amount of rain in our experiment was still less than 400 mm. By contrast, the control enclosures received only natural rain. The increased rainfall was evenly distributed biweekly throughout the growing season (*SI Appendix*, Fig. S1) for a period of 1 or 2 h each for the R50 and R100 treatment groups, respectively. Because we did not find significant differences in population abundance between the control and rainfall increase groups during Phase 1, we decided to increase the rainfall amount as well as shift more rainfall to May and June (i.e., the early breeding season of voles) in the next phase. Phase 2 ran from 2016 to 2018 and aimed to examine the combined roles of timing shift and more rainfall (increases averaging 56 and 112% compared to the natural rainfall amount; *SI Appendix*, Table S1) on vole populations. From 1980 to 2015, extreme rainfall events occurred frequently at our study site, and the maximum annual rainfall was 511.7 mm in 2012. According to the maximum value of rainfall in our region, we increased the amount of added rainfall to 130 and 260 mm (termed the ER130 and ER260 treatment groups, respectively; *SI Appendix*, Fig. S1) and allocated more rainfall (61.5% of the total added rain) to the early growing season (May to June). In Phase 2, we found that rainfall increases of both 130 and 260 mm in May to June increased vole abundance, but there was no difference between the 130 and 260 mm treatments, suggesting that excessive rainfall increase had no extra positive effect on vole density. Thus, we decided to further test the effects of rainfall timing on the population density of voles by fixing the total amount of added rainfall to 130 mm in the next experiment. Phase 3 was designed to last 2 y (2019 to 2020) but was disrupted in 2020 due to the coronavirus outbreak at the end of 2019 in China. In 2019, there were two rainfall increase treatments, with most water (61.5%) applied to enclosures in the early growing season (termed the ER130 treatment group) or the late growing season (termed the LR130 treatment group; *SI Appendix*, Fig. S1).

Animal Trapping and Vegetation Surveys. Some voles can survive through winter in our experimental enclosures, but the quality and number of these voles were different from enclosure to enclosure. To minimize the differences of founder voles in different treatment groups, at the beginning of experiment each year, we removed the overwintered voles from all enclosures and introduced new voles that captured from the field in the current year into enclosures. In April each year from 2010 to 2019, we conducted live-trapping for 7 consecutive days to remove all overwintered individuals in the enclosures and then introduced 15 (2010 to 2011) or 13 (2013, 2015 to 2019) pairs of voles from the field into each enclosure to create a new founder population. The size of the founding population was the same in all enclosures, which helped to exclude any confounding effects of initial population size. Voles were given 10 to 15 d to acclimatize to their enclosure before the manipulative experiments started. In 2014, the overwintered individuals were not removed and continued to be observed for another year to test the carryover effect of rainfall increase on vole populations. Capture-Mark-Recapture (CMR) was used to investigate the demography of vole populations biweekly (2010 to 2013) or monthly (2014 to 2019) from May to October. Each newly trapped vole was marked with a numbered aluminum ear tag (2010 to 2017) or implanted subcutaneously with a passive integrated transponder tag (2018 to 2019). Information on sex, body mass, reproductive status, and family location was recorded before individuals were released at their capture sites. A vegetation survey was also conducted each month following the census of the vole population. We randomly selected five quadrats (1 × 1 m) in each enclosure and measured the density (number of plants in each quadrat) and dry biomass of each plant species. For a more detailed description of the experimental procedure, see refs. 29, 58, and 60. Based on the relative preference index for each plant species consumed by Brandt's vole (60), all plant species within enclosures were a priori categorized into two groups: preferred plant species (i.e., *L. chinensis*, *Setaria viridis*, *Agropyron cristatum*, and *Medicago sativa*) and less preferred plant species (i.e., *Stipa krylovii*, *Lepidium apetalum*, *Corispermum declinatum*, *Carex tristachya*, *Cleistogenes squarrosa*, *Neopallasia pectinate*, and *Phlomis dentosa*).

Data Analysis. The population density in each enclosure was estimated using the CMR data with program CAPTURE (61) and expressed as numbers per 0.48 ha (i.e., the size of the enclosures). Recruits were defined as individuals that were born in the current year. Because it was difficult to identify the exact birth date for each individual, we pooled yearlings together each year in our analysis. Generalized linear models were performed to compare differences in the total number of recruits (conforming to negative binomial distribution) and yearling body mass in October between different rainfall treatments (62). Differences in population density and biomass of food resources for voles between different rainfall treatments were analyzed with linear mixed models, with enclosure specified as random effect. Bonferroni corrections were performed to adjust the *P* values for multiple comparisons. We used the permutational multivariate ANOVA with Bray-Curtis distance matrices (adonis function in R) to compare the differences in recruitment pattern of vole population during breeding seasons between different rainfall treatments (29). All statistical analyses were performed using R (63), version 3.5.1. All individuals in the enclosures were classified into two cohorts, overwintered and YOY, for analysis of survival. The CJS models implemented in program MARK were also used to estimate apparent survival to confirm the effect of rainfall increase.

Data Availability. Excel data have been deposited in Figshare (https://doi.org/10.6084/m9.figshare.14495178.v1) (64).

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