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Warming reduces the production of a major annual forage crop on the Tibetan Plateau

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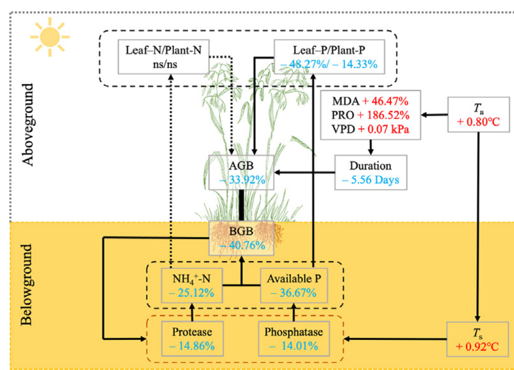
HIGHLIGHTS

- Experimental warming reduces the production of forage oat on the Tibetan Plateau.
- Warming increase VPD, damages the oxidant system, shortens growth duration, and exacerbates phosphorus limitation.
- There is an urgent need for advanced agronomic practices to meet the increasing demand for livestock forage crops.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate warming has been proposed to increase primary production of natural grasslands in cold regions. However, how climate warming affects the production of artificial pastures in cold regions remains unknown. To address this question, we used open-top chambers to simulate warming in a major artificial pasture (forage oat) on the cold Tibetan Plateau for three consecutive years. Surprisingly, climate warming decreased aboveground and belowground biomass production by 23.1%–44.8% and 35.0%–46.5%, respectively, without a significant impact on their ratio. The adverse effects on biomass production could be attributed to the adverse effects of high-temperatures on leaf photosynthesis through increases in water vapor pressure deficit (by 0.05–0.10 kPa), damages to the leaf oxidant system, as indicated by a 46.6% increase in leaf malondialdehyde content, as well as reductions in growth duration (by 4.7–6.7 days). The adverse effects were also related to exacerbated phosphorus limitation, as indicated by decreases in soil available phosphorus and plant phosphorus concentrations by 31.9%–40.7% and 14.3%–49.4%, respectively, and increases in the plant nitrogen: phosphorus ratio by 19.2%–108.3%. The decrease in soil available phosphorus concentration could be attributed to reductions in soil phosphatase activities (by 9.6%–18.5%). The findings of this study suggest an urgent need to advance agronomic techniques and cultivate more resilient forage genotypes to meet the increasing demand of forage for feeding livestock and to reduce grazing damage to natural grasslands on the warming-sensitive Tibetan Plateau.

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1. Introduction

Climate warming is one of the dominant drivers of global change (Ganjurjav et al., 2018). Previous studies have demonstrated that the Tibetan Plateau has been subjected to distinct warming in recent decades (Chen et al., 2013; Guo and Wang, 2012). The annual temperature on the Tibetan Plateau has increased dramatically at a rate twice the global average over the past half-century (Liu et al., 2018b). Alpine grasslands on the Tibetan Plateau are considered to be susceptible to warming (Jiang et al., 2016). A growing number of natural grassland experimental studies have explored how plant phenology, productivity, and soil properties respond to climate warming (Ganjurjav et al., 2016; Liu et al., 2018b); however, few studies have investigated the response of artificial pasture to warming in the alpine meadows of the Tibetan Plateau (Zhang et al., 2013).

The stability of grassland production on the Tibetan Plateau is crucial to the livelihood of native nomads, but the production in this region is susceptible to climate warming (Dong et al., 2020; Liu et al., 2019a). Most modeling studies based on remotely sensed data and long-term time-series datasets of the normalized difference vegetation index (NDVI) showed an increasing vegetation growth, although the changes varied with geographical heterogeneity and at spatial and temporal scales (Liu et al., 2018b; Ma et al., 2017). Field observations have shown that climate warming affects plant biomass (Zhou et al., 2019), but the signs of these effects have been inconsistent. For example, while most studies have shown increases in grass biomass (Ganjurjav et al., 2016; Gao et al., 2016; Ma et al., 2017), others have reported a decline (Klein et al., 2007) in response to climate warming. Some studies have suggested that more than 50% of the natural grasslands have degraded in the past few decades, primarily due to climate change and anthropogenic activities (Luo et al., 2018; O'Reagain et al., 2014). Anthropogenic activity-induced instability of plant biomass and land degradation can constrain the sustainable development of animal husbandry and hence the income of herders (Yang et al., 2020). One promising approach to mitigate the adverse effects is to establish artificial pastures in arable areas to reduce anthropogenic pressures on the natural grasslands (Duan et al., 2019).

A variety of ecosystem state variables and processes can respond to climate warming. For example, plant physiology is typically sensitive to temperature (Shen et al., 2020), which can be indicated by antioxidant enzyme activities, such as superoxide dismutase (SOD) activity and proline (PRO) and malondialdehyde (MDA) content. SOD is a major antioxidant enzyme that can reduce reactive oxygen species (ROS) accumulation, thereby regulating the level of lipid peroxidation (Apel and Hirt, 2004). PRO facilitates the scavenging of ROS and acts as an osmolyte. MDA content can indicate the level of membrane injury in plant cells in response to stress (Zhou et al., 2018). Thermal conditions can also be affected by climate warming, which may favor plant production (Liu et al., 2019b; Yang et al., 2015). Moreover, soil microbial activities such as the production of extracellular enzymes, are also sensitive to climate warming (Jing et al., 2014; Sinsabaugh and Follstad Shah, 2012). Early studies found that N supply limited plant productivity on the Tibetan Plateau (Liu et al., 2018a; Liu et al., 2013), while recent studies found that P can also affect plant productivity in this region (Ren et al., 2017; Xu et al., 2014). When plant production is limited by N or P, it can be indicated by the community-level leaf N:P ratio (Deng et al., 2015; Fay et al., 2015; Wang et al., 2015). Therefore, plant growth duration, plant physiology, soil extracellular enzyme activities (EEAs), soil nutrients and plant N:P stoichiometry are all useful for understanding how plant production responds to climate warming.

Forage oat (*Avena sativa*) is a cold-adapted crop that has been widely cultivated on the Tibetan Plateau as the most important source of forage, covering 70% of the artificial pasture in alpine meadows because of its short growth duration and excellent tolerances to leanness, saline-alkali, dry, and cold conditions (Xu et al., 2017). Therefore, we chose this species as our model plant to investigate the effects of warming on artificial pastures. We hypothesized that (H_1) experimental warming would ease cold constraints on plant growth, and increase the length of the growing season (Li et al., 2016; Nasim et al., 2016) and that (H_2) experimental warming would stimulate soil extracellular enzymatic activities and nutrient mineralization, consequently increasing forage oat production (Meng et al., 2020; Yue et al., 2018).

2. Materials and methods

2.1. Study site and experimental design

The present study was conducted over three consecutive years from 2017 to 2019 at the Lhasa Agricultural Experiment Station, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, on the Tibetan Plateau of China (29°40′N, 91°20′E, 3688 m above the sea level). Since forage oat is an annual crop and was sowed every year, the three-year experiment can be taken as three replicates, i.e., the experiment was repeated for three times. This area experiences a semi-arid temperate plateau monsoon climate with a mean annual temperature of 7.4 °C and solar radiation of 7026.6 MJ m^{-2}. The mean annual precipitation is 439 mm yr^{-1}, of which 90% falls during the growing season from May to September (Cui et al., 2016). The soil at the agricultural experiment station is sandy loam, according to the USDA classification system (Staff, 2010), with a pH value of 7.2, bulk density of 1.36 g cm^{-3}, organic matter content of 18.2 g kg^{-1}, total N of 0.84 g kg^{-1}, and total P of 0.66 g kg^{-1} in the 0–20 cm layer (Cui et al., 2017). The precipitation, daily mean air temperature, total radiation, and relative humidity were recorded by an automatic meteorological station from a weather station 0.1 km away.

Our field experiment comprised two treatments, warming treatment (warming) and ambient temperature treatment (ambient), which were established in April 2017. The ambient treatment experimental plots were 12 m^2 (3.0 m × 4.0 m), and the warming treatment plots were 4 m^2 (2.0 m × 2.0 m) in warming treatments, both with 0.25 m spacing between sowing rows. Our field experiment was a completely randomized block design with two treatments and three replications. We used open-top chambers (OTCs) to warm the plots at the Lhasa Agricultural Experiment Station. Owing to the limited conditions in this area, we modified the hexagonal ITEX OTC design described in Marion (1996) onto vertical polycarbonate walls with an angle iron frame according to the actual situation. The chamber dimensions were 2.1 m tall × 2 m long × 2 m wide with a top opening (the four corners were curved). We used transparent polycarbonate sheets with a thickness of 0.4 cm to allow high solar transmittance and natural ultraviolet conditions (Aronson and McNulty, 2009). A door of 0.5 m length and 1.5 m height was also installed on the leeward side of each OTC to allow access to the inside but was kept closed during the experiment.

All plots were plowed to a depth of 30 cm using a rotary cultivator and then irrigated with equal amounts of water before sowing according to the local field management practices. From 2017 to 2019, forage oat (*Avena sativa* L. Baiyan No. 7) was planted on May 2 (2017), May 6 (2018), and May 5 (2019). Seeds of forage oat were sown by hand at a depth of 3 cm, at a sowing density of 800 plants m^{-2}. Manual weeding was done twice, at 3 and 7 weeks after sowing. Field management was in accordance with local customs, and each plot was separated by a 2 m-wide buffer zone. We irrigated according to the rainfall during the growing season, keeping the amount of irrigation constant each time and only when necessary to ensure plant growth. Given that the rainfall may be insufficient during the growing season, we irrigated the plants after several continuous sunny days to maintain the normal growth of forage oats. Therefore, the number of irrigation events per year was determined by the amount of rainfall in the area during the growing season. We irrigated four, two, and five times in 2017, 2018, and 2019, respectively, and irrigated each plot with 146 mm of water each time. Before sowing in each year, base fertilizers (i.e., 75 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, and 30 kg K_2O ha^{-1}) were applied each year.

2.2. Canopy air temperature, relative humidity, soil temperature and moisture

To test the effectiveness of simulated warming under OTC cover, daily data for the plant canopy air temperature (T_a) were recorded by Hobo Pro v2 temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) every 30 min at the experimental site from sowing to harvest in 2017–2019. During the growing season, the height of the temperature data loggers was continuously adjusted as the plants grow upward to monitor the temperature of the canopy. Soil volumetric water content (VWC, SM) and soil temperature (T_s) at 7.6 cm soil depth was monitored on sunny days at each phenological stage (heading, filling, milking, and maturity) from 8:00 to 18:00 with a 2 h interval in 2018 and 2019, using a time-domain reflectometer (TDR350; Spectrum Technologies, Inc., Plainfield, IL, USA). The mean air temperature, air relative humidity, and rainfall during the growing season (May to September) from 2017 to 2019 are listed in Table S1.

2.3. Plant height, biomass, and phenological phase

Plant height was measured as the distance from the soil to the uppermost organ of the oat plant at the maturity stage with 10 plants randomly selected per plot. A 1 m × 1 m quadrat in each experimental plot was randomly selected 50 cm from the plot edge to determine the plant biomass at the maturity stage in each year. The heading and filling stages were counted from sowing until 50% of the plants in each subplot reached the phenological phase. Days to maturity were counted from sowing until maturity.

2.4. Soil extracellular enzyme activities (EEAs), P and N concentrations

Each year, soil samples were collected after the forage oats were harvested. At each plot, surface litter was removed and three soil cores (5 cm in diameter) at 0–10 cm soil depth were randomly collected and pooled as one soil sample. Soil samples were sieved through a 2 mm mesh and separated into two parts. One part was stored at −20 °C until analysis of soil enzyme activities, and the other part was air-dried for the determination of soil P and N concentrations.

Soil phosphatase activity was determined according to Tabatabai and Bremner (1969), using a spectrophotometer (Shimadzu, Kyoto, Japan). Soil protease activity was determined according to the method described by Hofmann (1965). Briefly, a soil suspension was distilled through centrifugation at 11,000 ×*g* for 10 min instead of filtering after incubation and then photometrically measured (Sardans et al., 2008). Enzymatic activity was expressed as milligram of substrate per gram of dry soil per day.

Air-dried soil samples were ground and sieved in a laboratory. Subsequently, soil total phosphorus (TP) concentration was determined using molybdenum antimony blue calorimetry (Murphy and Riley, 1962). Soil available phosphorus (AP) concentration was measured by treatment with 0.5 mol L^{-1} $NaHCO_3$, followed by molybdenum blue colorimetry (Pansu and Gautheyrou, 2007). Soil NH_4^+-N and NO_3^--N were extracted with 2 mol L^{-1} KCl (solution/soil 5:1, v/v) and measured using a Bran + Luebbe Auto Analyser 3 ((Bran+Luebbe, Norderstedt, Germany)) (Liu et al., 2008).

2.5. Leaf physiological properties

Leaf physiology is most active at the heading stage; therefore, we sampled the fresh green leaves at the heading stage to determine the MDA and PRO contents and SOD activity of leaves in 2018. Lipid peroxidation was determined by measuring MDA formation using the thiobarbituric acid (TBA) method described by Rao and Sresty (2000). Free proline content was determined according to the method described by Bates et al. (1973). SOD activity was assayed spectrophotometrically by the inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971).

We collected leaf samples after the forage oat harvest in 2017 and 2018 and collected aboveground plant samples after harvesting in 2019. All plant samples were oven-dried at 105 °C for 30 min and then dried at 80 °C to a constant weight. Plant samples were ground in a ball mill (Retsch MM200; Microtrac Retsch GmbH, Haan, Germany). Foliar P and whole plant P concentrations were measured spectrophotometrically after digestion with sulfuric acid (H_2SO_4), and N concentrations were determined using the Kjeldahl method (Wang et al., 2013).

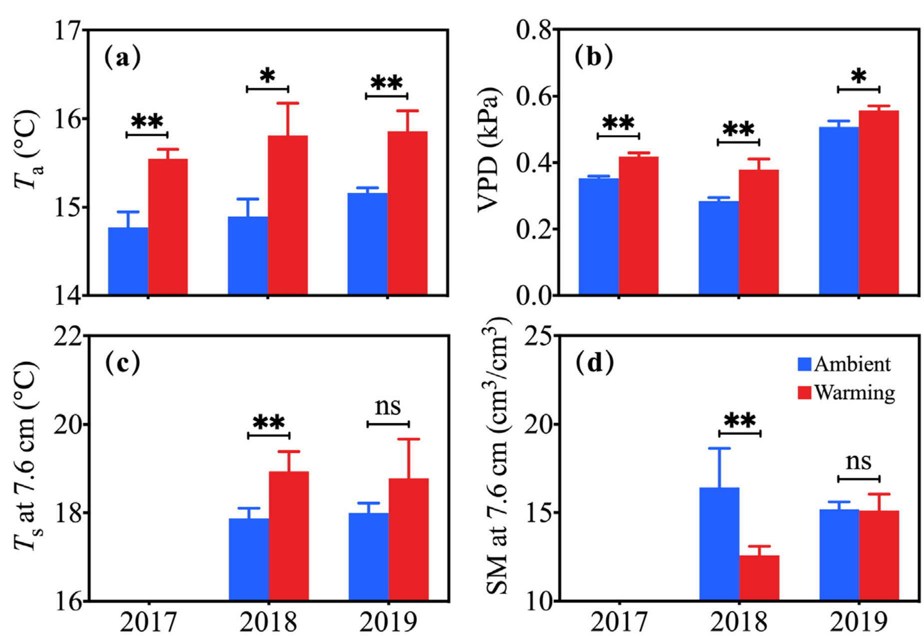

Fig. 1. Warming effects on the physical environment. Warming effects on (a) air temperature (T_a), (b) water vapor pressure deficit (VPD), (c) soil temperature at 7.6 cm (T_s), and (d) soil moisture content at 7.6 cm (SM). Treatments include ambient conditions (in blue) and warming treatment (in red). ** $P < 0.01$; * $P < 0.05$; *ns*, not significant. Values are means ± *SE* (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Statistical analysis

The comparisons of plant traits and soil properties between warming and ambient conditions were performed by one-way (warming effects within the year) analysis of variance at $P < 0.05$, using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). We assessed the plant N and P concentrations, and N:P ratio response ratio to warming following the meta-analysis method to compare the trend between leaf and whole plant concentrations (Luo et al., 2006). We analyzed the linear relationships between plant traits and soil properties with T_a or T_s, plant traits and soil properties, and plant biomass with the soil available N:P ratio or growth duration, and soil nutrients with soil extracellular enzymatic activities. The multiple linear relationships between the growth duration, aboveground biomass (AGB), and belowground biomass (BGB), and the T_a, T_s, SM, soil protease activity, soil phosphatase activity, available P, NH_4^+-N, NO_3^-N, and soil N:P ratio were also analyzed.

3. Results

3.1. Warming effects on the physical environment

On average, warming significantly increased T_a by 0.8 ± 0.1 °C (Figs. 1a and 7). The increase was 0.8 °C, 0.9 °C, and 0.7 °C in 2017, 2018, and 2019, respectively ($P < 0.01$; Fig. 1a, Table S2). The OTCs were a passive warming method; thus, the greatest magnitude of warming was exhibited in the daytime (1.0 ± 0.2 °C, 1.7 ± 0.3 °C, and 1.3 ± 0.2 °C in 2017, 2018, and 2019, respectively; $P < 0.01$; Table S2). At the same time, the warming treatment resulted in extremely high temperatures during the forage oat growing season (32.8, 37.4, and 38.6 °C in 2017, 2018, and 2019, respectively; Table S3). Warming significantly increased the VPD by 0.07, 0.10, and 0.05 kPa in 2017, 2018, and 2019, respectively ($P < 0.01$; Fig. 1b). Warming significantly changed T_s (from 17.9 ± 0.2 °C to 18.9 ± 0.4 °C) and soil moisture content (from 16.4 ± 1.8 cm^3 cm^{-3} to 12.6 ± 0.4 cm^3 cm^{-3}) in 2018 but not in 2019 (Fig. 1c, d). The lack of significant changes in 2019 was attributed to the high-frequency irrigations used (five times vs. twice in 2018) to offset the low precipitation (425.0 vs. 586.4 mm in 2018; Table S1), which may have counteracted warming effects on soil temperature and moisture content in 2019.

3.2. Warming effects on plant production

Warming significantly decreased forage oat AGB by 33.9% ± 5.2% on average ($P < 0.001$; Figs. 2a and 7). The BGB was significantly decreased by warming (40.8% ± 6.1%; $P < 0.001$; Figs. 2b and 7), while warming did not affect the AGB/BGB ratio (Fig. 2c). The plant height of forage oat was significantly lower (−21.3% ± 2.9%) in the warming treatment than that in the ambient plots ($P < 0.001$; Fig. 2d). A greater decrease in AGB, BGB, and plant height was observed in 2018 with a higher increment of T_a (Fig. 2).

3.3. Warming effects on plant physiology and phenology and soil nutrients

Forage oat growth duration was significantly shortened by warming, averaging −5.6 ± 0.6 days for the warming treatment throughout the experimental period ($P < 0.001$; Figs. 2e and 7). Compared with ambient temperature, warming significantly increased foliar MDA and PRO content by 46.5% ± 5.1% and 186.5% ± 15.8%, respectively ($P < 0.01$ and $P < 0.001$, respectively; Fig. 3a, c). Warming also increased SOD activity by 41.6% ± 10.0% ($P < 0.05$; Figs. 3e and 7). The negative responses of plant P concentrations to the warming treatments were observed every year ($P < 0.05$; Figs. 4a and S1), while warming did not affect plant N concentrations throughout the experimental period (Figs. 4b and S1). The positive responses of the plant N:P ratio to

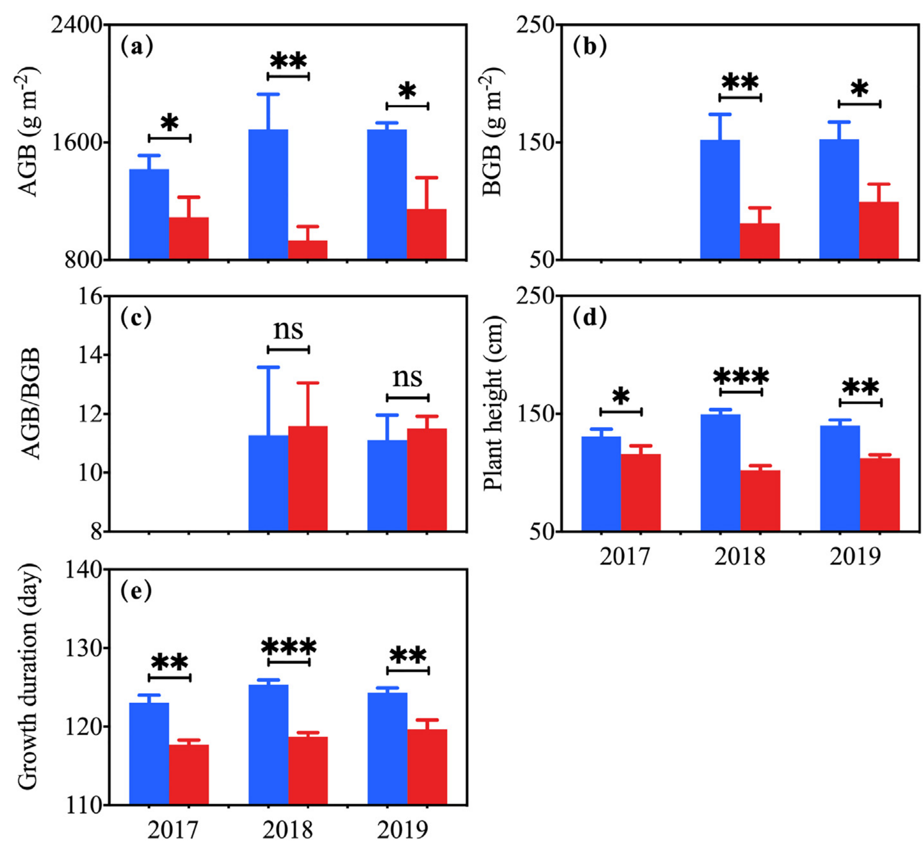

Fig. 2. Warming effects on forage oat production and growth duration. Warming effects on (a) aboveground biomass (AGB), (b) belowground biomass (BGB), (c) AGB/BGB ratio, (d) plant height, and (e) growth duration. Treatments include ambient conditions (in blue) and warming treatment (in red). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; *ns*, not significant. Values are means ± *SE* (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

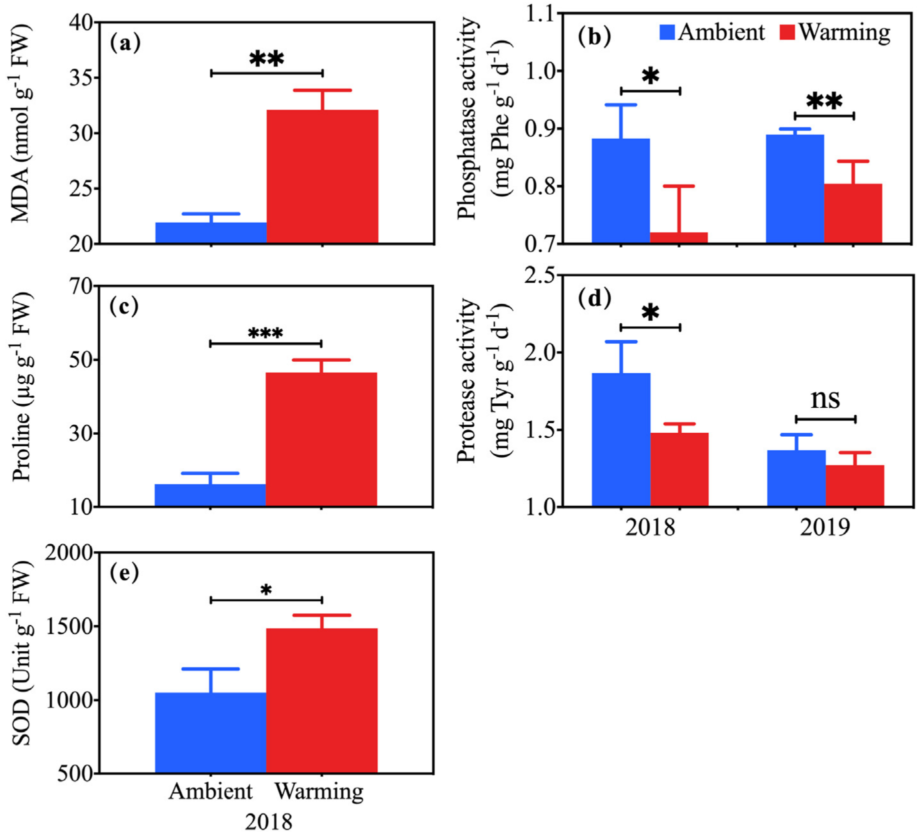

Fig. 3. Warming effects on forage oat antioxidant system and soil extracellular enzyme activities. Warming effects on (a) leaf malondialdehyde concentration (MDA), (c) proline concentration (PRO), (e) superoxide dismutase activity (SOD), (b) soil phosphatase activity, and (d) soil protease activity. Treatments include ambient conditions (in blue) and warming treatment (in red). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; *ns*, not significant. Values are means ± *SE* (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

warming were caused by the decreased plant P concentrations (Figs. 4 and S1), and a greater increase in the N:P ratio was observed in leaves (108.6% vs 19.4%; Fig. S1).

Soil phosphatase activity was significantly decreased by warming (−14.0% ± 3.4%; $P < 0.01$; Fig. 3b), and a greater increase in temperature led to a greater decrease in soil phosphatase activity (−18.2% ± 6.8% in 2018; $P < 0.05$; Figs. 1a and 3b). Warming significantly decreased soil protease activity in 2018 (−20.7% ± 6.4%; $P < 0.05$; Fig. 3d), while warming did not affect it in 2019 (Fig. 3d). Soil available P and NH_4^+-N concentrations significantly decreased in warming treatments relative to ambient values over three years (−36.7% ± 8.7%, $P < 0.01$ and −25.1% ± 4.1%, $P < 0.001$, respectively; Fig. 5b, d). Both soil TP and NO_3^-N concentrations significantly decreased in 2019 (−14.8% ± 4.9%, $P < 0.05$ and −20.6% ± 7.1%, $P < 0.05$, respectively; Fig. 5a, c), while no significant change was observed in 2017 and 2018. Warming only significantly increased the soil available N:P ratio in 2017 (47.8% ± 11.8%; $P < 0.05$; Fig. 5e).

3.4. Relationships among biomass production, leaf physiological properties, and soil properties

The N:P ratio of leaves and whole plants increased significantly with increasing T_a (Fig. S2i, k). Soil protease activity, TP, soil NO_3^-N, and available N:P ratio were not significantly correlated with T_a (Fig. S2e, m, s, u). AGB, BGB, soil phosphatase activity, NH_4^+-N, and growth duration showed a significantly negative relationship with T_a (Fig. S2a, c, g, q, w), while the soil available P concentrations showed a marginally negative relationship with T_a ($P = 0.08$; Fig. S2o). The leaf N:P ratio significantly increased with T_s (Fig. S2j). The soil protease and phosphatase activity, whole-plant N:P ratio, TP, NO_3^-N, and soil available N:P ratio were not significantly correlated with T_s (Fig. S2f, h, l, n, t, v). The AGB, BGB, NH_4^+-N, and growth duration showed a significantly negative relationship with T_s (Fig. 4b, d, r, x), while the soil available P showed a marginally negative relationship with T_s ($P = 0.06$; Fig. S2p).

The soil available P and NH_4^+-N concentrations were significantly positively correlated with soil phosphatase and protease activity, respectively (Fig. 6a, b). The AGB increased significantly with the soil available P, soil NH_4^+-N, and growth duration (Fig. 6c, d, f), whereas AGB showed a significantly negative relationship with soil available N:P ratio (Fig. 6e). The BGB, leaf P, and whole plant P concentrations increased significantly with soil available P (Fig. S3a, d, g). The BGB showed a significant positive relationship with soil NH_4^+-N (Fig. S3b), whereas leaf N and whole plant N were not significantly correlated with soil NH_4^+-N concentrations (Fig. S3e, h). BGB, leaf N, and whole plant N were not significantly correlated with the soil NO_3^-N concentrations (Fig. S3c, f, i).

The mean air temperature (T_a) in growing season and the soil phosphatase activity together substantially explained the variations in growth duration (coefficient = −3.68, $R^2 = 0.76$, $P < 0.01$; and coefficient = 16.72, $R^2 = 0.13$, $P < 0.05$, respectively; Table 1). The soil available P, VPD, mean soil temperature (T_s), and soil NO_3^-N partially explained the variation in AGB (coefficient = 86.84, $R^2 = 0.698$, $P < 0.001$; coefficient = −1729.12, $R^2 = 0.18$, $P < 0.001$; coefficient = −243.15, $R^2 = 0.06$, $P < 0.01$; and Coefficient = −162.05, $R^2 = 0.06$, $P < 0.01$, respectively; Table 1). The soil NH_4^+-N concentrations and mean air temperature (T_a) in the growing season partially explained the variations in BGB (coefficient = 11.96, $R^2 = 0.73$, $P < 0.05$, and coefficient = −36.31, $R^2 = 0.12$, $P < 0.05$, respectively; Table 1).

4. Discussion

Experimental warming led to a decrease, instead of an increase, in forage oat biomass production on the Tibetan Plateau, which did not

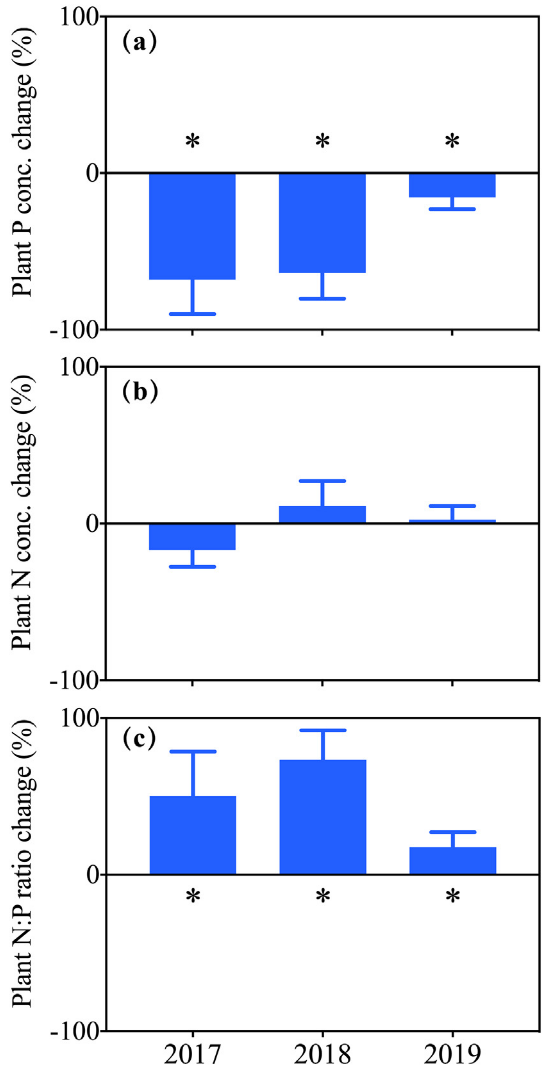

Fig. 4. Warming effects on forage oat nutrient status. Plant (a) P concentration, (b) N concentration, and (c) N:P ratio in response to experimental warming. * $P < 0.05$; *ns*, not significant. Values are means ± *SE* (n = 3). Leaf measurements in 2017 and 2018, aboveground biomass measurements in 2019.

support our first hypothesis that experimental warming would increase the production of forage oats. Our results contradict those of several previous studies (Guo et al., 2018; Natali et al., 2012) that reported a positive or non-significant effect of warming on plant production in alpine natural grasslands, but was consistent with a few other studies that found an adverse effect of warming on plant production (Fu et al., 2018; Hu et al., 2013). We propose three mechanisms to explain our findings. First, increasing temperatures may reduce stomatal conductance by increasing VPD, resulting in reductions in leaf photosynthesis rate (Novick et al., 2016) and plant production (Tsechoe et al., 2021). This hypothesis was supported by our results that showed VPD was positively correlated with T_a (Fig. 1b, Table S4) and negatively correlated with forage oat production (Tables 1 and S4), which is consistent with some previous studies (Broeckx et al., 2014; Novick et al., 2016).

Second, experimental warming increased the maximum temperature and number of hot days, both of which can reduce plant production. Although the mean annual temperature was low at the study site (7.4 °C), daytime temperatures in summer (i.e., July and August) can be higher than 25 °C and occasionally >28 °C (Tables S1 and S3), which are likely higher than the optimum temperature for growth of the cold-adapted forage oat species (Hossain et al., 2013; Huang et al., 2019). This hypothesis was supported by our result that experimental warming damaged leaf oxidant system (Fig. 3). In fact, summer in Tibet coincides with the reproductive growth period of forage oats, which is known to be the period that is particularly sensitive to heat stress (Klink et al., 2014).

Moreover, increased temperature during the reproductive stage can hasten crop senescence and decrease kernel weight and grain yield potential (Asseng et al., 2011). Schelling et al. (2003) found that high mean daily temperatures during the grain-filling period shortened that period, resulting in below-average barley and oat yields. Similarly, experimental warming shortened the growth duration in our study (Figs. 2e and 7). Regression analysis confirmed that growth duration was negatively correlated with the average T_a and T_s (Fig. S2, Table S4), and there was a positive correlation between forage oat production and growth duration (Fig. 6f, Table S4). Although the average temperature increases under warming treatment throughout the growing season was only 0.8 °C, the temperature increased to 33 °C on several occasions, resulting in temperature values that were much higher than the maximum temperature recorded on the Tibetan Plateau during the past two decades (Tables S2 and S3). These extremely high temperatures likely reduced the leaf photosynthesis rate and the production of forage oats (Zhang et al., 2019). The negative warming temperature effects may further result in a shorter plant life cycle (Fig. 2, life span), which was found by several previous studies (Hasanuzzaman et al., 2013; Schelling et al., 2003; Tuteja and Singh, 2012; Zhang et al., 2019).

Third, the negative warming effect on forage oat production could be attributed to warming-exacerbated P limitation. Warming may exacerbate P limitation through several mechanisms that are not exclusive (Hou et al., 2021). First, our results demonstrated that warming adversely affected soil P availability on the Tibetan Plateau (Figs. 3 and 5), which is consistent with the findings of a recent study on native grassland soils in North America (Siebers et al., 2017). Our results further suggest that warming may reduce soil P availability by reducing the activity of soil P-acquired enzymes (Figs. 5 and 7). Another study suggested that high temperatures may also reduce soil P availability by decreasing soil organic P pools and primary mineral P, which are potentially available to plants (He et al., 2008). Warming-reduced soil P availability may further lead to the lower plant P concentrations in the warming treatment than in the control treatment (Figs. 4, 7, and S1). Second, the warming effect on P limitation may be exacerbated by the high P demand of forage oats. The P demand of forage oats is very high, because of the high proportion (up to >50%) of P-enriched seeds in aboveground biomass production and the high aboveground biomass production (1327.0 g m^{-2} yr^{-1} vs. an average of 274.7 g m^{-2} yr^{-1} in global natural grasslands) (Sun et al., 2020). Indeed, a recent global meta-analysis showed that P limitation is more widespread and much stronger than previously estimated and can occur in many cold regions such as the Tibetan Plateau (Hou et al., 2020).

In contrast to our second hypothesis, warming decreased, instead of increasing, the activities of N- and P-acquired enzymes. This finding may be explained by three facts. First, under nutrient deprivation, microbes tend to invest more in catabolism rather than in anabolism (i.e., biomass synthesis), thus decreasing extracellular enzyme production (Zhu et al., 2021). Second, enzymes are perishable proteins that do not continuously accumulate in soils, and warming-induced enzyme inactivation would reduce overall enzyme activities (Alvarez et al., 2018). Third, the negative warming effect on plant growth (Fig. 2) may result in a decline in the carbon (C) input into the soil for soil microbes to produce and secrete EEA (Sardans et al., 2006). Consistent with the responses of EEAs to warming, soil NH_4^+-N and AP were also reduced in the warming treatment throughout the three consecutive years. However, NO_3^-N was reduced by warming only in 2019 (Fig. 5), probably because that warming also promotes nitrification, converting NH_4^+-N to NO_3^-N (Chapin et al., 1993). Moreover, plant N concentration was not significantly affected by warming, which could be attributed to the promotion of plant N acquisition by warming (Li et al., 2010). In general, plant and soil N concentrations were less affected by warming

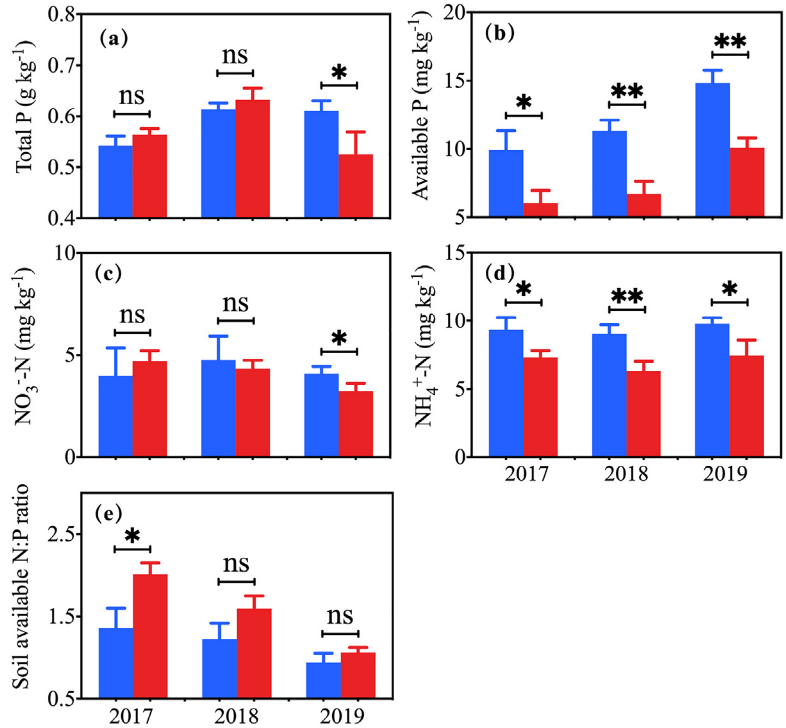

Fig. 5. Warming effects on soil nutrients. Warming effects on (a) soil total phosphorus concentrations (TP), (b) soil available phosphorus concentrations (AP), (c) soil nitrate-nitrogen concentrations (NO_3^-N), (d) soil ammonium nitrogen concentrations (NH_4^+-N), and (e) soil available N:P ratio. Treatments include ambient conditions (in blue) and warming treatment (in red). $^{**}P < 0.01$; $^{*}P < 0.05$; *ns*, not significant. Values are means ± *SE* (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

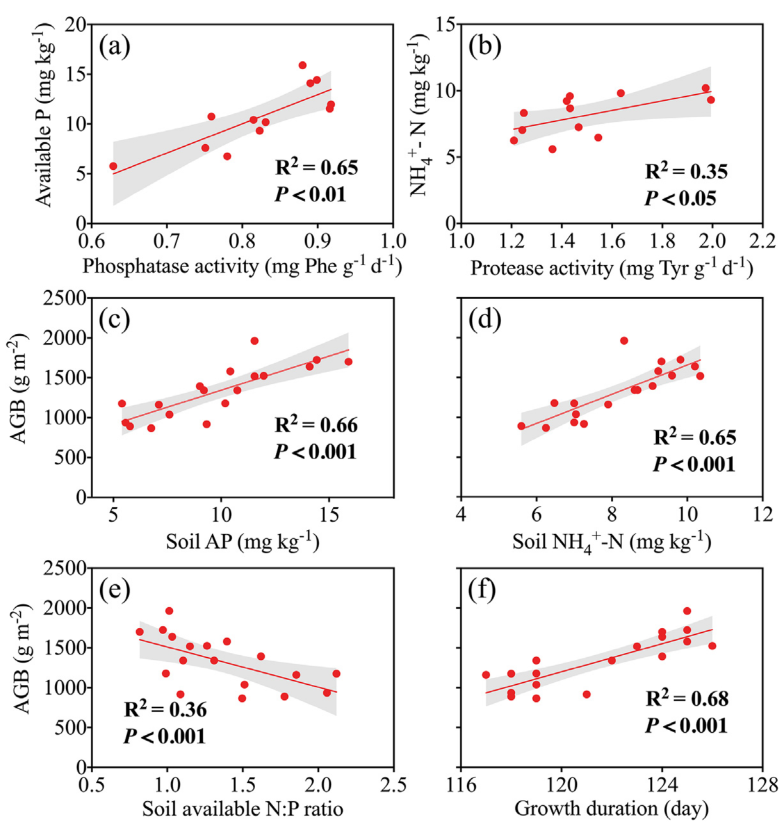

Fig. 6. Selected relationships among soil nutrients, soil enzyme activities, aboveground biomass (AGB), and growth duration. Relationships (a) between soil available phosphorus (AP) and the soil phosphatase activity, (b) between the NH_4^+-N and the soil protease activity, (c) between the AGB and the soil AP, (d) between the AGB and the soil NH_4^+-N, (e) between the AGB and soil available N:P ratio, and (f) between the AGB and the growth duration. Each point represents one replicate in different years.

Table 1
Summary of regressions of experimental duration, aboveground biomass (AGB), and belowground biomass (BGB) against temperature, VPD, and soil properties. T_a indicates air temperature, T_s indicates soil temperature, soil AP indicates soil available phosphorus, and VPD indicates water vapor pressure deficit.

Variable		Coefficients	Partial correlation	R^2	P
Growth duration	Intercept	164.95			**<0.001**
	T_a	−3.68	−0.79	0.76	**<0.01**
	Soil phosphatase activity	16.72	0.73	0.13	**<0.05**
AGB	Intercept	6314.82			**<0.001**
	Soil AP	86.84	0.97	0.69	**<0.001**
	VPD	−1729.12	−0.94	0.18	**<0.001**
	T_s	−243.15	−0.91	0.06	**<0.01**
	Soil NO_3^-N	−162.05	−0.88	0.06	**<0.01**
BGB	Intercept	584.29			**<0.05**
	Soil NH_4^+-N	11.96	0.70	0.73	**<0.05**
	T_a	−36.31	−0.68	0.12	**<0.05**

than plant and soil P concentrations, resulting in higher plant and soil N:P ratios in the warming treatment than in the control treatment (Fig. 5). These results further highlight the critical role of P in mediating the warming effect on the production of forage oats on the Tibetan Plateau, which was consistent with the results of a global standardized factorial nutrient addition experiment (Fay et al., 2015).

Our results have important implications for sustainable artificial pasture management. First, plant breeding is needed to produce new cultivars of forage oats and other crops that are more adaptable and resilient to rising temperatures. Second, an adaptation of agronomic practices, such as adjusting sowing times and fertilization according to cultivar traits and yield potential, can also help increase forage oat production on the Tibetan Plateau. In addition, intercropping or mixed cropping may be an effective method to improve forage production, and has been shown to provide greater total production while also increasing the use efficiency of resources such as land, water, and soil nutrients (Li et al., 2020). Finally, long-term warming experiments are needed to further examine the temporal dynamics of the warming effects on forage oat production. Given the significant heterogeneity in climate and soil conditions across the Tibetan Plateau (Huang et al., 2016), our conclusions may not be applicable to the whole Tibetan Plateau and should be verified by more manipulative warming experiments at various sites under different environmental conditions in the future.

5. Conclusion

We showed that experimental warming reduced the production of forage oats in OTCs on the Tibetan Plateau. The reduction was accompanied by increased VPD, damaged forage oat antioxidant system, and shortened growth duration. The reduction was also related to exacerbated phosphorus limitation, as evidenced by decreases in plant P and soil available P concentrations and an increase in the plant N:P ratio. Our results, therefore, suggest the urgent need for advanced agronomic techniques, more resilient genotypes, and improved fertilization management in the face of climate warming.

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CRediT authorship contribution statement

Design of the research, Fuqiang Wang, Xiaoming Shao; **Performance of the research**, Fuqiang Wang, Jiwang Tang, Jie Xiang, Liwei Wang, Li Tian; **Data analysis, collection and interpretation**, Fuqiang Wang, Jiwang Tang, Zhaolei Li, Lifen Jiang, Yiqi Luo, Enqing Hou, Xiaoming Shao; **Supervision**, Enqing Hou, Xiaoming Shao; **Writing manuscript**, Fuqiang Wang, Zhaolei Li, Yiqi Luo, Enqing Hou, Xiaoming Shao.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

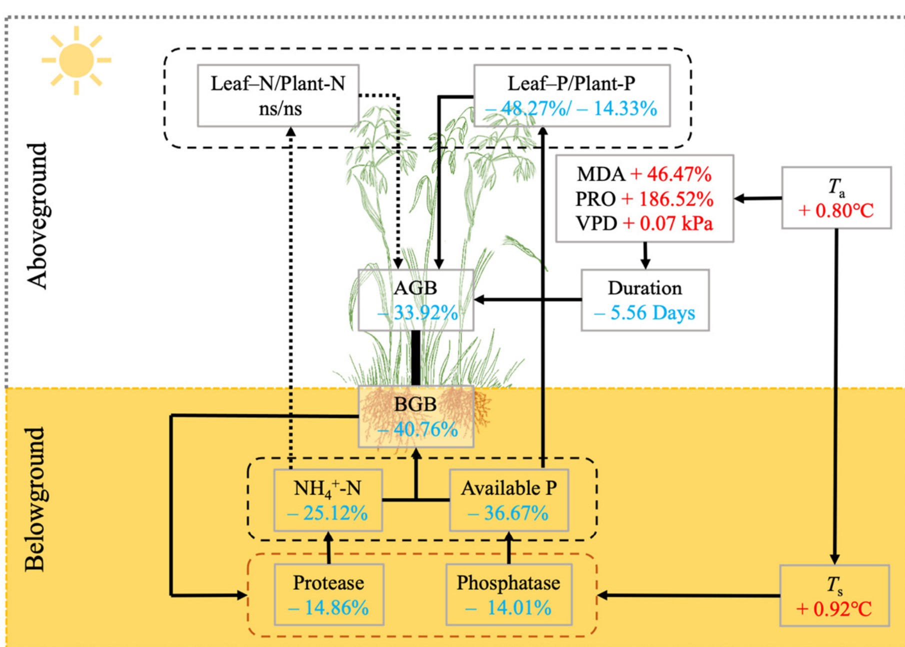

Fig. 7. Schematic diagram illustrating mechanisms underlying warming impacts on forage oat production. If the warming effect on a variable is statistically significant ($P < 0.05$), the percentage change of the variable is shown underneath the variable (positive in red, and negative in blue); if not, ns is shown. T_a indicates air temperature, T_s indicates soil temperature, MDA indicates malondialdehyde concentration, PRO indicates proline content, VPD indicates water vapor pressure deficit, AGB indicates aboveground biomass, and BGB indicates belowground biomass. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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