Functional traits rather than abiotic factors determine the response of flowering phenology to biodiversity loss and nitrogen addition

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Abstract

1. Numerous evidence agree that global changes have altered plant phenology, abiotic factors and functional traits are center drivers linking phenology. However, few studies have considered the joint effects of these factors on flowering phenology under nitrogen (N) inputs and biodiversity loss. 2. A common garden experiment with two N addition and six plant diversity levels was established in Beijing. We assessed the effects of N addition and plant diversity loss on three flowering phenology events of Medicago sativa via functional traits and abiotic factors. 3. The first flowering day (FFD) delayed, the last flowering day (LFD) advanced, and flowering duration (FD) shortened after N addition. While FFD advanced, LFD delayed, and FD extended an average of 0.31, 0.64, and 0.95 days per species lost, respectively. Importantly, three analysis methods had been used to prove that the contributions of functional traits for the variance in flowering phenology changes was significantly larger than abiotic factors under biodiversity loss and N addition. 4. Our findings illustrate the non-negligible effects of functional traits on flowering phenology, and highlight the importance of including functional traits in phenology models to improve predictions of the response of plant phenology to N inputs and biodiversity loss.

1 | Introduction

Human activities such as agricultural production and fossil fuel combustion add ˜200 Tg/year of reactive nitrogen (N) to global ecosystems, which is approximately equal to that provided by natural N fixation (Galloway et al., 2004; Schlesinger, 2009; Fowler et al., 2013). Apart from N deposition increasing, biodiversity loss is another global change issues that human being confront, which is mainly induced by land use change, climate change, and N deposition increase (Sala et al., 2000; Gossner et al., 2016; Harpole et al., 2016). Recent study showed that the world's seed-bearing plants have been disappearing at a rate of nearly 3 species a year since 1900, which is up to 500 times higher than would be expected as a result of natural forces alone (Ledford, 2019). It has been proved that the plant life cycles and ecosystem functions is significantly changed under N deposition increase and plant diversity loss (Hautier et al., 2014; Grace et al., 2016; Wolf et al., 2017; Pennekamp et al., 2018), yet how N addition, biodiversity loss, and its interactions influence on plant phenology remains uncertain.

Flowering phenology, including flowering date and duration, is important developmental stages in plant phenology as well as a sensitive indicator of global changes (Hovenden et al., 2008; Hulme 2011; Wang et al., 2014; Suonan et al., 2017; Piao et al., 2019). Many factors control the flowering phenology in grassland

ecosystems, N deposition increase and biodiversity loss are two of the most important (Sanz et al., 2011; Smith et al., 2012; Xia & Wan, 2013; Xi et al., 2015; Wolf et al., 2017; Wang & Tang, 2019a). While numerous studies reveal that N addition delayed the flowering phenology of plant (Smith et al., 2012; Xia & Wan, 2013; Wang & Tang, 2019a), and plant diversity loss advanced flowering events (Wolf et al., 2017). Specifically, increasing N inputs lead to an increase in available soil N (Smith et al., 2012), and then could delay flowering phenology indirectly through extending the length of vegetative growth (Wang & Tang, 2019a). While plant diversity loss could influence flowering phenology indirectly, via effects on abiotic processes (soil moisture and temperature) and resource availability (available soil N), or directly, via biotic interactions (plant density) (Wolf et al., 2017; Du et al., 2019). The projected N addition and plant diversity loss could change abiotic and biotic processes in the community and thus impact plant flowering phenology.

Functional traits of a plant are measurable biotic properties related to adaptation to environment (Enquist et al., 2015; He et al., 2018; Gustafsson & Norkko, 2019). Compared with abiotic factors, the alteration in biotic factors (e.g. functional traits) may be the more directly and better indicator of ecosystem function changes under N addition and plant diversity loss (Dickson et al., 2014; Cadotte, 2017). Given that soil N availability often alters the switches from the growth to reproduction stage (Cleland et al., 2006), and thus increasing N could delay flowering phenology (Wang & Tang, 2019a); available soil N increase under plant diversity loss, and then plant species flowered earlier, with peak flowering date advancing an average of 0.6 days per species lost (Wolf et al., 2017). However, previous studies showed that the indirectly effects of abiotic factors on phenology through altering plant morphological and physiological traits (König et al., 2018; Pérez-Ramos et al., 2019). Plant light and nutrient acquisition traits could elucidate linkages between plant phenology and environmental changes (Jia et al., 2011; Dorji et al., 2013; König et al., 2018; Pérez-Ramos et al., 2019). For instance, traits associated with resource acquisition, such as rooting depth and life history, mediate plant reproductive phenology responses to changing climatic conditions in an alpine grassland (Dorji et al., 2013); traits related to competition and growth rate, like plant height, specific leaf area and leaf dry matter content, had substantial explanatory power in the effects of climate changes on the first flowering day at the global scale (König et al., 2018; Pérez-Ramos et al., 2019). Therefore, our knowledge regarding the linkages between changes in flowering phenology and functional traits is crucial for better understanding the regulating mechanisms of N enrichment and plant diversity loss on plant phenology and predicting grassland community dynamics under increasing N deposition and biodiversity loss scenarios.

Here, we report on a common garden experiment investigating the influence of N deposition increase and plant diversity loss on three flowering phenology of Medicago sativa in an assemblage grasslands (Supplementary Fig. 1). Specifically, we explored the effects of N addition and multiple plant diversity levels on the first flowering day, the last flowering day, flowering duration, flower numbers, environmental factors, and a series of plant functional traits. We used partial correlation, variation partitioning, and structural equation modelling analysis to quantify the direct and indirect effects of N addition and plant diversity loss on flowering phenology and flower numbers. We hypothesized that (a) increased N inputs and plant diversity would delay flowering phenology; (b) increased N inputs promote light acquisition traits, and decreased plant diversity promote light and nutrient acquisition traits; and (c) changes in abiotic factors and functional traits would together drive the response of plant flowering phenology to N addition and plant diversity loss.

2 | Materials and Methods

2.1 | Study site and experimental design

Our study site is located at the field research station of Beijing Research & Development Center for Grasses and Environment (40°10' 45" N, 116°26'13" E) on Xiaotang Mountain in Beijing, China. The mean annual temperature is 11.8 °C (2000-2018). The mean annual precipitation is 526 mm (2000-2018), with >80% of the precipitation falling in the growing season (June-September). Soil organic carbon of top soil (0-10 cm) is around 1%, and the average pH value is 7.47. The study area is dominated by perennial plants, including

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E. nutans and *T. mongolicum* .

Our experimental plots were established within an area of 8×12 m in 2019, using a randomized block design with nitrogen (N) addition and plant diversity gradient as main treatment factors. Each block contained twelve treatments, crossing two levels of N addition (no nitrogen addition and add 60 kg N ha^{-1} yr^{-1}as urea) and six levels of plant diversity (0, 1, 2, 4, 6, and 8). Each diversity level had four plant assemblage types, except for 0 and 8 (Supplementary Table 1). Each treatment had four replicates, resulting in 144 polyvinylchlorid bottom sealed pipes (Supplementary Fig. 1b). Each pipe is 30 cm in diameter and 50 cm in height, three holes were drilled into the bottom to provide drainage, filled with uniformly mixed soil and sand (soil-sand ratio is 3:1), and then buried into soil (Supplementary Fig. 2). Soil inside and outside of pipe was 5 cm lower than its upper edge. According to the germination rate of each species (Supplementary Table 1), seeds was sown at the depth of 1 cm in each plot after fully watered in March, and maintained 120 seedlings in each plot. The eight species we selected were *Poa annua* , *Carex breviculmis* , *Medicago sativa* , *Astragalus adsurgens Pall* ,*Dianthus barbatus* , *Penstemon campanulatus* ,*Chrysanthemum maximum* , and *Allium schoenoprasum* , the assemblage types at each diversity level were showed in the Supplementary Table 1. The amount of N addition is twice the background deposition in Beijing (Yu et al., 2019), and urea was applied by spraying on 1 May.

2.2 | Phenology monitoring

To track flowering phenology of *M. sativa* , phenology was monitored every 3–4 days during the growing season from May to September in 2019. Three individuals for each plot were randomly selected, marked, and monitored for the growing season. The first and last date a flower was observed for each of the marked individuals was recorded as the first and last flowering day, the periods between the first and last flowering day was recorded as the flowering duration. Flower number was counted for each of the marked individuals. Flowering phenology events and flower numbers were averaged for three individuals of each plot.

2.3 | Functional traits and abiotic factors measurements

Light acquisition traits (plant height and relative height, leaf mass and area, leaf length and width, and specific leaf area) and nutrient acquisition traits (Leaf carbon (C) and nitrogen (N) content, leaf C/N ratio, biomass and abundance, relative biomass and abundance) are closely related to plant phenology (Lavorel & Grigulis, 2012; Grigulis et al., 2013). Consequently, we determined these traits to explore the mechanisms underlying regulating the response of flowering phenology to experimental N addition and plant diversity gradients. Before the measurements, we investigated the abundance and height of each species in the plot. *M. sativa* is the predominant species (relative abundance >40% in each pine) and 6 healthy mature individuals were selected to measure the species-level traits in each plot. The functional traits were quantified using standard methods proposed by Pérez-Harguindeguy et al. (2013). The specific leaf area was calculated as the ratio of leaf area to its dry weight. To measure leaf area, length, width, and maximum width, spread leaves were scanned and analyzed Li-Cor 310 (Li-Cor Inc. USA), and then leaves were oven-dried to a constant weight. Finally, the oven-dried leaf samples were ground to determine leaf carbon and nitrogen with an elemental analyzer (PE 2400 II, USA). To measure the biomass of community and individual species, the aboveground part of each plot was clipped in early September (the peak of growing season). Plants clipped from each plot were pooled together, sorted to species, and then oven-dried to a constant weight.

Soil temperature and moisture at the depth of 10 cm were measured every week from April to October with W. E. T sensor kit (Delta-T Devices Ltd, UK). Three soil cores were collected in each plot in early September at the depth of 10 cm, and then mixed together into one sample. Available soil N (Ammonium (NH_4^+) and nitrate (NO_3^-)) contents in the extracts were determined colorimetrically by automated segmented flow analysis (Bran + Luebbe AAIII, Germany)

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2.4 | Statistical analyses

We analyzed experimental data with the following three steps. First, we scaled the species-level height to the community level by calculating the mean of the abundance distributions (Equation 1, Gross et al., 2009):

$\text{Mean}_j = \sum_i^n p_i T_i$ (1)

where p_i and T_i are the relative abundance and the plant height of the species j , respectively, and n is the number of species.

Second, we examined how N addition and plant diversity affected environmental factors, the functional traits and flowering events of*M. sativa* . We applied linear mixed effects models using "lme4" function (package "NLME", Pinheiro *et al* ., 2007) to test the effects of N addition and plant diversity loss on soil temperature and moisture separately in 2019. We set treatments as fixed effects, block and time as a random effect in each model to account for variation among repeated measurements of temperature or moisture. Linear mixed effects models were also used to examine the effect of N addition and plant diversity loss on flowering phenology (first and last flowering day, flowering duration, and flower numbers) and functional traits (leaf and community traits). Treatment was treated as fixed effects, and block was treated as a random effect to account for variation within block.

Third, partial correlation was conducted to evaluate the relationships between the flowering events and the various factors (Chen et al., 2019). For example, after controlling N addition and plant diversity levels, we examined the relationships of the flowering events with light acquisition traits, nutrient acquisition traits, and abiotic factors. Variation partitioning analysis that partitioned the variance shared by all factors was then used to quantify the unique contribution of each group of factors (Chen et al., 2019). Structural equation modelling analysis was employed to evaluate the hypothesized underlying factors that influence flowering phenology (Wang & Tang, 2019b) using the package '*piecewise-SEM* ' in R software (Shipley, 2000). The model was assessed by *Fish C* statistic, Akaike information criterion (AIC), and P values.

All statistical analyses and graphs were prepared in R 3.2.2 (R Core Team, 2018). Differences were considered to be statistically significant at P [?] 0.05.

3 | Results

3.1 | Soil environmental conditions

In our common garden experiment, soil temperature and moisture (at the depth of 10 cm) was significantly influenced by N addition and plant diversity loss (Fig. 1). Soil temperature (ST) significant increased but soil moisture (SM) had no change after N addition. Soil temperature and moisture changed markedly along the plant diversity gradient, but the changing trend was contrary under different N addition level (Fig. 1c and d). ST decreased but SM increased with decreasing species richness at the control treatments, while ST increased and SM decreased with plant diversity loss at the N addition treatments (Fig. 1c and d).

3.2 | The effects of plant diversity loss and N addition on phenology

The flowering phenology of *M. Sativa* was significantly affected by N addition and plant diversity loss, but the effects was varied with phenological stages (Fig. 2). N addition delayed the first flowering day (FFD) of *M. Sativa* by 1.9 days, but plant diversity loss had no effects on it (Fig. 2a). The last flowering day (LFD) was earlier by 1.2 days after N addition, but it was delayed under plant diversity loss (Fig. 2b). LFD was delayed by on average of 0.51 and 0.77 days per species lost at the control and N addition treatment, respectively (Fig. 2b). Obviously, the delay of FFD and the advance of LFD inevitably shorten of the flowering duration (FD). N addition shortened FD of*M. Sativa* by 3.2 days, while FD was extended under plant diversity loss (Fig. 2c). FD was extended by 1.08 and 0.82 days per species lost at the control and

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N addition treatment, respectively (Fig. 2c). Moreover, our study also showed that flower numbers (FN) significantly decreased under N addition and plant diversity loss (Fig. 2d).

3.3 | The effects of N addition and plant diversity loss on functional traits

Six different leaf traits of *M. Sativa* was measured in our study. N addition significantly increased leaf mass and area, but had no effects on specific leaf area, leaf carbon and nitrogen content, and leaf C/N ratio (Fig. 3). However, with losing of plant diversity, specific leaf area and leaf nitrogen content significantly increased, leaf C/N ratio decreased, but leaf mass, leaf area, and leaf carbon content had no changes (Fig. 3).

The relative biomass, abundance, and height of *M. Sativa* were used to represent the plant traits in our study. We found that N addition had no effects on plant traits, but plant traits was significant changed under plant diversity loss (Fig. 4). The biomass (Fig. 4a), relative biomass (Fig. 4b), and relative abundance (Fig. 4c) of *M. Sativa* were significantly increased with plant diversity loss, while relative height significantly decreased (Fig. 4d).

3.4 | Ecological factors influencing flowering phenology

Three statistical methods, including partial correlation, variation partitioning, and structural equation modelling analysis, were used to discern the influence of ecological factors on flowering phenology. Partial correlation analysis showed that with controlling N addition and plant diversity levels, FFD was closely correlated with leaf carbon content, C/N ratio, and available soil N; LFD was closely correlated with leaf nitrogen, C/N ratio, and ST; FD was correlated with relative abundance and ST; FN was closely correlated with leaf mass, C/N ratio, and plant height.

Variation partitioning analysis indicated that light and nutrient acquisition traits explained a much greater portion of the variance in FFD (21% and 40%), LFD (41% and 62%), FD (57% and 73%), and FN (52% and 64%), respectively (Figure 6). Structural equation modelling analysis showed that the changes in FFD was positive correlated with available soil N and negative correlated with leaf carbon content (Fig. 7a). Leaf nitrogen content and ST jointly explained 44% of the variance in LFD, and the negative effects of plant diversity on FFD was mainly through its negative effects on leaf nitrogen content (Fig. 7b). ST and relative abundance jointly explained 38% of the variance in FD, and the negative effects of plant diversity on FD was mainly through its negative effects on relative abundance (Fig. 7c). Leaf mass and leaf C/N ratio jointly explained 30% of the variance in FN, and the positive effects of plant diversity on FN was mainly through its negative effects on leaf C/N ratio (Fig. 7d).

4 | Discussion

Many studies have explored the response of flowering phenology to global change. Three aspects of our study, however, distinguish it from these previous studies. First, while a few studies of the effects of global changes on phenology have considered one factors, our study examines the effects of N addition, plant diversity loss, and their interactions on plant flowering phenology in a single experiment. Second, our study explores the effects of N addition and plant diversity loss on multiple flowering phenology events. Third and most importantly, through three different analysis methods, we confirmed that N addition and plant diversity loss altered flowering phenology through its effects on functional traits rather than abiotic factors.

4.1 | The effects of N addition and plant diversity loss on flowering phenology

The flowering phenology is one of the more important factors determining the reproductive success of plants, because the timing of flowering means when plants expose it reproductive organs to the changing biotic and abiotic environments (Rathcke & Lacey, 1985; Kudo & Hirao, 2006). In our study, N addition delayed the first

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flowering day (FFD) but advanced the last flowering day (LFD), and then resulted in a shortened flowering duration (FD), which consistent with those of previous manipulative experiments in natural grasslands (Smith et al., 2012; Xi et al., 2015). Nevertheless, the 1.92 d delay in FFD at the N addition plots is shorter than that reported from the alpine grasslands in North America (Smith et al., 2012); the 1.2 d advance in LFD and 3.2 d shortened in FD is longer than that reported from the alpine grasslands in China (Xi et al., 2015). Which suggesting that the effects of N addition on flowering phenology varies among ecosystems and phenological stages.

Different from the effects of N addition, plant diversity loss advanced the FFD but delayed LFD, which resulted in an extension in FD. The 0.31 d advance per species lost in FFD is similar to the results (ranging from an advancement of 1.8 d per species lost to a delay of 0.7 d per species lost, average is 0.6 d advance) that reported from the serpentine grasslands in North America (Wolf et al., 2017), suggesting that the effects of plant diversity loss on FFD varies among species. Moreover, we noticed that the amplitude of changes (0.64 d) in LFD is more than that in FFD (0.31 d) after per species lost, which revealing that LFD may be more sensitive to plant diversity loss.

4.2 | The effects of N addition and plant diversity loss on functional traits

We found evidence to support our second hypothesis that increased N inputs promote light acquisition traits. Specifically, leaf mass and area of M. sativa was promoted after N addition. These results were inconsistent with that reported from the alpine grasslands in China (Liu et al., 2017; Zhang et al., 2019), which may be induced by the differences in species. M. sativa, a legumes, can fix N by means of symbionts to relieve their N limitation (Bordeleau & Prevost, 1994), and thus the nutrient acquisition traits of M. sativa had no changes under N addition. Meanwhile, the relief of N limitation accompany with the increasing demand of light (Hautier et al., 2009). Hence, the changes in light demand of M. sativa under N addition could lead to an increase in leaf mass and area.

Our findings for plant diversity loss also support our second hypothesis that both light (specific leaf area) and nutrient (leaf nitrogen content and relative abundance) acquisition traits was promoted under plant diversity loss. These results were inconsistent with the effects of plant diversity loss on grass species that reported from the grasslands in Germany (Gubsch et al., 2011), which may be caused by the differences in species. Closed canopies are characterized by pronounced gradients in spectral light quality and quantity (Jones, 1992). In the present study, plant diversity loss impacts on morphological traits associated with light acquisition were mainly attributable to the changes in relative height of M. sativa. Relative height decreased with plant diversity loss, indicating higher efforts for light acquisition should be allocated by plants (Spehn et al., 2005; Lorentzen et al., 2008). Hence, M. sativa exhibited high specific leaf area in adjustment to increasing competition for light. Moreover, nitrogen is not the limitation resource of M. sativa. Higher light acquisition traits are accompanied with higher nutrient acquisition traits (Hautier et al., 2009; Zhang et al., 2019), which resulted in an increase in leaf nitrogen content and relative abundance under plant diversity loss.

4.3 | Functional traits rather than abiotic factors determine flowering phenology

Our findings not support for our third hypothesis that functional traits and abiotic factors co-diver the response of flowering phenology to N addition and plant diversity loss, the contributions of functional traits for changes in flowering phenology was far more larger than abiotic factors (Fig. 5, 6, and 7). Specifically, the advance in FFD under biodiversity loss was mainly induced by the decrease in available soil N, which was consistent with the result from Wolf et al. (2017) in American serpentine grassland. Lower available soil N advanced the plant switches from the growth to reproduction stage (Wang & Tang 2019), and then indirectly caused the advancement in FFD at the monocultures.

However, the response of LFD and FD to biodiversity loss and N inputs was mainly driven by leaf nitrogen content and relative abundance. It has been proved that plant morphological and physiological traits could

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elucidate the changes in plant phenology (Jia et al., 2011; Konig et al., 2018; Perez-Ramos et al., 2019) and the alteration of living strategies of plants (Wilson & MacArthur, 1967; He et al., 2008; Perez-Ramos et al., 2019). At the monocultures, higher efforts for nutrients (leaf nitrogen content and relative abundance) acquisition means increase in intraspecific competition for resource among individuals of M. sativa (Lorentzen et al., 2008; Liu et al., 2017), and K-strategy with higher survive rate was chosen by M. sativa at the monocultures, which resulted in delaying LFD, extending FD, and decreasing FN (Fig. 2). As species richness increase, the intraspecific competition for resource decrease, but the interspecific competition among different species increase (Hector et al., 1999; Tilman et al., 2001). However, because the predominant competition advantage of M. sativa (high relative abundance and plant height, Fig. 4) at the mixed-cultures, r-strategy was chosen by M. sativa with more flower numbers and lower survival rate (Guiz et al., 2018), which resulted in advancing LFD, shortening FD, and increasing FN (Fig. 5b, c, and d). Therefore, the gradually shifts from r-strategy to K-strategy of M. sativa led to the changes in flowering phenology under plant diversity loss.

5 | Conclusions

Our study highlighted that the influence of functional traits on flowering phenology is far more greater than abiotic factors under N addition and plant diversity loss in an assemblage grasslands through a common garden experiment, and the driver of flowering phenology was varied with phenological stages, which suggests that functional traits should include in phenology models to improve prediction of the response of phenology to global changes. Moreover, advance in the last flowering day and shortened in flowering duration is accompanied with the increase of flower numbers, which indicated that changes in functional traits not only altered the flowering phenology, but also heralded the changes in reproductive strategy of plants under global changes.

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Author contributions

CW and JW planned and designed the research. CW, XL, WZ, RS, RF, and CL performed the research. CW and YT analyzed the data. CW wrote the manuscript.

Data availability statements

The data that support the findings of this study will be openly available at Dryad after the manuscript accepted.

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Figure legends

Figure 1 Soil temperature (°C, 10 cm) (a) and soil moisture (%, 10 cm) (a) under different plant diversity and N addition level from April to October 2019. Mean (+-*se,* $n = 4$ or 8) soil temperature (c) and soil moisture (d) for the whole growing season under different plant diversity and N addition level in 2019. We applied the linear mixed effects models to test the effects of treatments (plant diversity and N addition) on soil temperature and soil moisture. We set treatments as fixed factors, time and block as random factors in each model to account for variation among repeated measurements of soil temperature and moisture. Points and lines with different shape represent different nitrogen addition levels, points with different colour represent different plant diversity levels. N represents nitrogen addition, D represents plant diversity.

Figure 2 Average first flowering day (a), last flowering day (b), flowering duration (c), and flower number (d) under different plant diversity and N addition levels. All the analyses were performed using the linear mixed effects models to test the effects of treatments (plant diversity and N addition) on flower phenology. Treatments were treated as fixed factors, block was treated as a random factor. Points and lines with different shape represent different N addition levels, points with different colour represent different plant diversity levels. N represents nitrogen addition, D represents plant diversity.

Figure 3 Average leaf mass (a), leaf area (b), specific leaf area (c), leaf carbon content (d), leaf nitrogen content (e), and leaf C/N ratio (f) under different plant diversity and N addition levels. All the analyses were performed using the linear mixed effects models to test the effects of treatments (plant diversity and N addition) on leaf traits. Treatments were treated as fixed factors, block was treated as a random factor. Points and lines with different shape represent different N addition levels, points with different colour represent different plant diversity levels. N represents nitrogen addition, D represents plant diversity.

Figure 4 Average *M. Sativa* biomass (a), relative biomass (b), relative abundance (c), and relative height (d) under different plant diversity and N addition levels. All the analyses were performed using the linear mixed effects models to test the effects of treatments (plant diversity and N addition) on plant traits of *M. Sativa* . Treatments were treated as fixed factors, block was treated as a random factor. Points and lines with different shape represent different N addition levels, points with different colour represent different plant diversity levels. N represents nitrogen addition, D represents plant diversity.

Figure 5 Partial correlations between the flowering events and the light acquisition traits, nutrient acquisition traits, and abiotic factors after controlling N addition and plant diversity levels. The x-axis shows the factors of which the correlations with the flowering events are examined. The size and colour of the circles indicate the strength and sign of the correlation. Differences in circle size and colour indicate the level of dependency of the correlation between the flowering events and the examined factor after controlling N addition and plant diversity levels. FFD, first flowering day; LFD, last flowering day; FD, flowering duration; FN, flowering numbers; LM, leaf mass; LA, leaf area; LL, leaf length; LW, leaf width; SLA, specific leaf area; LN, leaf nitrogen content; LC, leaf carbon content; LCN, leaf carbon/ nitrogen ratio; AB, aboveground biomass; PA, plant abundance; PH, plant height; RB, relative biomass; RA, relative abundance; RH, relative height; AN, available soil nitrogen; SM, soil moisture; ST, soil temperature. $*P < 0.05$; $**P < 0.01$; $***P < 0.01$.

Figure 6 Relative contributions of light acquisition traits, nutrient acquisition traits, and abiotic factors to flowering events. Variation partitioning analysis was to identify the variance in the first flowering day (a), the last flowering day (b), flowering duration (c), and flowering numbers (d) explained by these three groups of biotic and abiotic factors.

Figure 7 A structural equation modelling of plant diversity loss and N addition on the first flowering day (a), the last flowering day (b), the flowering duration (c), and flower number (d). Red and black arrows represent significant positive and negative pathways, respectively. Solid and dashed arrows indicate significant and non-significant pathways, respectively. Numbers near the arrow indicate the standardized path coefficients indicating the effect size of the relationship with * indicating $P < 0.05$, ** indicating$P$

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< 0.01, and *** indicating $P < 0.001$. Arrow width is proportional to the strength of the relationship. R^2 represent the proportion of variance explained for each dependent variable. The goodness-of-fit statistics for the structural equation modelling are shown below each model. N, nitrogen.

Figures

Figure 1

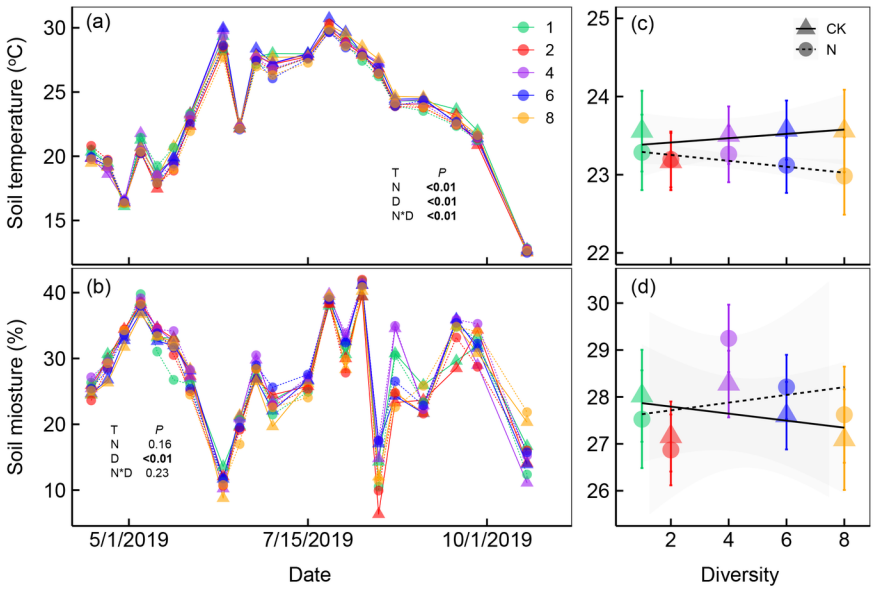

Figure 2

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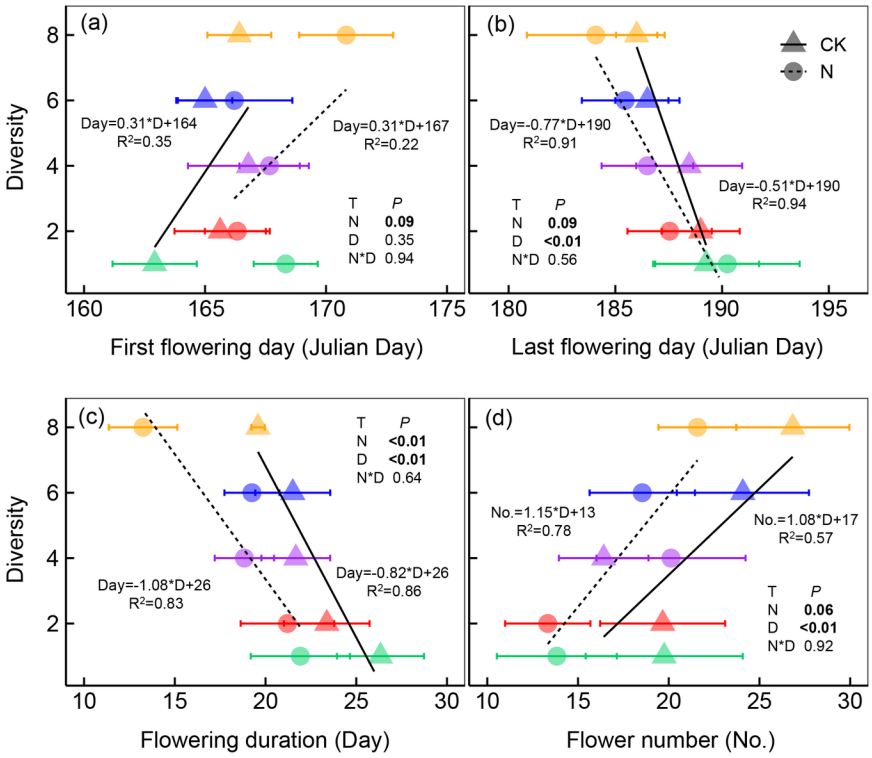

Figure 3

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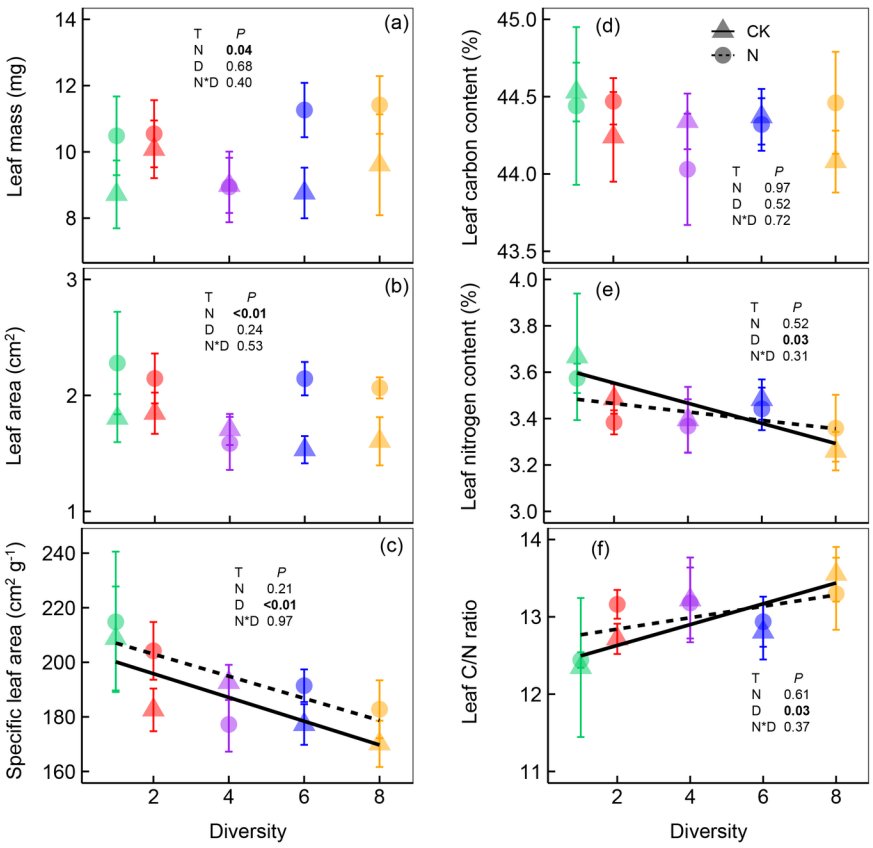

Figure 4

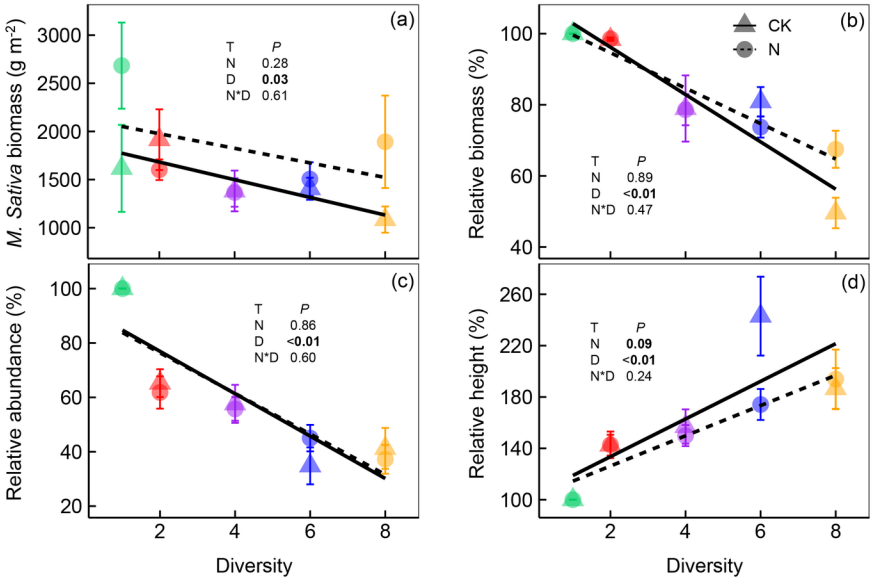

Figure 5

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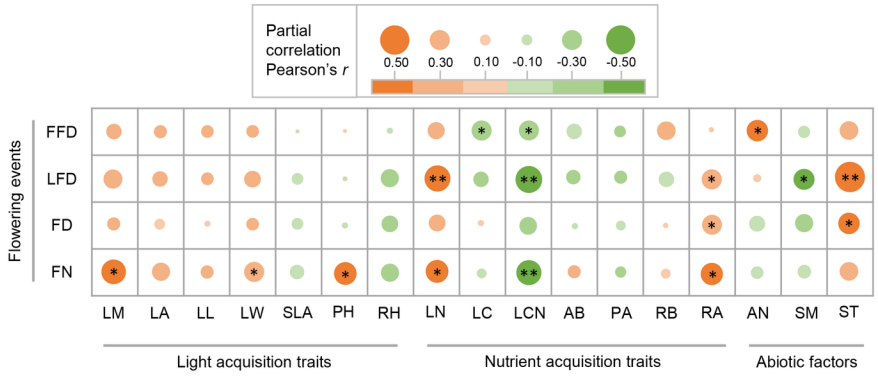

Figure 6

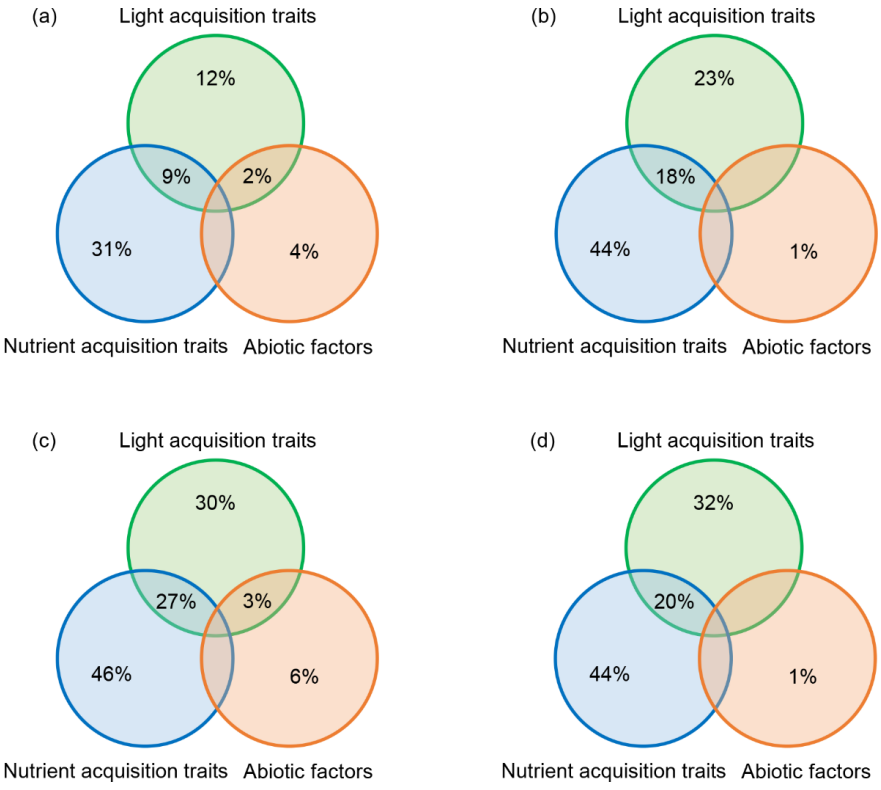

Figure 7

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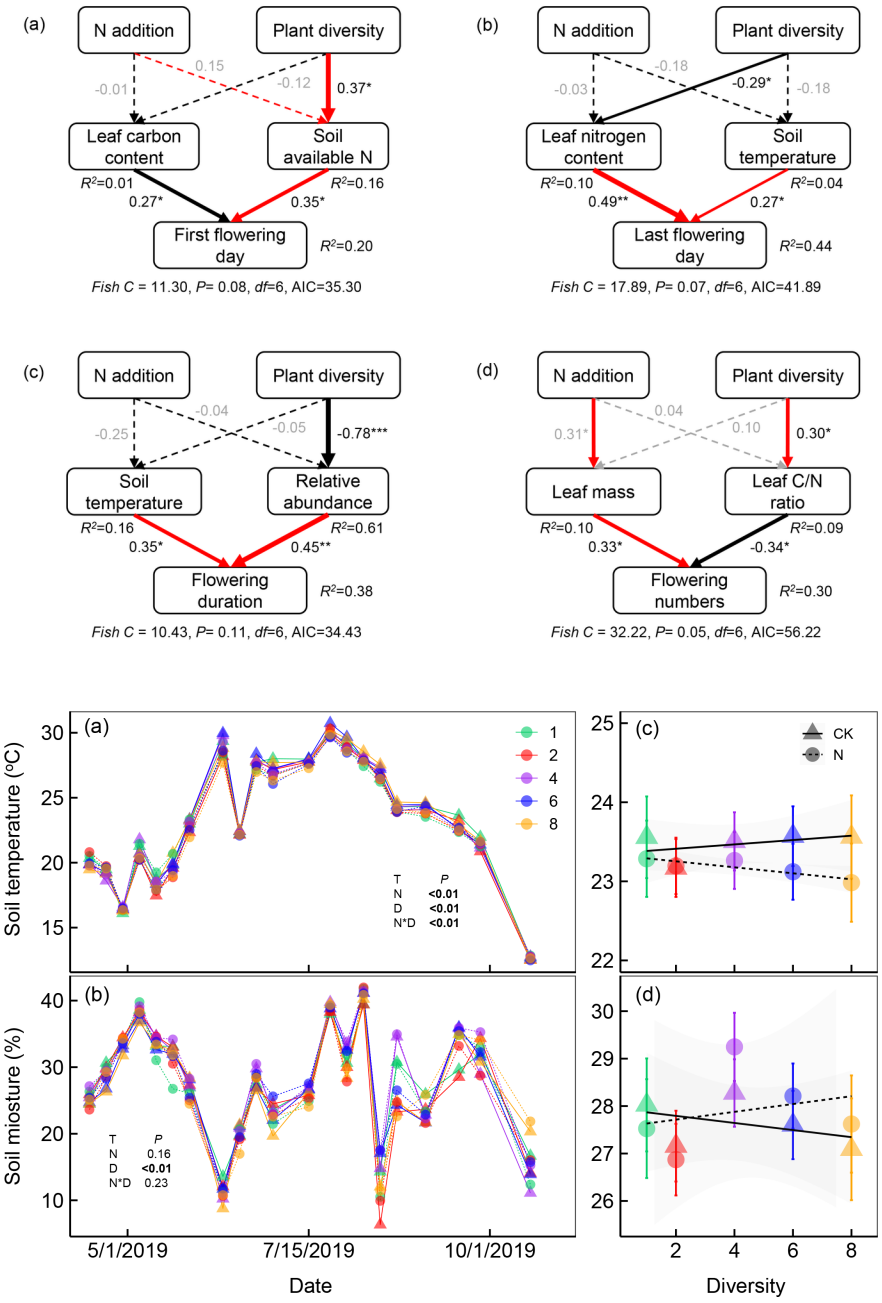

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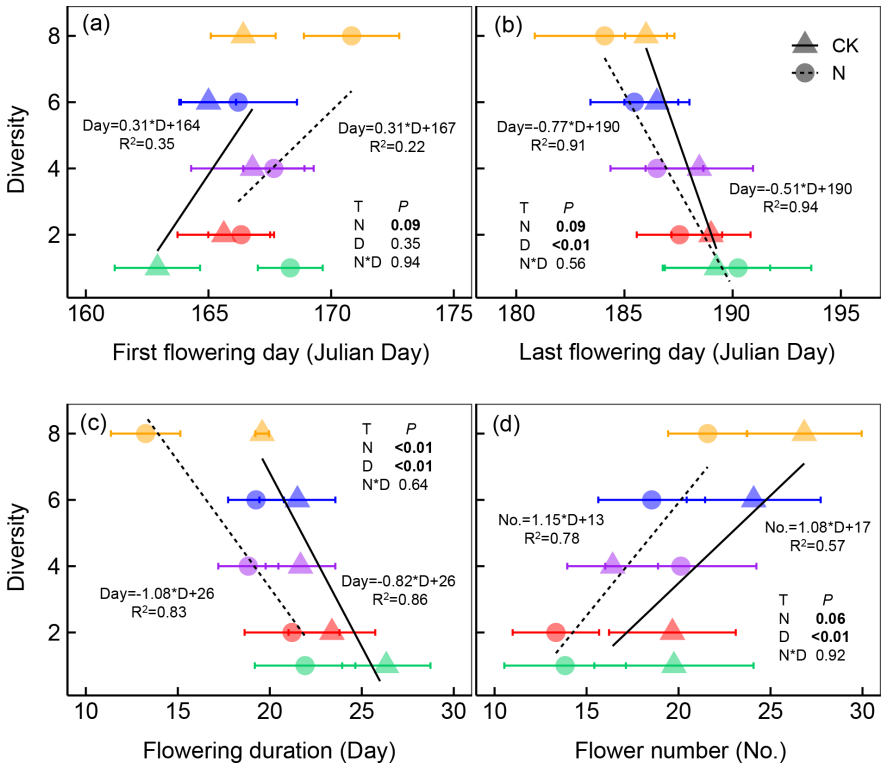

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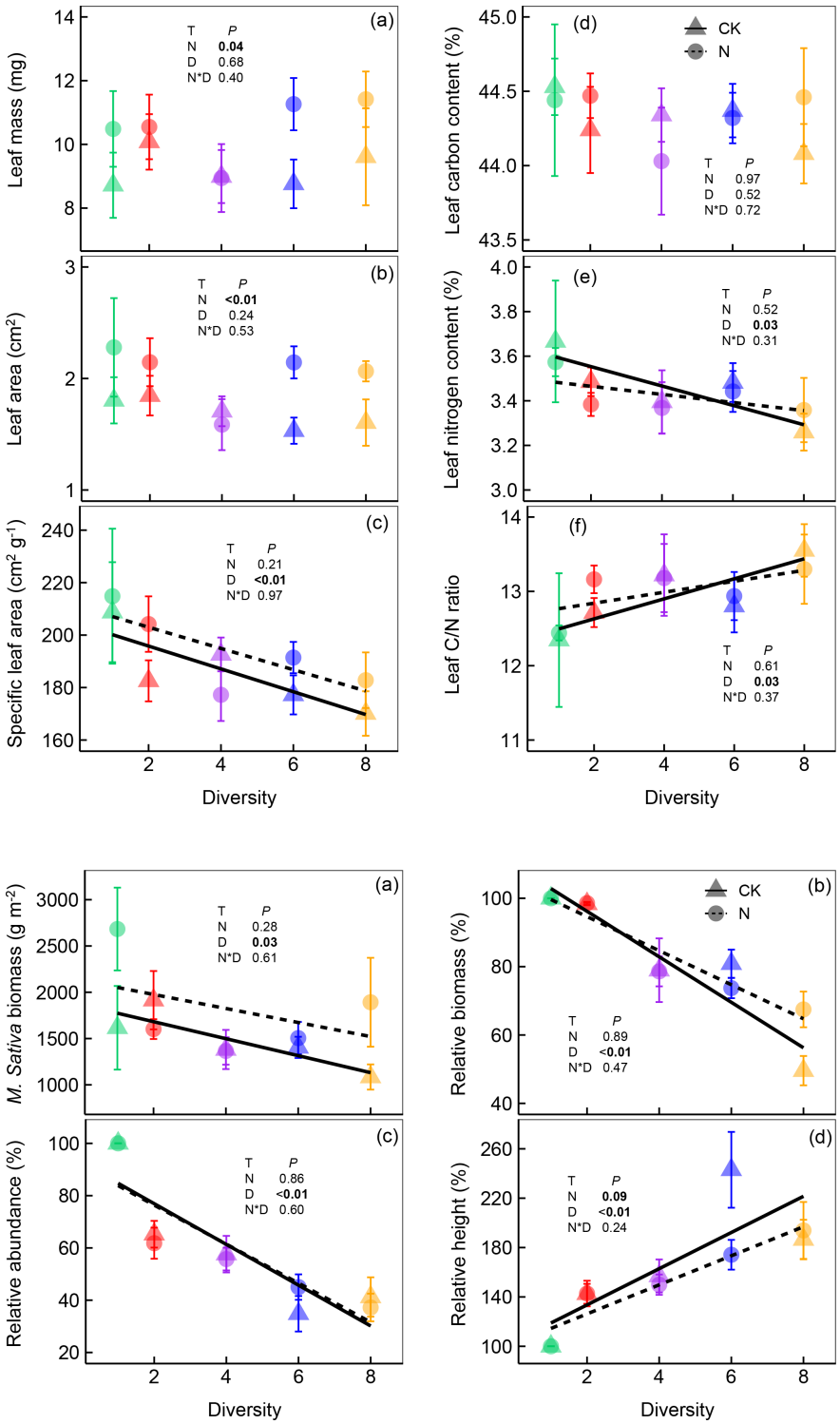

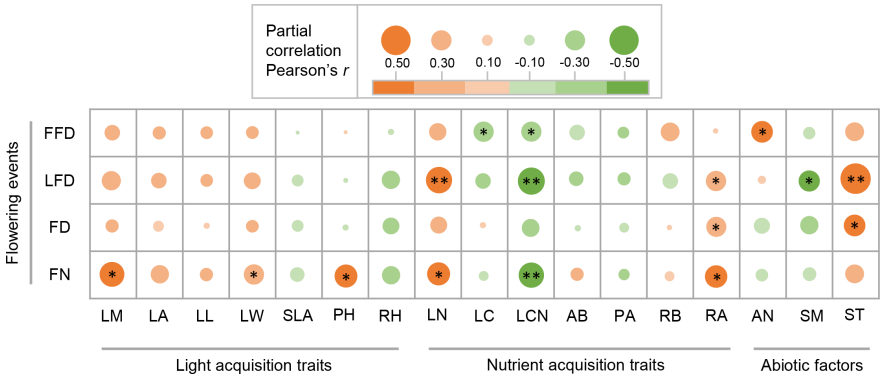

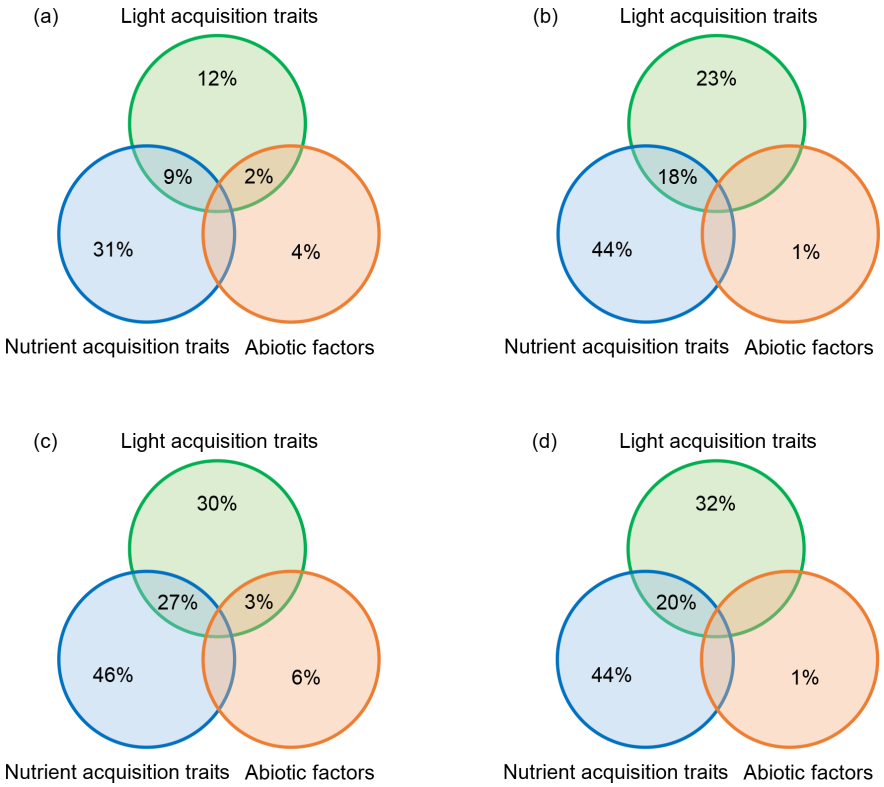

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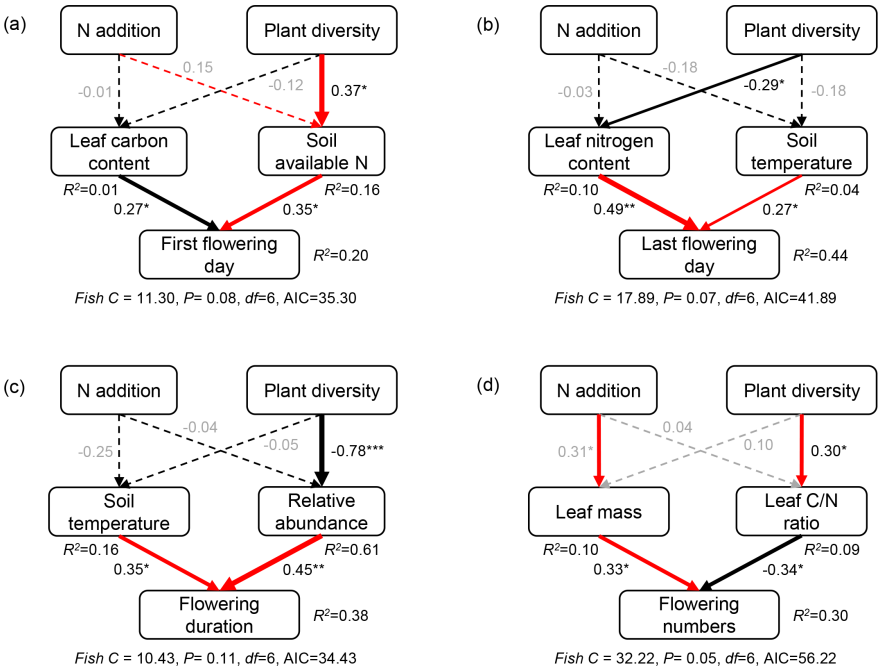

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