



Suppression of arbuscular mycorrhizal fungi decreases the temporal stability of community productivity under elevated temperature and nitrogen addition in a temperate meadow

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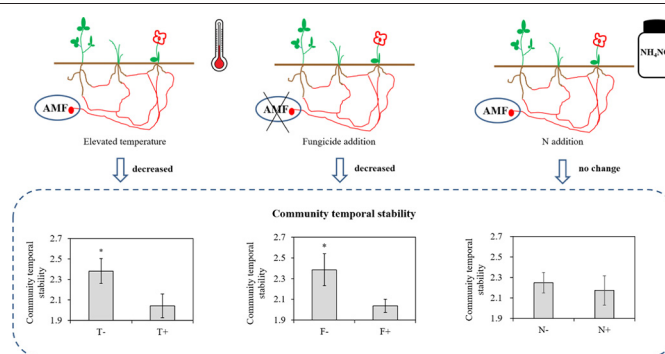
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HIGHLIGHTS

- Elevated temperature and AMF suppression decreased community temporal stability, but N addition had no effect.
- Dominant species stability was the main factor driving community temporal stability.
- AMF richness affected community temporal stability via community asynchrony and the temporal mean of community productivity.
- Mycorrhizal colonization increased community temporal stability via the temporal mean of community productivity.
- Plant species richness and Simpson's dominance did not affect community temporal stability.

GRAPHICAL ABSTRACT



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ABSTRACT

Global change alters how terrestrial ecosystems function and makes them less stable over time. Global change can also suppress the development and effectiveness of arbuscular mycorrhizal fungi (AMF). This is concerning, as AMF have been shown to alleviate the negative influence of global changes on plant growth and maintain species coexistence. However, how AMF and global change interact and influence community temporal stability remains poorly understood. Here, we conducted a 4-year field experiment and used structural equation modeling (SEM) to explore the influence of elevated temperature, nitrogen (N) addition and AMF suppression on community temporal stability (quantified as the ratio of the mean community productivity to its standard deviation) in a temperate meadow in northern China. We found that elevated temperature and AMF suppression independently decreased the community temporal stability but that N addition had no impact. Community temporal stability was mainly driven by elevated temperature, N addition and AMF suppression that modulated the dominant species stability; to a lesser extent by the elevated temperature and AMF suppression that modulated AMF richness associated with community asynchrony; and finally by the N addition and AMF suppression that modulated mycorrhizal colonization. In addition, although N addition, AMF suppression and elevated temperature plus AMF suppression reduced plant species richness, there was no evidence that changes in community temporal stability were linked to changes in plant richness. SEM further showed that elevated temperature, N addition and AMF suppression regulated community temporal stability by influencing both the temporal mean and variation in

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community productivity. Our results suggest that global environmental changes may have appreciable consequences for the stability of temperate meadows while also highlighting the role of belowground AMF status in the responses of plant community temporal stability to global change.

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1. Introduction

Temporal stability (the constancy of ecological variables, such as biomass, over time) of the plant community is one of the most fundamental properties of any ecological system (Pimm, 1984). However, many ecosystems are experiencing significant changes in environmental conditions (IPCC, 2018; Stevens, 2019), potentially leading to changes in community temporal stability (Li et al., 2018; Stevens, 2018) and thus affecting the sustainable provisioning of ecosystem functions and services (Tilman et al., 2014).

Global warming and increased nitrogen (N) deposition are two of the most prominent global change stressors (Gruber and Galloway, 2008; IPCC, 2018; Stevens, 2019). The global mean surface temperature increased by 0.87 °C during the decade of 2006–2015 (IPCC, 2018). Elevated temperature has been demonstrated to increase the biomass of plant functional groups and subsequently increase community temporal stability (Shi et al., 2016). Moreover, the abundance and stability of dominant species may change under elevated temperature if they suffer largely from the detrimental effect of elevated temperature-induced drought stress (Yang et al., 2016), which could subsequently influence community temporal stability. Due to relief from the limitation of cold temperatures in alpine grasslands, elevated temperature enhances the synchronous growth of plants and therefore decreases community temporal stability (Ma et al., 2017), indicating that species asynchrony may be important in determining community temporal stability under elevated temperature. Hence, the elevated temperature that alters community functional productivity, the stability of dominant species and the degree of species asynchrony may have the potential to alter community temporal stability.

Concurrently, many temperate ecosystems are predicted to experience rates of atmospheric N deposition as high as 2–5 g m⁻² y⁻¹ above the preindustrial rates during this century (Galloway et al., 2004). Anthropogenic terrestrial total N inputs are predicted to reach levels that are almost three times those due to human activities from 1850 to 2050 (Kanakidou et al., 2016). Nitrogen enrichment can also influence community temporal stability through various mechanisms. First, N addition can increase aboveground net primary production (Stevens et al., 2015) and thus could potentially increase community temporal stability via the overyielding effect. On the other hand, losses of plant species induced by N addition can also lead to a reduction in community temporal stability (Yang et al., 2012; Hautier et al., 2015). Moreover, species asynchrony contributes to stabilizing community productivity over time when declines in the biomass of some species are compensated for by increases in the biomass of other species (Loreau and de Mazancourt, 2008; Isbell et al., 2009). Therefore, the reduced species asynchrony resulting from N enrichment may lead to a less stable community (Xu et al., 2015; Chen et al., 2016; Zhang et al., 2016b). Finally, community temporal stability may also be affected by dominant species stability, especially when communities are dominated by a small number of species. Natural communities are composed of a few dominant species and many rare species (McGill et al., 2007). Rare species typically contribute little to community productivity, while dominant species contribute the most (Grime, 1998). Thus, dominant species stability plays an important role in regulating community temporal stability in response to N enrichment (Yang et al., 2011b; Chen et al., 2016). However, although there are studies on the individual effects of elevated temperature (Ma et al., 2017) and N deposition (Chen et al., 2016; Zhang et al., 2016b) on community temporal

stability, the combined effects of elevated temperature and N addition are unclear.

Most studies on community temporal stability have focused on aboveground factors, although belowground microbial activity can also influence community temporal stability (Yang et al., 2014). Arbuscular mycorrhizal fungi (AMF) can promote the acquisition of mineral nutrients (N, phosphorus (P)) by plants and redistribute resources among plant species, thus enhancing plant growth and promoting the coexistence of plant species (O'Connor et al., 2002; Collins and Foster, 2009). However, competition for N between AMF and plants in the case of substantial N demand does exist, and plant performance could thus be reduced in N-limited soil, which has been shown in grassland ecosystems (Blanke et al., 2005; Püschel et al., 2016). Therefore, suppression of AMF may relieve competition between AMF and plants for nutrients and thus positively influence plant growth (Wang et al., 2018). However, suppression of AMF can also decrease compensatory dynamics among plant species and functional groups, which are important for maintaining community temporal stability (Yang et al., 2014). In addition, global changes, such as global warming and N deposition, profoundly affect the abundance, diversity, and composition of AMF species (Johnson et al., 2003; Kim et al., 2015; Zhang et al., 2016a; Jiang et al., 2018). It is unknown how AMF suppression affects competition between AMF and plants and to what extent competition may constrain community temporal stability under conditions of nutrient addition and elevated temperature.

Here, we examine the extent to which AMF affect community temporal stability under elevated temperature and nutrient addition in a temperate meadow in northern China. Benomyl, which inhibits AMF enzyme activities and mycorrhizal colonization (Fitter and Nichols, 1988; Larsen et al., 1996), has been recommended as a treatment to reduce (but not fully eliminate) AMF colonization in the field (Fitter and Nichols, 1988; O'Connor et al., 2002; Yang et al., 2014). We thus applied fungicide (benomyl) in a four-year field experiment with elevated temperature and N addition. We hypothesized that (1) elevated temperature and N addition would reduce the community temporal stability, (2) suppression of AMF would increase plant performance due to relieved competition between AMF and plants for resources, thus reducing plant productivity, but that community temporal stability would be reduced because of decreased compensatory dynamics among plant species, (3) elevated temperature, N addition and AMF suppression would interactively affect community temporal stability, and (4) elevated temperature, N addition and AMF suppression would affect community temporal stability via mutually nonexclusive ecological factors (e.g., dominant species stability and community asynchrony) that operate independently or in combinations of two or more.

2. Materials and methods

2.1. Experimental field site and design

The experiment was conducted at the Songnen Grassland Ecological Research Station (44°45'N, 123°45'E), Northeast Normal University, Jilin Province, northeast China. The grasslands are widely distributed at the eastern end of the Eurasian steppe zone, which covers an area of approximately 1.7 × 10⁵ km² in the central part of northeastern China (Wang et al., 2007), and are characterized as temperate meadows. The average altitude of this flat region is 141 m above sea level, varying from 138 m to 145 m (Wang et al., 2007). The mean

annual precipitation is approximately 400 mm, 90% of which occurs in the growing season from May to October. The annual average air temperature is 4.9 °C, and the annual average land surface temperature is 6.2 °C. The soil in this area is soda-saline soil, and the soil pH is approximately 9.0, with 3–4% organic matter in the soil surface layer. The plant species *Leymus chinensis* (Trin. Tzvel) and *Puccinellia tenuiflora* (Griseb. Scribn. et Merr.) are dominant species at the experimental site.

In April 2015, we set up a field experiment in which grassland plants growing in 48 experimental plots were exposed to four air/soil temperature and soil N level treatments, i.e., no elevated temperature and background soil N level = C, elevated temperature without N addition = T, nitrogen addition without elevated temperature = N and elevated temperature and nitrogen addition in combination = TN with (F+) and without (F-) fungicide treatment. This resulted in a total of eight different treatment combinations ($n = 6$ per treatment; $N = 48$ experimental plots, Fig. S1). Hereafter, the abbreviations C, T, N and TN in F+ and F- are used in the text, figures and tables whenever referring to field experiment treatments. Each plot was 2.5 m wide \times 1 m long, and plots were 1 m apart in each block. Blocks were 2 m apart from each other. The average air temperature in northeastern China has increased by 1.70 °C in the past 50 years, and it will be a great challenge to limit the global increase in temperature to ≤ 2 °C (Chu et al., 2017; IPCC, 2018). Therefore, an elevated temperature of approximately 1.7 °C (average soil temperature with sensors from 0 to 30 cm, CR1000 Datalogger, Campbell Scientific Inc., Logan, UT, USA) was achieved by applying infrared heaters (MSR-2420, Kalgio Electronics Inc., Bethlehem, PA, USA) suspended over the plot center at a height of 2.25 m. Dummy heaters of the same shape and size were installed in the other plots. N deposition levels in Northern China averaged $6.06 \text{ g N m}^{-2} \text{ y}^{-1}$, and the deposition levels showed spatial and temporal variation in the range of $2.85\text{--}10.04 \text{ g N m}^{-2} \text{ y}^{-1}$ (Pan et al., 2012). Therefore, for N addition treatment, we added ammonium-nitrate ten times with 1 g N m^{-2} each time from May to September every two weeks each year for a total of $10 \text{ g N m}^{-2} \text{ y}^{-1}$. We chose this higher rate to simulate future N deposition rates, as the N deposition rate has increased year by year in this region. An identical amount of water (without N) was added to the other plots. Fungicide-treated plots received benomyl as a soil drench (18 g m^{-2} active ingredient in 10 L water) every two weeks from May to September each year, and an identical amount of water (without benomyl) was added to the other plots. The level of benomyl addition was determined based on a greenhouse experiment evaluating the efficacy and side effects of benomyl. Benomyl was chosen for the AMF suppression treatment, as it has been shown to suppress colonization of plant roots by glomalean fungi without altering other soil fungi and bacteria in field plots (Hartnett and Wilson, 1999; O'Connor et al., 2002; Yang et al., 2014) and having only negligible effects on plants and nontarget fungi (O'Connor et al., 2002; Yang et al., 2014). In our pot experiment, we found that the benomyl addition decreased mycorrhizal colonization the most when applied at 18 g m^{-2} (Table S1, Fig. S2). No detectable effect of benomyl addition on productivity (Table S1, Fig. S3) or soil total N concentration (Table S1, Fig. S4) for any rate of benomyl addition under sterilized soil was found. However, benomyl addition decreased productivity when applied at 18 g m^{-2} but increased productivity when applied at 72 g m^{-2} under non-sterilized soil.

2.2. Soil and root sampling for soil microbial and AMF analyses

In each plot in mid-September of 2017 and 2018, we collected, sieved ($\leq 2 \text{ mm}$) and mixed three soil cores (15 cm in depth, 5 cm in diameter) to create a composite soil sample. The fresh soil samples were frozen and stored at -80 °C for phospholipid fatty acid (PLFA) analysis in 2018. Roots from each plant species in each plot were cut into approximately 1-cm lengths and thoroughly mixed to estimate root mycorrhizal colonization.

AMF spores were extracted from soil samples using wet sieving and sucrose centrifugation (McKenney and Lindsey, 1987), mounted on glass slides and examined microscopically. The species or genus of

each spore was identified using taxonomic criteria (Schenck and Perez, 1990), the information published by INVAM (<http://invam.caf.wvu.edu/>) and Schüßler's Glomeromycota Species List (<http://www.lrz.de/~schuessler/amphylo/>).

Extraradical hyphae from soil samples were extracted and stained with trypan blue and hyphal length density was quantified microscopically (Jakobsen et al., 1992). Roots were cut into 1-cm segments and thoroughly mixed, and then a subsample of 0.5 g was cleared with 10% (w/v) KOH at 90 °C for 2 h and stained in 0.05% trypan blue in lactic acid glycerin solution (Kormanick and McGraw, 1982). Mycorrhizal colonization was then examined microscopically (Giovannetti and Mosse, 1980).

PLFAs were used to test the effects of benomyl on soil bacteria and fungi according to modified Bligh and Dyer methodology (Frostegard et al., 1991). PLFA analyses were performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and SHERLOCK software (MIDI Inc., Newark, NJ, USA). The fatty acids i14:0, i15:0, a15:0, 15:0, i16:0, 16:1 ω 7c, i17:0, a17:0, 17:0cy, 17:0, 18:1 ω 7c and 18:1 ω 5c were chosen to represent bacterial PLFAs. 16:1 ω 5 was selected to represent the AMF PLFAs, and 18:2 ω 6.9 and 18:1 ω 9c were taken to represent the PLFAs of other fungi (Schnoor et al., 2011).

2.3. Plant community survey

A permanent quadrat of $1 \text{ m} \times 1 \text{ m}$ was established in each subplot in May 2015. From 2015 to 2018, we recorded each plant species within each quadrat in mid-July. In early September from 2015 to 2018, all aboveground tissues within randomly selected $0.25 \text{ m} \times 0.25 \text{ m}$ quadrats in each plot outside the permanent quadrat were clipped, classified into three different groups (dominant, common and rare species) and oven-dried at 65 °C for 48 h to determine peak aboveground biomass. The abundance curve for aboveground biomass vs species rank across all the plots was used to define dominant species with relative abundance $>5\%$, common species with relative abundance ranging from 1% to 5% and rare species with relative abundance $<1\%$ (Ma et al., 2017). This classification regime yielded 4 dominant species, 6 common species and 23 rare species (Table S2, Fig. S5).

We quantified the temporal stability of community productivity in the field experiment as the ratio of the temporal mean of community productivity (μ) to its temporal standard deviation (s.d.) (σ) in each plot over the sampling period (2015–2018) (Tilman, 1999; Hautier et al., 2014). The temporal stability of individual species was calculated using the same method but applied to each species within a community. Species richness was defined in each plot as the total number of species recorded in a $1 \text{ m} \times 1 \text{ m}$ permanent quadrat within a year. We also calculated Simpson's dominance index (Smith and Wilson, 1996). The degree of species asynchrony was quantified by the community-wide species asynchrony index (Loreau and de Mazancourt, 2008), defined as:

$$1 - \Phi_x = \frac{1 - \sigma^2}{\left(\sum_{i=1}^S \sigma_i\right)^2}$$

where Φ_x is species synchrony, σ^2 is the temporal variance of community biomass and σ_i is the s.d. of biomass of species i in a plot with S species. No significant temporal trend in community productivity was detected during the experimental period; thus, no detrending was conducted.

2.4. Data analysis

All analyses were conducted using R version 3.6.0. (R Core Team, 2019). We performed linear mixed-effects models (*nlme* package; Pinheiro et al., 2020) to test the effects of elevated temperature, N addition, fungicide addition and their interactions on spore density, AMF species richness, hyphal length density, mycorrhizal colonization, PLFA concentrations (AMF, fungi and bacteria), temporal mean and standard

deviation of community productivity, plant species richness, Simpson's dominance, community temporal stability, community-wide species asynchrony, species asynchrony and temporal stability of productivity for each of the three different abundance groups (dominant, common and rare species). Elevated temperature, N addition and fungicide addition were treated as fixed factors, and plots nested into blocks were treated as random factors. Bonferroni's post hoc test was used in the case of significant treatment interactions (*emmeans* package; Lenth, 2020). Additionally, for the main effects and two-way interactions, we use the terms T+ vs T- for the temperature main effect, N+ vs N- for the N addition main effect and F+ vs F- for the fungicide main effect; for two-way interactions, e.g., T x N: T+ vs T- in N- and N+, and then N+ vs N- in T- and T+, we tested both T and N effects in each combination. All of the data met assumptions of normality and homogeneity of variances.

We performed linear mixed-effects models to assess relationships between spore density, AMF species richness, hyphal length density, mycorrhizal colonization, AMF PLFA concentrations, community-wide species asynchrony, species asynchrony of the three abundance groups (dominant, common and rare), species richness, Simpson's dominance, the stability of productivity of the three abundance groups (dominant, common and rare), temporal mean of community productivity, temporal standard deviation of community productivity and community temporal stability. Linear mixed-effects models were also used to assess whether their relationships differed with and without fungicide addition because we wanted to determine whether the suppression of AMF could change the relationship between community temporal stability and its regulators.

Structural equation modeling (SEM) was used to explore the pathways by which elevated temperature, N addition and fungicide addition affected community temporal stability. Here, we focused on two components of community temporal stability: temporal mean and temporal standard deviation of community productivity. Based on linear mixed-effects model results and knowledge from the literature, we constructed an a priori conceptual structural equation model (Fig. S6) depicting the direct and indirect effects of elevated temperature, N addition, fungicide addition, AMF species richness, mycorrhizal colonization, dominant plant species asynchrony, community-wide plant species asynchrony, dominant plant species stability, Simpson's dominance and plant species richness on the two components of community temporal stability. SEM was performed by using linear mixed models ('lme' function of the *nlme* package) with block as random factor (*piecewiseSEM* package; Lefcheck, 2016). The model was then simplified by stepwise exclusion of variables with nonsignificant weights and nonsignificant covariance until a minimal adequate model showing specific linkages remained, estimated by the lowest Akaike information criterion (AIC). The model fit was evaluated by χ^2 ($P > 0.05$) and Fisher's C test (when $0 < \text{Fisher's } C/df < 2$ and $0.05 < P < 1.00$), and we report the standardized coefficient for each path, as well as the R^2 values calculated for each linear mixed-effects model.

Table 1

Results of linear mixed-effects models (LMEs) testing the effect of elevated temperature (T), nitrogen addition (N), fungicide addition (F) and their interactions on spore density, hyphal length density (HLD), mycorrhizal colonization, AMF species richness and biomass of AMF, fungi (no AMF) and bacteria estimated through phospholipid fatty acid (PLFA) analysis.

	Spore density		HLD		Colonization		Richness		AMF PLFA		Other fungi PLFA		Bacteria PLFA	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
T	4.22	0.04	7.98	0.01	2.06	0.16	4.39	0.04	0.03	0.87	1.67	0.21	1.68	0.20
N	1.35	0.25	0.01	0.94	4.36	0.04	30.03	<0.001	0.01	0.94	0.42	0.52	0.53	0.47
F	28.14	<0.001	54.56	<0.001	40.86	<0.001	13.74	<0.001	11.53	<0.01	3.36	0.08	2.79	0.10
T x N	0.04	0.83	4.53	0.04	0.35	0.55	0.23	0.63	0.22	0.64	0.83	0.37	0.85	0.36
T x F	1.20	0.27	2.27	0.13	0.49	0.49	2.60	0.12	0.01	0.91	0.00	1.00	0.20	0.66
N x F	0.20	0.65	0.63	0.43	3.94	0.05	4.39	0.04	4.95	0.03	0.28	0.60	0.84	0.37
T x N x F	0.00	0.95	0.15	0.70	3.29	0.07	0.94	0.34	0.35	0.56	1.74	0.20	1.67	0.20

Significant results ($P < 0.05$, $P < 0.01$, $P < 0.001$) are bolded.

3. Results

3.1. Soil microclimate

Table S4 presents all the main and interaction effects of F- and P-values. Both T+ vs T- and N+ vs N- increased the soil temperature by 1.7 °C and 0.5 °C, respectively, across the four growing seasons (Fig. S7). T+ vs T- in N- and N+ significantly decreased soil moisture on average by $13 \pm 1\%$ and $20 \pm 2\%$, respectively (Fig. S7). N+ vs N- in W- increased soil moisture on average by $6 \pm 1\%$ (Fig. S7).

3.2. Elevated temperature, N addition and fungicide effects on soil microbes and AMF

Table 1 shows all the main and interaction effects of F- and P-values. After four years of application, T+ vs T- increased AMF spore density but decreased AMF richness on average by $30 \pm 4\%$ (Fig. 1a) and $20 \pm 3\%$ (Fig. 1b), respectively. F+ vs F- decreased AMF spore density and hyphal length density on average by $49 \pm 4\%$ (Fig. 1c) and $23 \pm 2\%$ (Fig. 1d), respectively. A significant T x N effect was detected for hyphal length density (Fig. 1e). T+ vs T- in N- increased the hyphal length density by $16 \pm 3\%$, but T+ vs T- in N+ had no significant impact on the hyphal length density (Fig. 1e). A significant N x F effect was detected for AMF richness, mycorrhizal colonization and AMF PLFA concentrations (Fig. 1f, g, h). F+ vs F- in N- decreased AMF richness, mycorrhizal colonization and AMF PLFA concentrations by $23 \pm 4\%$ (Fig. 1f), $60 \pm 4\%$ (Fig. 1g) and $42 \pm 12\%$ (Fig. 1h), respectively. F+ vs F- in N+ did not affect AMF richness (Fig. 1f) but decreased mycorrhizal colonization and AMF PLFA concentrations by $36 \pm 6\%$ (Fig. 1g) and $46 \pm 6\%$ (Fig. 1h), respectively. N+ vs N- in F- decreased AMF richness and mycorrhizal colonization by $23 \pm 4\%$ (Fig. 1f) and $31 \pm 3\%$ (Fig. 1g), respectively but had no significant effect on AMF PLFA concentrations (Fig. 1h). N+ vs N- in F+ only decreased AMF richness by $16 \pm 3\%$ (Fig. 1f) but did not affect mycorrhizal colonization (Fig. 1g) or AMF PLFA concentrations (Fig. 1h). The concentrations of other fungal and bacterial PLFAs were not significantly affected by any of the treatments, and no signs of pathogenic fungi or their effects were observed through visual inspection of the roots (Table 1).

3.3. Elevated temperature, N addition and fungicide effects on the grassland community and its stability

Table 2 presents all the main and interaction effects of F- and P-values. T+ vs T- decreased Simpson's dominance, and N+ vs N- decreased plant species richness by an average of $20 \pm 3\%$ (Fig. 2a) and $15 \pm 2\%$ (Fig. 2b), respectively. A significant T x F effect was detected for plant species richness (Fig. 2c). F+ vs F- in T- and T+ decreased plant species richness by $21 \pm 3\%$ and $31 \pm 3\%$, respectively (Fig. 2c). A significant N x F effect was detected for community productivity (Fig. 2d). F+ vs F- in N- increased community productivity

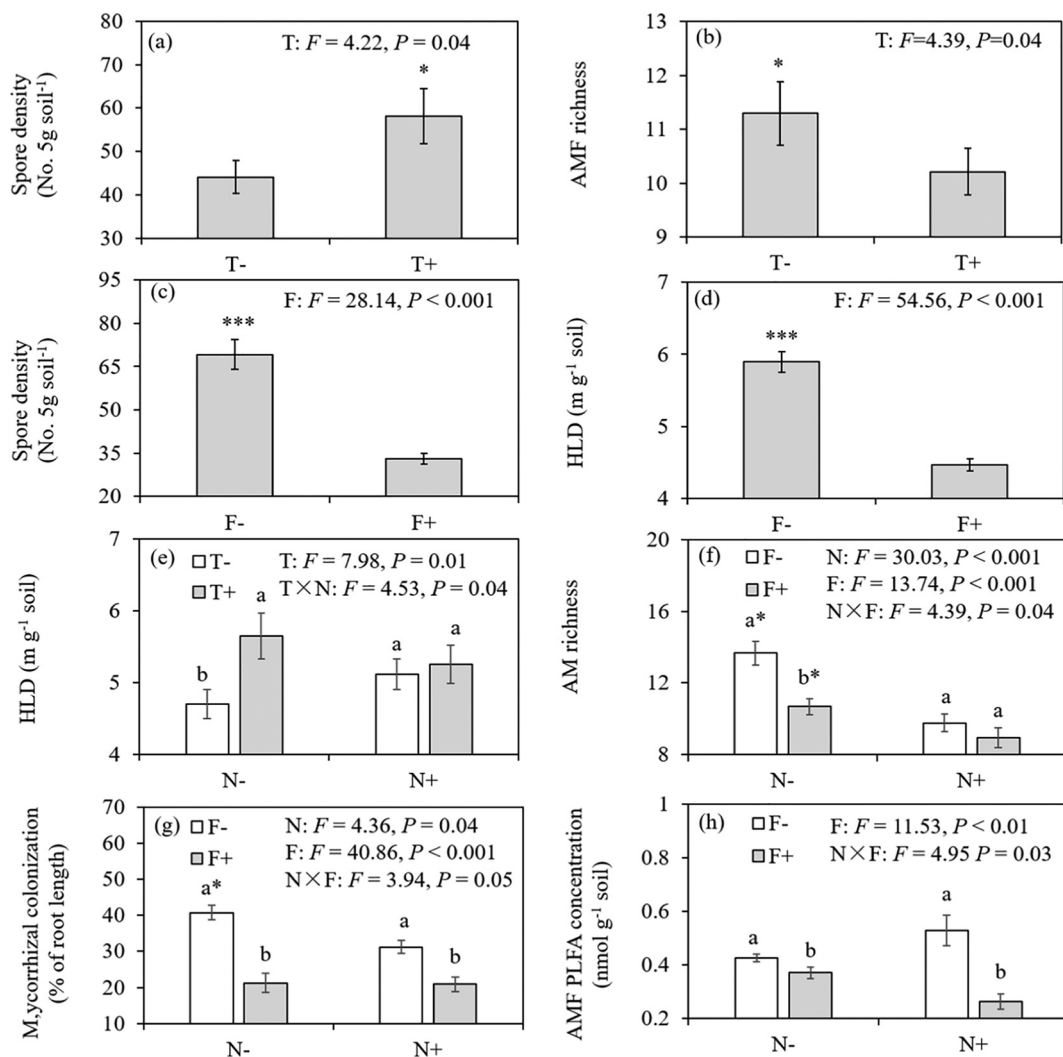


Fig. 1. Effect of T+ vs T- on spore density (a) and AMF richness (b). Effect of F+ vs F- on spore density (c) and hyphae length density (HLD) (d). Interactive effect of elevated temperature (T) and N addition (N) on HLD (e). Interactive effect of N addition (N) and fungicide addition (F) on AMF richness (f), mycorrhizal colonization (g) and AMF PLFA concentration (h). Asterisks indicate significant differences among treatments at $P \leq 0.05$ (*) and $P \leq 0.001$ (***) (a–d). Asterisks indicate a significant difference between N+ vs N- in F- and F+ at $P \leq 0.05$ (*) (f, g). Different lowercase letters indicate significant differences between T+ vs T- in N- and N+ at $P \leq 0.05$ (e). Different lowercase letters indicate significant differences between F+ vs F- in N- and N+ at $P \leq 0.05$ (f, g, h). The data are the means \pm standard errors.

by $24 \pm 4\%$, but F+ vs F- in N+ had no effect on community productivity (Fig. 2d). N+ vs N- in F- increased community productivity by $22 \pm 3\%$, but N+ vs N- in F+ did not affect community productivity (Fig. 2d). No treatment effect on Simpson's diversity was found (Fig. S8).

T+ vs T- and F+ vs F- reduced the community temporal stability by an average of $27 \pm 4\%$ (Fig. 3a) and $25 \pm 3\%$ (Fig. 3b), respectively. N+ vs N- decreased dominant species stability by an average of $24 \pm 4\%$ (Fig. 3c). A significant T \times F effect was detected for dominant species stability (Fig. 3d). F+ vs F- in T- decreased dominant species stability by $29 \pm 5\%$, while F+ vs F- in T+ did not affect dominant species stability (Fig. 3d). T+ vs T- in F- decreased dominant species stability by $28 \pm 4\%$, but T+ vs T- in F+ did not affect dominant species stability (Fig. 3d). Dominant species stability accounted for $82 \pm 3\%$ of the community temporal stability across all treatments. We only found that T+ vs T- increased rare species asynchrony by an average of $42 \pm 8\%$ and F+ vs F- decreased common species asynchrony by an average of $65 \pm 7\%$ across all treatments (Table 2). We did not find interactive effects of any of the treatments on community temporal stability, and no treatment effect on community asynchrony was detected (Table 2).

3.4. Environmental and ecological factors influencing community temporal stability

The relationships between community temporal stability and dominant species stability ($F_{1,39} = 5.13, P = 0.02$; Fig. 4a), community asynchrony ($F_{1,39} = 4.29, P = 0.04$; Fig. 4b) and standard deviation of community productivity ($F_{1,39} = 5.86, P = 0.02$; Fig. 4c) differed between F+ and F- treated plots. The community temporal stability increased with the level of dominant species stability and community asynchrony, but this relationship was much steeper without fungicide addition (Fig. 4a, b). The community temporal stability decreased with the level of temporal standard deviation of community productivity, with much lower community temporal stability under fungicide addition (Fig. 4c). Community temporal stability has no significant correlation with the temporal mean of community productivity (Fig. 4d). Only dominant species asynchrony, AMF richness and mycorrhizal colonization were related to community temporal stability, and no relationship between plant species richness, Simpson's dominance, common species asynchrony (stability), rare species asynchrony (stability), spore density, hyphae length density, AMF PLFA concentrations or community temporal stability was detected (Table S3).

Table 2 Results of linear mixed-effects models (LMEs) testing the effect of elevated temperature (T), nitrogen addition (N), fungicide addition (F) and their interactions on temporal mean of community productivity, temporal standard deviation (s.d.) of community productivity, species richness, Simpson's dominance, community temporal stability, dominant species stability, common species stability, community asynchrony, dominant species asynchrony, common species asynchrony and rare species asynchrony.

	Productivity (g m ⁻²)			Richness			Simpson's Dominance			Community Temporal Stability			Dominant Species Stability			Common Species Stability			Rare Species Stability			Community Asynchrony			Dominant Species Asynchrony			Common Species Asynchrony			Rare Species Asynchrony			
	F	P	s.d.	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P			
	T	0.30	0.58	4.67	0.04	0.60	4.46	0.04	4.41	0.04	16.75	< 0.001	4.00	0.05	0.53	0.47	1.60	0.21	0.17	0.69	0.02	0.90	4.44	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
N	1.87	0.17	3.93	0.05	8.43	0.33	0.56	0.31	0.58	4.76	0.04	0.29	0.59	1.99	0.17	1.31	0.26	1.20	0.28	0.62	0.62	0.07	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	
F	10.89	< 0.01	6.13	0.02	37.95	0.47	0.49	5.28	0.03	8.87	0.01	0.37	0.55	1.07	0.31	0.12	0.73	0.49	0.49	5.02	0.03	0.12	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	
T × N	0.26	0.61	0.69	0.41	1.31	0.26	0.00	0.99	0.41	0.53	1.08	0.31	0.26	0.11	0.65	0.43	0.36	0.55	0.83	0.03	0.87	0.78	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	
T × F	1.05	0.31	2.66	0.11	5.23	0.79	0.37	2.94	0.10	4.00	0.05	0.95	0.34	1.12	0.30	0.51	0.48	0.20	0.66	0.00	0.92	0.10	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	
N × F	4.92	0.03	1.05	0.31	0.01	0.93	0.00	0.97	0.11	0.74	0.06	0.81	0.37	1.20	0.28	0.06	0.86	0.81	0.39	0.54	0.18	0.67	2.11	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	
T × N × F	0.12	0.72	0.86	0.36	0.01	0.96	0.33	1.68	0.20	0.00	0.98	1.91	0.18	0.31	0.58	0.03	0.86	0.01	0.92	2.08	0.16	0.05	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83

Significant results (P<0.05, P<0.01, P<0.001) are bolded.

SEM explained 81% of the variance in community temporal stability (Fig. 5a). Elevated temperature, N and fungicide additions induced multiple and sometimes opposite pathways affecting community temporal stability (Fig. 5a). Elevated temperature, N and fungicide additions reduced the positive effect of dominant species stability on community temporal stability. Elevated temperature and fungicide addition reduced the positive effect of AMF richness on community temporal stability. N addition and fungicide addition reduced the positive effect of mycorrhizal colonization on community temporal stability. Additionally, elevated temperature indirectly increased community temporal stability via an increased temporal mean. N addition directly increased community temporal stability. Fungicide addition directly reduced community temporal stability and indirectly increased community temporal stability via an increased temporal mean.

Standardized total effects derived from the SEM revealed that community temporal stability was mainly driven by dominant species stability, followed by AMF richness, community asynchrony, and mycorrhizal colonization; the overall effects of elevated temperature and fungicide addition on community temporal stability were negative, whereas the overall effect of N addition on community temporal stability was neutral (Fig. 5b).

4. Discussion

4.1. Elevated temperature, N addition and fungicide addition affected AMF functions

Elevated temperature increased spore density but decreased AMF richness; N addition decreased AMF richness and mycorrhizal colonization; and fungicide addition decreased spore density, hyphal length density, AMF richness, mycorrhizal colonization and AMF PLFA concentrations. The positive effect of elevated temperature on AMF spore density may result from increases in root C allocation for AMF sporulation (Bai et al., 2012), or the formation of large amounts of spores may be a strategy adopted by AMF for survival in response to such environmental stress (Kim et al., 2015). However, species sporulating little and/or rarely were reduced by elevated temperature, and AMF richness was thus reduced (Sun et al., 2013). In contrast, the negative effect of N addition on AMF richness and mycorrhizal colonization may be explained by enhanced competition for carbohydrates among fungal species, as plants allocate less carbon to mycorrhizal symbioses due to relieved limitation of N resources (Liu et al., 2012). The negative effect of fungicide addition on AMF without alteration of the PLFA concentrations of other soil fungi and bacteria was observed, verifying that AMF function was reduced by fungicide addition and excluding pathogenic fungi effects, in accordance with previous results in a tall-grass prairie and temperate steppe (Hartnett and Wilson, 1999; Yang et al., 2014). In addition, elevated temperature-induced increases in hyphal length density were hampered by N addition, resulting in no changes in hyphal length density, which indicated antagonism between these two factors. N addition can also interact with fungicide addition to reduce AMF richness, mycorrhizal colonization and AMF PLFA concentrations. These results indicated that N addition exerts a more negative effect on AMF than elevated temperature and that fungicide addition can suppress AMF functions effectively.

Moreover, some previous studies showed that fungicide (benomyl) is a risk to soil invertebrates which may influence nutrient absorption and plant growth. For example, benomyl had subtle and sublethal toxic effects on earthworm giant nerve fibers (Drewes et al., 1987) and increased migration by promoting earthworm movement over the surface of the ground more than normal activity (Christensen and Mather, 2004). Moreover, the fecundity of ants was reduced by benomyl application, which might be responsible for the altered species composition of ant assemblages (Pech and Heneberg, 2015). Since humid and warmer conditions might enhance the toxicity of some pesticides by increasing penetration through the skin of animals (Römbke

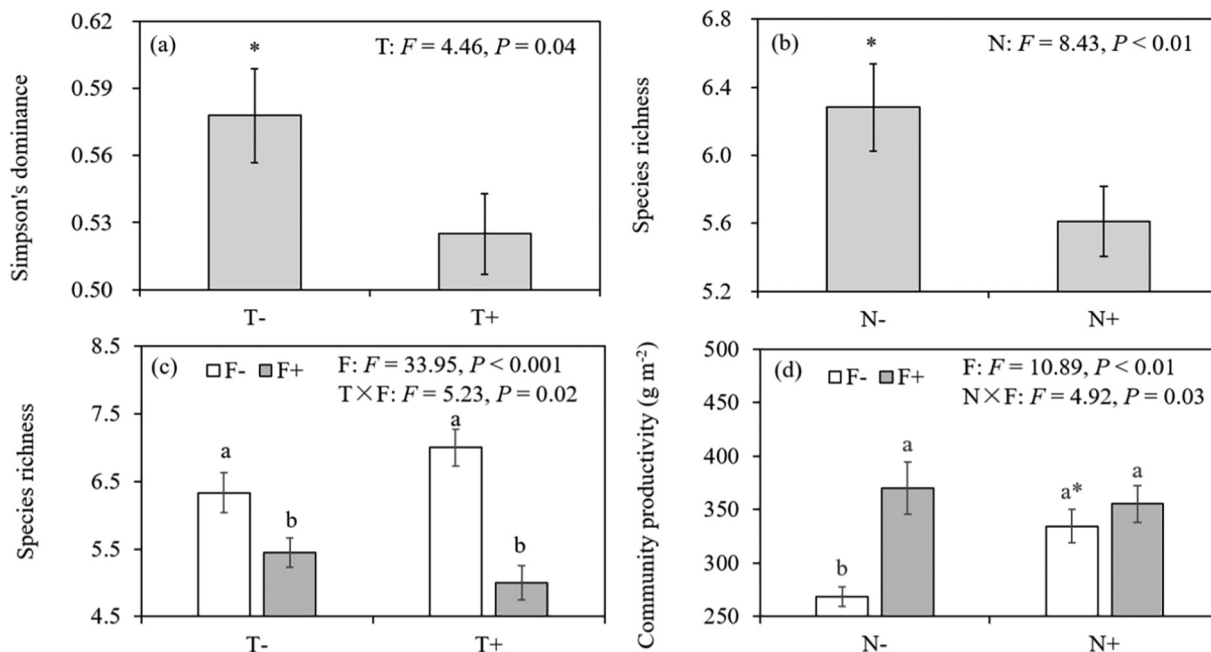


Fig. 2. Effect of T+ vs T- on Simpson's dominance (a). Effect of N+ vs N- on plant species richness (b). Interactive effect of elevated temperature (T) and fungicide addition (F) on plant species richness (c). Interactive effect of N addition (N) and fungicide addition (F) on community productivity (d). Asterisks indicate significant differences among treatments at $P \leq 0.05$ (*). Different lowercase letters indicate significant differences between F+ vs F- in T- and T+ at $P \leq 0.05$ (c). Different lowercase letters indicate significant differences between F+ vs F- in N- and N+ at $P \leq 0.05$ (d). Asterisks indicate a significant difference between N+ vs N- in F- at $P \leq 0.05$ (*). The data are the means \pm standard errors.

et al., 2007), the toxicity of benomyl may lessen to neutral in our temperate steppe due to drought stress. However, studies have also shown that the total numbers of enchytraeids and nematodes were not affected by benomyl and that their influence on plant growth was negligible (Martikainen et al., 1998).

4.2. AMF suppression and N addition affected community productivity

After 4 years, F+ vs F- in N- increased community productivity, while F+ vs F- in N+ had no effect on community productivity, which

is consistent with the previous result showing that the application of fungicide increased maize yield under conditions of low N availability but not under conditions of high N availability (Wang et al., 2018). AMF acquire significant amounts of N from the soil for their growth, and the N concentration of extraradical mycelium hyphae is 4–7 times as high as that of plant shoots and 10 times that of the roots, which would induce competition for N between AMF and host plants (Hodge and Fitter, 2010). Suppression of AMF alleviated N immobilization in AMF mycelium and enhanced plant performance under N-limiting soils. Moreover, fungal-derived mineral and organic N resources from

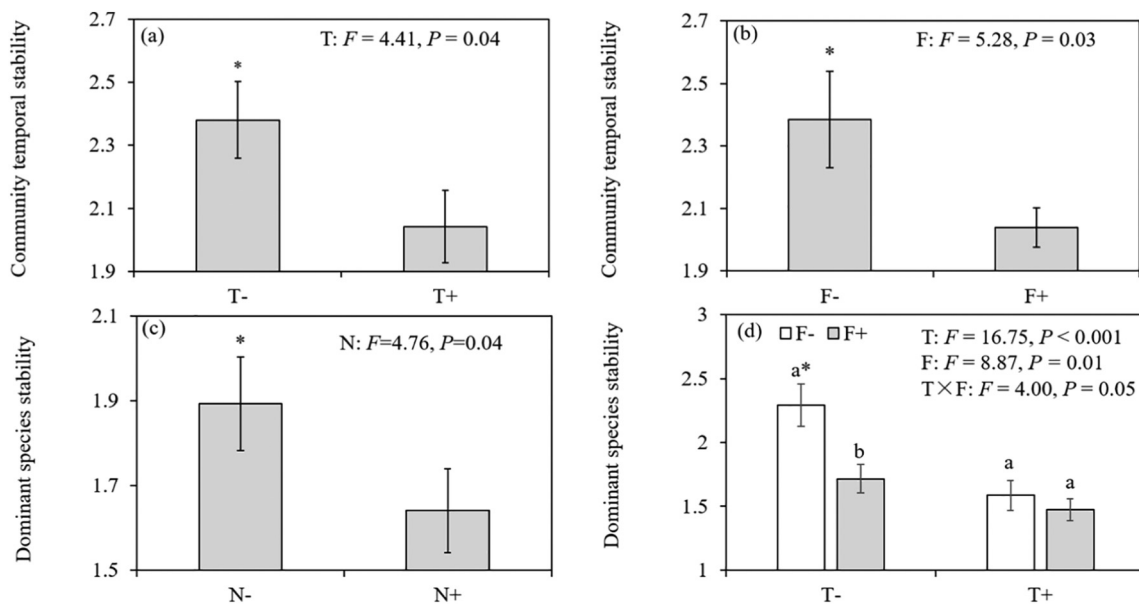


Fig. 3. Effect of T+ vs T- (a) and F+ vs F- (b) on community temporal stability. Effect of N+ vs N- on dominant species stability (c). Interactive effect of elevated temperature (T) and fungicide addition (F) on dominant species stability (d). Asterisks indicate significant differences among treatments at $P \leq 0.05$ (*). Asterisks indicate a significant difference between T+ vs T- in F- and F+ at $P \leq 0.05$ (*). Different lowercase letters indicate significant differences between F+ vs F- in T- and T+ at $P \leq 0.05$. The data are the means \pm standard errors.

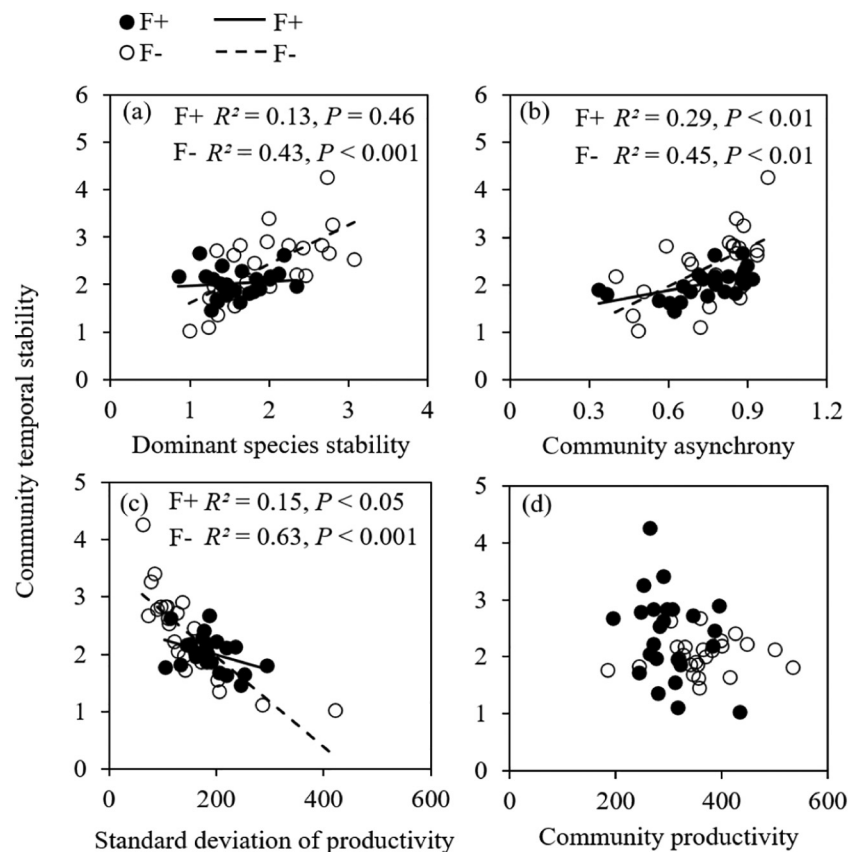


Fig. 4. Relationships between community temporal stability and (a) plant dominant species stability, (b) community asynchrony, (c) temporal standard deviation of productivity and (d) temporal mean of productivity with F+ (solid lines) and F- (dotted lines). The relationships between community temporal stability and plant dominant species stability (a), community asynchrony (b), and temporal standard deviation of productivity (c) were different between F+ and F- ($P \leq 0.05$). Community temporal stability was not related to the temporal mean of community productivity (d).

senescing fungal mycelium are released with fungicide addition (Veresoglou et al., 2011) and ‘translate’ into increased biomass production. Moreover, N+ vs N- in F- increased community productivity, while N+ vs N- in F+ did not affect community productivity. These results are in accordance with previous study that N addition increased maize yield without fungicide application but not with fungicide application (Wang et al., 2018). N addition did not change community productivity in the absence of AMF because of soil P limitation, but increased community productivity in the presence of AMF due to alleviated P limitation since AMF can promote absorption of P for plants, which has been demonstrated before (Grogan and Chapin, 2000).

In addition, as water is a limiting factor for plant growth, especially in temperate meadows (Gong et al., 2015) and there was no control treatment without water in this study, water may exert its potential beneficial effects on our results. However, the amount of water we added was equivalent to ~2 mm of rainfall each time and ~20 mm in total, which was a 5% increase in ambient annual precipitation (400 mm) in this area. A previous study also found that a 20% increase in ambient precipitation had no impact on community cover and plant species richness at the site (Zhong et al., 2019). Therefore, the effect of water on our results could be neglected or otherwise weaken our treatment effect because of the lack of solvent. Actually, in this study, elevated temperature and the combination of elevated temperature and N addition decreased soil moisture and offset the promoting effect on plant growth due to an accelerated growth rate and thus had no impact on community productivity owing to drought stress, which is in accordance with previous results showing that elevated temperature-induced decreases in soil water content suppress plant growth by stimulating evapotranspiration

(Niu et al., 2008). Thus, side effects of elevated temperature on the plant community will be more important than direct effects, especially in water-limited regions (Yang et al., 2011a).

4.3. Elevated temperature, N addition and AMF suppression affected community temporal stability by changing dominant species stability, AMF richness and mycorrhizal colonization

Our results showed that community temporal stability was determined by dominant species stability, AMF richness, which was associated with community asynchrony and mycorrhizal colonization under elevated temperature, N addition and AMF suppression. First, dominant species stability tends to positively affect the temporal stability of community productivity (Yang et al., 2016; Ma et al., 2017). In our study, elevated temperature, N addition and AMF suppression decreased dominant species stability and then decreased community temporal stability. The relative abundance of dominant species accounted for 83%, and its stability was positively associated with community temporal stability, but that of common and rare species, which accounted for only 17% of all species, had no impact on community temporal stability, supporting the ‘‘mass ratio’’ hypothesis and highlighting the role of dominant species in ecosystem stability, a role that could be more important than species richness in determining community temporal stability (Grime, 1998). Second, species asynchrony has been verified to determine community temporal stability (Yang et al., 2011b; Ma et al., 2017). In this study, we also found that community asynchrony positively correlated with community temporal stability. This result highlights the importance of compensatory mechanisms, in which the declines in the biomass

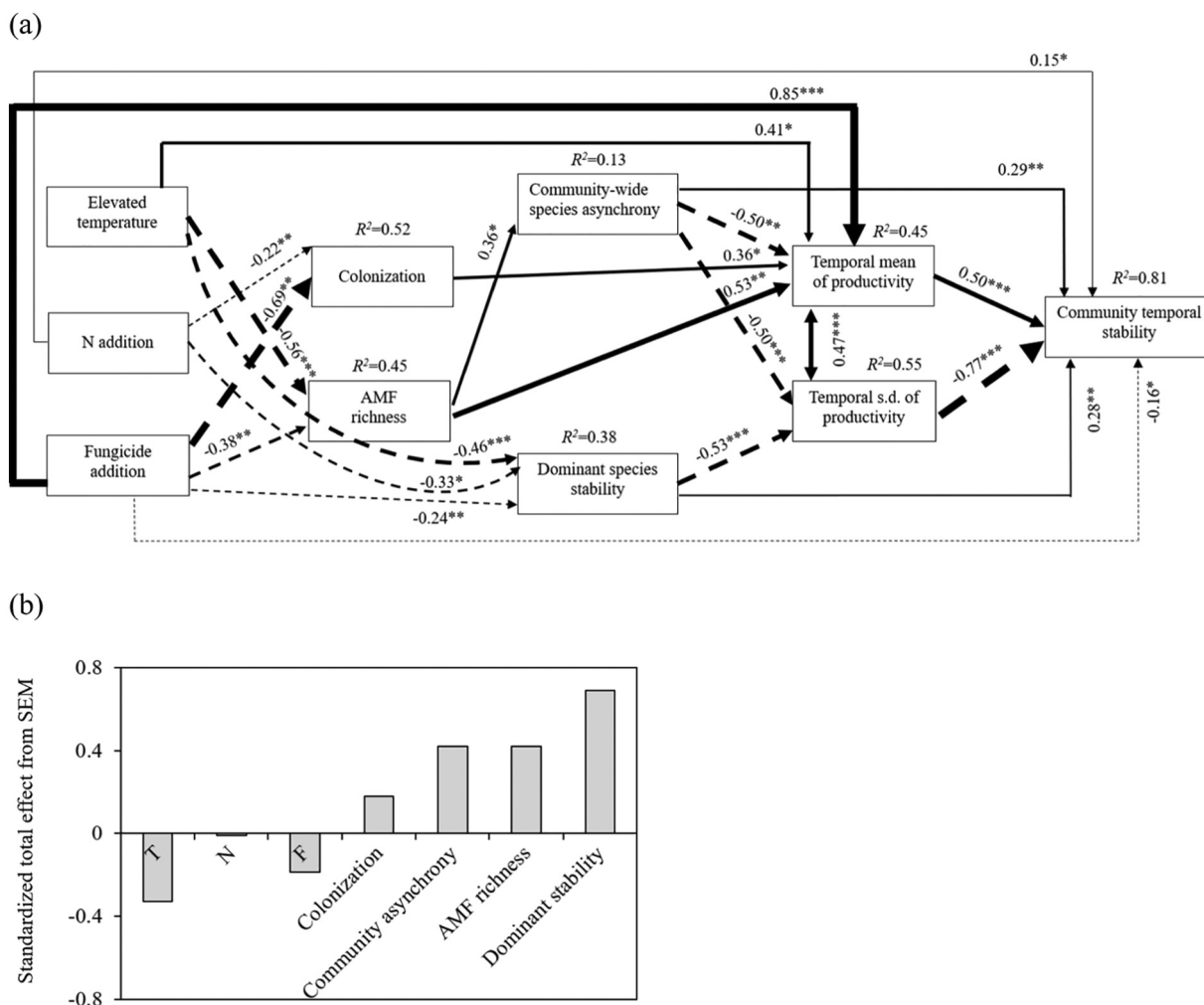


Fig. 5. (a) Results of structural equation modeling (SEM) showing the pathways through which elevated temperature, nitrogen and fungicide addition influence the community temporal stability and their standardized path coefficients ($\chi^2 = 43.03$, $df = 38$, $P = 0.27$; Akaike information criteria (AIC) = 115.03). (b) Standardized total effects (direct plus indirect effects) of each treatment, mycorrhizal colonization, AMF richness, community-wide species asynchrony and dominant species stability for community temporal stability. Boxes indicate measured variables entered in the model. Single-headed arrows represent causal relationships, and double-headed arrows represent covarying variables. Positive and negative paths ($P \leq 0.05$) are shown as solid and dashed arrows, respectively, with the thickness of the paths (arrows) scaled to the magnitude of the path strength. Path strengths are designated by arrows and R^2 indicates the proportion of variance explained. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

of some species could be compensated by the increases of other species, alleviating temporal fluctuation in the productivity of the whole community (Hautier et al., 2014). Community asynchrony and dominant species asynchrony were not affected by any of our treatments, while elevated temperature increased rare species asynchrony, and fungicide addition decreased common species asynchrony (Table 2), which is in disagreement with other studies reporting that community asynchrony is increasingly suppressed by global change (Xu et al., 2015; Ma et al., 2017). One possible explanation is that our studied grassland has lower species richness than alpine grassland (Ma et al., 2017), making it difficult to detect the effects of species asynchrony on community temporal stability. This is probably also because temperature is not a limiting factor in temperate meadows, but precipitation is (Yang et al., 2011a). Additionally, the longer the term of the study project, the greater the possibility of asynchrony. Third, AMF species richness can influence community temporal stability by changing community asynchrony under elevated temperature and fungicide addition. Because AMF can change competition among plant species and alter the coexistence of plants and resource partitioning between co-occurring plants (van der Heijden et al., 2003; Wagg et al., 2011; Yang et al.,

2014), AMF can mediate plant interactions and finally increase compensatory effects among plant species and functional groups (van der Heijden et al., 2003; Wagg et al., 2011; Yang et al., 2014).

Community temporal stability was defined as the ratio of the mean of community productivity to its standard deviation over the experimental period. Changes in community temporal stability can therefore result from changes in both variables (temporal mean and temporal variation). In this study, dominant species stability and AMF richness affected community temporal stability via changes in the temporal variation of community productivity, while AMF richness and mycorrhizal colonization changed community temporal stability through changes in the temporal mean of community productivity.

Community temporal stability has also been shown to be positively associated with plant diversity in previous studies (Tilman et al., 2006; Cardinale et al., 2012; Hautier et al., 2015; Zhang et al., 2018). However, in the present study, although N addition and AMF suppression decreased plant species richness, plant species richness had little effect on community temporal stability. This finding is consistent with results from experiments on semiarid and wet grasslands varying from 7 to 18 years in length (Grman et al., 2010; Xu et al., 2015; Yang et al., 2016). A possible explanation for the

lack of a species diversity effect on community temporal stability in our study is that most of the lost or gained species in response to N addition and AMF suppression were rare species that contributed only to a small part of community productivity compared to dominant species, making species diversity an unimportant driver of community temporal stability, which is consistent with previous findings (Xu et al., 2015; Ma et al., 2017). Simpson's diversity was not found to be affected by each treatment, which may be attributed to the equal effect on species richness and evenness.

4.4. Elevated temperature, N addition and AMF suppression independently affected community temporal stability

The results of this study showed that elevated temperature and AMF suppression independently affected community temporal stability but interactively affected dominant species stability. A possible explanation is that both factors affected dominant species stability through the same mechanisms but that there were different mechanisms for community temporal stability. Specifically, elevated temperature and AMF suppression might mainly have a direct impact on dominant species stability but affect community temporal stability in both direct and indirect ways. We also noticed that AMF suppression decreased plant species richness under elevated temperature more than that under no temperature elevation, suggesting that suppressed AMF exacerbated plant species lost under a global change context, which may have a far-reaching effect on community temporal stability in the long-term.

5. Conclusions

Our study provides new empirical evidence that elevated temperature and AMF suppression decreased community temporal stability, while N addition was a neutral factor. Our results also highlight the different mechanisms associated with the effects of elevated temperature, increased N deposition and AMF suppression on community temporal stability in temperate meadows. Furthermore, dominant species stability, AMF richness and mycorrhizal colonization were common paths that these treatments used to affect community temporal stability, suggesting the importance of belowground AMF in stabilizing aboveground plant community temporal stability under a global change context. These findings point to the need for evaluating microbial performance in response to projected future environmental changes and their role in maintaining the structure and functions of the aboveground plant community.

CRedit authorship contribution statement

Xue Yang: Software, Writing - original draft, Visualization. **Pierre Mariotte:** Writing - review & editing, Visualization. **Jixun Guo:** Conceptualization, Methodology, Validation, Supervision. **Yann Hautier:** Writing - review & editing, Visualization. **Tao Zhang:** Conceptualization, Methodology, Validation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.143137>.

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