**Burn it or tolerate it: the negative correlation between shade tolerance and flammability in grasses**

Xiulin Gao and Dylan W. Schwilk

**Summary**

1. Persistence of sun-like grasses in savannas is dependent on recurrent fires to keep canopy open. Differences in microhabitat preference among grasses in relation to tree canopies, however, suggest different shade tolerance strategies. As an alternative strategy to shade tolerance in tree-associated grasses, flammability traits can be selected in sun-like grasses due to reduced light competition by fire. It is thus possible that flammability and shade tolerance are evolutionarily correlated in grasses. In this work, we aim to test if shade tolerant grasses are less flammable when compared to shade intolerant grasses.

2. We examined the relationship between shade tolerance and flammability by determining individual-level flammability and species shade tolerance of 17 grass species. We conducted a greenhouse shading experiment and indoor flammability trials to examine plant traits that influenced flammability and the post-fire responses of grasses.

3. Grass species mainly varied in the amount of heat released during burning. More shade tolerant species produced less heat at 50 cm above the ground. Biomass and live fuel moisture had greatest effects on heat release. However, the negative effect of live fuel moisture on heat release at the soil surface was weakened in plants with high specific leaf area. In addition to shade effect, increased live to dead biomass ratio also increased live fuel moisture, especially under full sunlight. Heat release at the soil surface negatively influenced post-fire survival rate and resprouting tiller number while pre-fire tiller number had positive effects on the two measures. Plants with more pre-fire tiller number, however, were less affected by heat release at the soil surface in terms of survival rate and resprouting strength.

4. *Synthesis*. More shade tolerant grasses produced less heat at 50 cm. Besides biomass effect, species phenology at the time of fire was also important in influencing grass flammability. Reduced heat release in shade tolerant grasses can result in less damage to coexisting trees. Such heterogeneity in grass flammability can help maintain tree-grass mixture in savannas. However, further field-scale experiments are necessary to determine the biological significance of altered fire behavior that due to differences in grass flammability.

**Keywords:** fire, grass, flammability, resprouting, shade tolerance, ecological strategy, savanna

**Introduction**

Savannas are fire-dependent ecosystems, where recurrent fires suppress tree cover and allow shade intolerant grasses to persist. In regions where climate conditions support closed-canopy woodlands (e.g. mesic savannas), disturbances such as fires control tree density (Van Nieuwstadt and Sheil 2005, Nano and Clarke 2010). Accumulation of fine dead biomass forms well-aerated, continuous fuel bed that burns frequently. Grass-fueled fires are fast-moving with short residence times that often result in little soil heating (Sharrow and Wright 1977). Such fires can damage or kill trees, yet spare grasses which have meristems well protected from heat at the soil surface or below ground. Fires remove dead aboveground biomass and open the canopy, create well‑lit environments, and provide a pulse of nutrients (Romanyà et al. 2001). These conditions favor post fire recovery in grasses. In fueling frequent and fast-moving fires, grasses thus may act as niche constructing species creating favorable environments for their propagules (Schwilk and Kerr 2002, Schwilk 2003). Grasses differ both in their tolerance of shading by trees and in their resilience to fire (Weltzin and Coughenour 1990, Naumburg et al. 2001, Ripley et al. 2015, Moore et al. 2019). It is possible that shade tolerance and fire response are correlated with one another and part of an overall ecological strategy: shade intolerant grasses might be more fire resilient given the benefit they gain from recurrent disturbances, but this conjecture has never been investigated. Recently, species specific variation in flammability has been shown for grasses (Fill et al. 2016, Simpson et al. 2016, Gao and Schwilk 2018). If grass species also differ in their flammability, then it is possible for selection to act on individuals that can maintain both the frequency of disturbance and the persistence of their population in post-disturbance habitats (Schwilk and Ackerly 2001).

The fact that multiple light environments exist in savannas suggests that this environments can support multiple grass shade tolerance strategies. The under canopy light intensity can be 50% of that in open area in most savannas (Belsky 1994, Ludwig et al. 2001). Furthermore, grass species composition varies between these light environments with some species prefer under canopy habitats while the others dominate in open areas (Whittaker et al. 1979, Weltzin and Coughenour 1990 and personal observation). Despite potential nursing effects by trees, this contrasting preference in microhabitats associated with tree cover also can be due to species variation in tolerance of shade (Cardoso et al. 2018). While shade tolerant grasses can occur under trees, the persistence of grasses preferring open habitats is dependent on recurrent fires to reduce canopy cover. As plant traits can influence fire behavior to alter fire regime, flammability traits can be selected in shade intolerant grasses in order to keep well-lit habitats and benefit from niche construction (Cardoso et al. 2018, Charles-Dominique et al. 2018). We thus set out to determine if shade intolerant grasses are more flammable than shade tolerant grasses in order to promote fire to interrupt the establishment of woody plants (Fig. 1).

Flammability is multidimensional; variation along flammability axes could indicate different plant strategies in a fiery ecosystem. Fire has long been an evolutionary agent and plants have evolved different flammability strategies to cope with specific fire regimes (Schwilk and Ackerly 2001, Pausas and Keeley 2009, He et al. 2012, Pausas et al. 2017). Flammability is a collective term consisting of various measurements that quantify fire behavior. Current empirical evidences suggest that there are 2 main axes of variation in flammability: heat release and rate of heat release (de Magalhães and Schwilk 2012, Engber and Varner 2012, Michelaki et al. 2020). With generally fast rate of spread and short combustion duration, grass fuels mainly vary in their heat release (Gao and Schwilk 2018). This specific combination of fire characteristics may depict a “kill thy neighbor” strategy: a species with high heat release, especially in the aboveground space, can be detrimental to coexisting plants meanwhile protects their surface or belowground meristems via reduced soil heating (D’Antonio and Vitousek 1992, Bond and Midgley 1995, Gagnon 2010, Bowman et al. 2014). Variation in heat release is primarily due to difference in biomass in grasses fuels (Simpson et al. 2016). However, recent work demonstrates that grass canopy architecture has additional effects on heat release (Fill et al. 2016, Gao and Schwilk 2018).

Our novel contribution with this work is to test if habitat preference and ecological strategies are correlated with flammability in grasses. We expect that species that prefer open habitats will be more resilient under frequent fire (e.g. fast regeneration rate) and benefit from increased flammability due to reduced light competition by fire. In contrast, species that prefer shadier microhabitats are less flammable and may decrease under frequent fire as increased light resource does not pay off energy required to resprout and outcompete sun-like grasses in open habitats. It is possible that species also vary in short term responses (e.g. survival rate) to fire. Although surface and belowground meristems are often protected by leaf sheaths or soil, prolonged soil heating can kill buds (Choczynska and Johnson 2009, Gonzalez et al. 2015). Moreover, grasses with more pre-fire tillers will have higher survival rate and stronger resprouting vigor (Moore et al. 2019).

Plant ecological strategies involve combinations of specific traits. Associations might arise from selection or from involved traits that have multiple effects: for example, shade tolerance traits might have direct effects on flammability. Shade response traits that might alter fire behavior include canopy architecture and leaf dimension. To efficiently capture light in shady environments, plants tend to develop fewer branches to reduce foliage aggregation thus reduce self-shading (Niinemets 2010). The resulting less densely packed biomass in turn can negatively influence heat release (Fill et al. 2016, Gao and Schwilk 2018). Moreover, leaves under low light are often thinner and broader with a high specific leaf area and surface to volume ratio, which can positively influence the rate of spread (de Magalhães and Schwilk 2012, Engber and Varner 2012, Poorter et al. 2019). In addition to the direct trait effects on flammability, reduced solar radiation in shady habitats creates wetter environments where fuel moisture is high and that reduces flammability (Cardoso et al. 2018). Furthermore, C3, cool season grasses are more likely to be shade tolerant than C4, warm season grasses (Pearcy and Ehleringer 1984). Change in dead to live biomass ratio during fire seasons between these two can vary dramatically given their different phenology, and thus affects fire behavior (Dimitrakopoulos and Bemmerzouk 2003, Pellizzaro et al. 2007a, Emery et al. 2020).

In this study we test if shade tolerant grasses are less flammable than shade intolerant grasses. We examine how fire behavior, species shade tolerance, and pre-fire tiller number influence post-fire survival and resprouting, and how biomass, fuel moisture content, canopy architecture and leaf traits influence flammability. We grew 17 different grasses species in greenhouse under full sunlight and 50% shade to test species shade response, then we measured plant traits and conducted in-door burning experiments to collect flammability data.  We hypothesized that: 1) shade intolerant grasses will produce more heat in comparison to shade tolerant grasses; 2) prolonged soil heating will negatively affect grass survival rate and resprouting; 3) grasses with lower shade tolerance, more pre-fire tillers will perform better after fire than grasses with greater shade tolerance and less pre-fire tillers; and 3) biomass density, and specific leaf area will positively influence flammability while live fuel moisture will decrease flammability.

**Materials and methods**

**Study species**

We selected 17 grass species from 16 genera and 9 tribes for our study (Table 1). Species were chosen according to 3 criteria: 1) perennial grasses that are common in grassy ecosystems in the southwestern United States; 2) potential shade tolerant and intolerant species according to species shade tolerance information from USDA plant database (<https://plants.usda.gov/topics.html>); 3) species from different genera and tribes to maximize variation in plant traits and enrich phylogenetic diversity.

**Greenhouse methods**

Seeds of these species were obtained by either purchasing from a seed company (Nature’s Seed, Lehi UT, USA) or requesting from the National Plant Germplasm System by United State Department of Agriculture (<https://www.ars-grin.gov/npgs/>). Seeds were germinated during April of 2018. All species germinated within 2 weeks with a minimum number of 50 available seedlings. We selected 10­­ – 12 seedlings per species that reached the height of 5 cm to measure initial total above ground biomass. We then selected 20 seedlings at simil­ar height for shading experiment.

­­­­To minimize the effect of spatial variation in temperature and relative humidity, we applied a split-block design to 5 blocks in the greenhouse and evenly split each block into 0% and 50% shade treatment. We chose 50% shade because it is the common shade level underneath tree canopy in savanna ecosystems (Belsky 1994, Ludwig et al. 2001). We constructed a frame with PVC pipes (2-1/2”), and covered each with polypropylene shade cloth at height of 1.2 m above the workbench surface. The frame without shade cloth was also built for the 0% shade treatment to control for shade cast by PVC pipes. A 20 cm wide gap between shade cloth and workbench surface was left for venting. We randomly assigned each seedling to one of the light treatments in each block with 5 replicates per light treatment per species in total. Seedlings were transplanted in 4.3 L pots with regular greenhouse potting soil.

To access species tolerance to 50% shade, we measured total aboveground biomass 4 months after treatment. The mean relative aboveground biomass gained by plants grew under 50% shade (aboveground biomass of plant under 50% shade divided by aboveground biomass of plant under 0% shade from the same block) was calculated for each species and used as an index for species shade tolerance. Given the destructive feature of biomass measurement and limited greenhouse space, we planted paired seedlings in each pot: one for biomass measurement, and the other one for the flammability experiment. Plants were randomly placed and relocated within the block every month to minimize any effect of variation in light intensity. Plants were watered regularly and fertilized every Friday (15-5-15 cal-mag at 150 ppm N).

Greenhouse temperature control was terminated in early November of 2018 to allow dormancy occur before flammability experiment.

**Plant trait measurements**

To determine trait effects on flammability and post fire response, we measured plant traits at individual, canopy, and leaf level (Table 2).

In July of 2018, we sampled three fully expand leaves from each plant for specific leaf area measurements, and randomly selected and marked one plant from each pot for destructive measurements. We scanned each leaf using a flatbed scanner (CanonScan LiDE 25) at a fixed resolution (300 dpi) along with a printed ruler as scale reference. We then calculated leaf area using Image J (Rasband 2018). To estimate leaf area for species with needle-like leaves, we took leaf sections with known lengths, and assumed each as a cylinder and measured two perpendicular diameters. All leaf samples then were oven dried to measure dry mass. For leaves of plants assigned for flammability trials, we also measured leaf thickness (Lth, excluding the midrib), and calculated leaf surface area to volume ratio as 1/ Lth (Roderick et al. 1999). In late August at the peak of biomass, all marked plants were cut at the soil surface for total aboveground biomass measurement.

To measure live fuel moisture and live to dead biomass ratio, a 10% of the total aboveground fresh biomass( 1 – 5 g) from both dead and live materials (proportional according to dead : live ratio) was taken from each plant prior to burn, weighted, separated into live and dead biomass, oven-dried and weighted again. To measure total aboveground fresh biomass, we measured initial plant weight (including weight of pot, soil, and plant) prior to ignition and final plant weight (including weight of pot, soil, and belowground biomass) at the end of flammability experiment after remove fuel that was left. The difference between the two measurements was total aboveground fresh biomass if we assume that combustion did not significantly change water content of soil and belowground biomass. Total aboveground biomass was then estimated as the product of live fuel moisture and total aboveground fresh biomass.

We measured plant height and width prior to flammability experiment to estimate plant volume (Supplementary Material S1). We then calculated bulk density by dividing total aboveground biomass by plant volume.

We counted the number of tillers for each plant the day prior to flammability trials to determine its effect on post fire survival and resprouting strength.

**Flammability trials and measurements**

From late March through early May of 2019, we conducted indoor flammability experiments at the Lubbock Fire Department for flammability measurements (Table 2). To mimic natural burning conditions, we drought stressed experimental plants 3 weeks before each burn. Measured soil moisture content varied within 11.6% ± 6.8%. Burning was conducted in an incomplete block design: we randomly chose the greenhouse block and 8 or 9 species from the block on each burning day, took plants from the two light treatments for chosen species for flammability trials. Flammability data were collected for all plants from the same block on 2 consecutive burning days or within one day. Flammability trials of plants from two different blocks were separated by a minimum of 3 days. To maintain an upright canopy for individuals of *D. californica* and *B. saccharoides*, we added a wire cylinder to all plants after the first trial, of which 16 tested plants (one replicate per treatment from eight species) did not vary significantly in flammability when compared to observations from later trials (Supplementary Material S1).

Individual plant was placed on a balance (with 0.01g readability) that was connected to a laptop to measure remained mass every second during combustion. A metal ruler was placed by the plant as reference for maximum flame height reading. We measured flame temperature very second by placing k-type thermocouples (0.8 mm diameter, Omega Engineering, INC, Norwalk CT) at multiple heights relative to soil surface: 0 cm, 10 cm, 50 cm, and 100 cm. We also placed a black aluminum disc (3.65 mm in thickness, 9.0 cm in diameter, 53 g in mass) at 50 cm height and the soil surface to estimate heat released at the two locations. We measured the temperature of the aluminum disc before ignition, after flame extinction, and when no ember was in vision with an infrared thermometer gun (TG56, FLIR Systems, Wilsonville OR). We ignited the plant base by igniting a cotton ball to which 10 ml pure ethanol was added and then placing the burning cotton ball by plant base for 45 s. We removed the cotton ball 5 s after ignition occurred. Ignition was counted as a failure if the plant did not ignite after exposure to the ignition source for 45 s. We recorded relative humidity and temperature every 5 minutes during combustion using HOBO temperature and humidity sensor (Onset, Bourne MA), and recorded ignition delay, flaming and smoldering combustion duration.

Because fuel moisture content varied prior to and after combustion, we could not measure consumability by dry mass directly but instead estimated it (Supplementary Material S2 ).

**Post fire recovery**

We returned all plants to the greenhouse after burning trials. They were kept in well-lit area in greenhouse, watered and fertilized regularly. All plants were relocated every 2 weeks. We assessed plant survival two months after the burn by counting the number of tillers that emerged: plants with post-fire tiller number larger than 0 were counted as survived individuals, plants were counted as dead otherwise. We then harvested aboveground biomass three months after the fire to assess resprouting strength. To control for plant size effect, we calculated percentage biomass recovered (post-fire aboveground biomass/pre-fire aboveground biomass) to assess biomass recovery of experimental plants.

**Data analysis**

Due to the correlation between measured variables, we conducted principal component analysis (PCA) on plant trait and flammability measurements to select variables for further analysis. We included total aboveground biomass, bulk density, live fuel moisture, specific leaf area, and leaf surface area to volume ratio in PCA. To do PCA for flammability measurements, we included biomass loss, heat absorbed by aluminum discs, maximum flame height, maximum mass loss rate, time to ignition, and combustion duration. We dropped integrated temperature (Supplementary Material S2) from further analyses because of the large number of zeros. Measurements were chosen for further analysis if: 1) trait measurements had large loadings on the first two PCA axes, and also fell into each of the categories including individual, canopy, and leaf level measurements; 2) flammability measurements with large loadings on PCA axes that not only represented heat release but also demonstrated distribution of heat release in space.

To determine if shade intolerant grasses are more flammable in comparison to shade tolerant grasses, we built two linear mixed effects models with shade tolerance and light treatment as fixed effects to predict each of the two selected flammability measurements: heat release at the soil surface and 50 cm height. Some species (e.g. *B. gracilis, N. tenuissima*, *N. viridula*, *C. latifolium*, and *A. purpurea*) under 0% shade did not reach the same size as mature plants we observed in the field (Sorensen et al. 2012, Peláez et al. 2013, Gao and Schwilk 2018). Variation in aboveground biomass can contribute to flammability variation that may not be observed in mature perennial grasses. Therefore, we also included biomass as a model covariate. The final linear mixed effects models included species shade tolerance, light treatment, and total aboveground biomass as fixed effects with all possible interaction terms. We allowed intercept to vary by species to account for other potential species specific variation and applied this to all the following models.

A logistic model was built to determine how fire behavior and plant traits influence post-fire survival rate. Species shade tolerance, pre-fire tiller number, and heat release at the soil surface were included in model as fixed effects with all possible interaction terms. We then built a linear mixed effect model with all the same predictors but only for observations that did resprout to examine how survived plants vary in the strength of resprouting in terms of percentage biomass recovered.

To determine plant trait effects on heat release at the soil surface and 50 cm height, . we built linear mixed effect models including biomass, live fuel moisture content, biomass density, and specific leaf area as fixed effects. To account for daily temperature variation we included temperature in each model as a fixed effect without associated interaction term. We included all possible 2-way and 3-way interactions among plant traits. Light treatment was not included in the model because we assumed that we included all important plant traits in our model that can vary under light treatments to influence flammability.

To disentangle the effects of leaf trait and species phenology on live fuel moisture from light effect, we built a linear mixed effect model including specific leaf area, live to dead biomass ratio, and light treatments as fixed effects with all possible interaction effects.

All mixed effects models were built using lme4 package in R (Bates et al. 2015, R Core Team 2019). Independent variables were standardized as z-scores to make fixed effects comparable. We log-transformed all dependent variables to avoid violating the assumption of the normal-distribution of residuals during linear regression. We examined significance of fixed effects using Anova() function from car package (Fox et al. 2013). Type III sums of squares were calculated for testing hypothesis including interaction effects (Shaw and Mitchell-Olds 1993, Hector et al. 2010). Approximate degree of freedom and p-value were calculated using Kenward-Roger approximation to avoid unacceptable type-I error (Luke 2017). Data and code can be found here: <https://github.com/XiulinGao/shade-flammability>.

**Results**

**Variation of flammability measurements and plant traits**

The first two principal components for flammability measurements accounted for 77.6% of total variance (Supplementary Material Figure S3-a). Measurements including total biomass combusted, heat absorbed by aluminum discs at the soil surface and at 50 cm height, and combustion duration had large loading values on the first two axes. Heat absorbed at the soil surface and 50 cm height were chosen for further analysis.

Accumulative variance of the first two principal components for plant trait measurements was 61.8%, and specific leaf area, fuel moisture content, bulk density, and aboveground biomass had large loading values on the first 2 axes (Supplementary Material Figure S3-b). We kept these four traits as candidate flammability traits for further analysis.

**Negative correlation between grass shade tolerance and heat release**

As expected, aboveground biomass positively influenced heat release at the soil surface (p = 0.009) and at 50 cm height (p < 0.001). In addition, plants grown under 50% shade produced less heat at both locations than did plants grown under 0% shade (soil surface: p < 0.001, Fig. 2; 50cm: p = 0.009, Fig. 3). However, species with greater shade tolerance produced less heat at 50 cm height (fixed effect p = 0.009, Fig. 3). Moreover, the positive effect of biomass on heat release at 50 cm depended on light treatment and species shade tolerance (three-way interaction p = 0.016). Under the 0% shade, the same amount of increase in biomass led to a larger increase in heat release at 50 cm for species with greater shade tolerance in comparison to those under 50% shade (interaction coefficient: 0.27, Fig. 3). There was no effect of shade tolerance on heat release at the soil surface.

**Relationship between soil heating, plant traits and fire response**

Heat release at the soil surface negatively influence both post-fire survival rate (p = 0.039,

Fig.4) and percentage biomass recovered (p = 0.005, Fig. 5). Although we did not detect any main effect of pre-fire tiller number on survival or biomass recovery, plants with more pre-fire tillers were less affected by increased soil heating in terms of percentage biomass recovered (interaction coefficient: 0.40, p = 0.029). Species shade tolerance did not affect post-fire response of experimental plants.

**Plant trait effects on heat release**

Plants with more biomass produced more heat at the soil surface ( p = 0.047, Fig. 6) and at 50 cm height (p < 0.001, Fig. 7). In contrast, higher fuel moisture decreased heat release at both locations (p < 0.001). There was no interaction effect between aboveground biomass and fuel moisture content on heat release at either location. However, fuel moisture content interacted with specific leaf area to influence heat release at the soil surface. For plants with thinner leaves, increasing fuel moisture content led to a slower decrease (interaction coefficient: 0.21, p = 0.005) in heat release at the soil surface when compared to the enhanced negative effect of fuel moisture content in plants with thicker leaves. No effect of bulk density was detected on heat release at either location.

We did further explore how specific leaf area, live to dead biomass ratio, and light environment influence live fuel moisture. Plants grew under 50% shade had higher moisture content at the time of fire than did plants grew under full sunlight (p < 0.001, Fig. 8). As live to dead biomass ratio increased so did live fuel moisture (p < 0.001). However, the positive effect of live to dead biomass ratio on live fuel moisture was strengthened in plants from 0% shade treatment (p < 0.001).

ANOVA and coefficient tables for all models can be found in Supplementary Material Table S4-S7.

**Discussion**

1. **Reduced flammability in tree-associated grasses helps maintain savannas**

We hypothesized that there are two different flammability strategies coping with light competition in savannas: shade tolerant, less flammable grasses that can coexist with trees, and shade intolerant, flammable grasses that burn frequently to keep canopy open. Our results were consistent with this hypothesis: we found that shade tolerant grasses produced less heat at 50 cm height than did shade intolerant grasses across both light treatments and the measured biomass range. Although, we found that under 50% shade treatment at the lower range of biomass (biomass < 5 g), grasses with high shade tolerance tended to produce more heat than grasses with low shade tolerance (Fig. 2). However, once individuals of shade intolerant grasses managed to maintain relatively higher biomass (biomass > 5 g) under 50% shade, they were more flammable than shade tolerant grasses of similar biomass. This shows that shade tolerance and flammability are correlated in grasses because of divergence in species specific traits other than biomass, and that differences in fire behavior are not due to plastic response to the environment.

Species specific flammability can potentially cause different plant responses to fire at small spatial scale that would help maintain savannas in long term. Fire-induced plant mortality is largely determined by heat exposure above lethal temperature in live plant tissues (Keyser et al. 2006, Catry et al. 2010, Bowman et al. 2017). As total heat release increases, so does plant mortality (Vilà et al. 2001). However, heat release at different locations can have different biological effects on plants. Prolonged soil heating that often results from extended fire residence time will decrease the survival rate of surface and belowground reproductive structures in both trees and grasses, and cause distortion and dysfunction of xylems in trees (Ryan and Frandsen 1991, Odion and Davis 2000, Balfour and Midgley 2006, Dickinson 2016). Increased heat release aboveground at higher locations is detrimental to trees as it damages cambium tissues or causes top-kill (Keyser et al. 2006, Catry et al. 2010, Smith et al. 2017). In contrast, given no important grass tissues will be exposed to such heat but only dead canopy biomass, canopy heating is less lethal to grasses. Therefore, fast spreading fires would have differentiated effects on trees and grasses if grasses are homogenously flammable, which will challenge the long term coexistence of these two. The reduced heat release at 50 cm height in tree-associated grasses, however, might lead to dynamic fire response in local plants by causing less damage to coexisting trees and help maintain the tree-grass mixture.

1. **Post-fire resprouting dynamic**

Experimental plants producing less heat at the soil surface were more likely to survive and recovered better after the fire. As perennial grasses resprout from surface and belowground meristems, heat release at the soil surface directly determines survival in surface meristems thus influences resprouting. Prolonged soil heating also increases heat transferred into the soil and increases the likelihood of belowground meristems being exposed to lethal temperature (Swezy and Agee 1991, Stephens and Finney 2002, Choczynska and Johnson 2009, Gonzalez et al. 2015). In contrast to previous work, we did not find higher survival rate in plants with more pre-fire tillers (Moore et al. 2019). However, increased pre-fire tillers mitigated the damage that extended soil heating could do to biomass regeneration, which is likely due to increased number of surviving buds that is resulted from a larger bud bank (Hendrickson and Briske 1997, Benson et al. 2004). In addition, increased number of tillers in defined area (e.g. the pot) probably can provide better heat isolation via reduced exposure surface in densely packed tillers, especially when combustion is fast.

Shade intolerant grasses were not more fire resilient than shade tolerant grasses. Previous work suggests that post-fire resprouting in grasses is related to phylogenetic lineage and fire history in natural habitat (Ripley et al. 2015). The majority of our study species are common in grassy ecosystems where fire is one of the predominant disturbances. As a common disturbance response trait, it is possible that resprouting only acts as a precondition for flammability to evolve in grasses preferring open habitats (Bond and Midgley 1995).

1. **Plant phenology influences flammability**

Aboveground biomass and live fuel moisture were key flammability traits influencing heat release, while specific leaf area weakened the negative effect of live fuel moisture on heat release at the soil surface. Biomass increases heat release and live fuel moisture decreases heat release because the former one acts as heat source while the latter one acts as heat sink. High specific leaf area indicates more exposure to heat of fuels, and large surface area where evaporation happens quickly. This can accelerate pre-heating process thus mitigate the negative effect of live fuel moisture on flammability. However, such interaction effect was not observed for heat release at 50 cm height. This may be due to the lower bulk density in canopy biomass, which counteracts heat transfer during combustion and offsets the leaf trait effect (Schwilk 2015). In contrast to previous work, we found that bulk density did not influence heat release at the soil surface (Fill et al. 2016, Gao and Schwilk 2018). It is possible that effect of bulk density on soil heating was negligible in live fuel, of which the high fuel moisture content severely suppresses combustion.

As one of the key flammability traits, live fuel moisture is determined by environmental factors, fuel traits, and plant phenology. Precipitation and drought events directly influence live fuel moisture. However, fuel moisture content at the time of fire is determined by the interaction between environment and fuel properties such as particle size (Pellizzaro et al. 2007b, Nelson and Hiers 2008, Jin and Chen 2012). Plant phenology controls the change in live to dead biomass ratio of a plant and thus influences live fuel moisture (Wittich 2011, de Angelis et al. 2012). Phenology varies between cool and warm season grasses: cool season grasses start growth in early spring and have summer dormancy, and the opposite occurs in warm season grasses (Peterson et al. 2002). Due to the advantage of C3 photosynthesis in cold and shady environments, C3 grasses are cool season grasses with better mechanism tolerating shade (Pearcy and Ehleringer 1984, Ehleringer and Monson 1993, Osborne and Freckleton 2009). The majority of grasses that were grouped into high shade tolerance in our study were cool season C3 grasses having higher live to dead biomass ratio in early spring when burning was conducted. Therefore, reduced flammability observed in shade tolerant grasses was due to different plant phenology that shade tolerant, C3 grasses had higher live to dead biomass ratio, thus higher live fuel moisture, at the time of fire.

1. **Future work**

It is possible that shade tolerance and flammability are two different plant strategies that evolutionarily correlated in grasses. Grasses evolved in the late Cretaceous with ancestor living in mesic, shady environments (Osborne and Freckleton 2009, Edwards and Smith 2010). Initial shift into open habitats in grasses possibly occurred in the early Eocene, and is linked to the evolution of disturbance resistant traits (Bouchenak-Khelladi et al. 2010, Strömberg 2011, Linder et al. 2018). Evolution of C4 photosynthesis may then enable the persistence of grasses in open-habitat via high water use efficiency (Edwards and Smith 2010). Fire in combination with drought then promoted the formation and expansion of C4-dominant grasslands and savannas during late Miocene at the expense of trees, during which evolution of flammability traits could occur through vegetation-fire feedbacks (Keeley and Rundel 2005, Anderson 2006, Keeley et al. 2011). Future experiment incorporating grass phylogeny and wider range of open- and closed-habitat species is highly suggested to further examine this hypothesis. The potential increase in fitness when being flammable to suppress tree cover in shade intolerant grasses is increased light resource. Although shade intolerant species produced more heat up in the canopy, such altered fire behavior however is laboratory-based observations. To further investigate the biological significance of such variations in flammability between species varying in habitat preference, field-scale burning experiment looking at both fire behavior and fire responses in trees is required.

1. **Conclusion**

We found a significant negative correlation between grass shade tolerance and flammability. Besides influence of biomass, observed variation in flammability is largely due to different light environments and plant phenology influencing live fuel moisture. Although heat release at the soil surface decreased survival rate and post-fire biomass recovery in grasses, plants with more pre-fire tillers had better chance to recover after fire. Our study is the first work examining how grasses varying in shade tolerance behave as fuels. The study will provide a novel perspective to understand fire-maintained stability of savannas by suggesting two different plant flammability strategies in grasses. The outcome will also benefit fire management and fire risk prediction of grassy ecosystems where species composition varies across space.

**Reference**

Anderson, R. C. 2006. Evolution and origin of the Central Grassland of North America : climate , fire , and mammalian grazers. Journal of the Torrey Botanical Society 133:626–647.

de Angelis, A., S. Bajocco, and C. Ricotta. 2012. Phenological variability drives the distribution of wildfires in Sardinia. Landscape Ecology 27:1535–1545.

Balfour, D. A., and J. J. Midgley. 2006. Fire induced stem death in an African acacia is not caused by canopy scorching. Austral Ecology 31:892–896.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal Of Statistical Software 67:1–48.

Belsky, A. J. 1994. Influences of Trees on Savanna Productivity: Tests of Shade, Nutrients, and Tree-Grass Competition. Ecology 75:922.

Benson, E. J., D. C. Hartnett, and K. H. Mann. 2004. Belowground bud banks and meristem limitation in tallgrass prairie plant populations. American Journal of Botany 91:416–421.

Bond, W. J., and J. J. Midgley. 1995. Kill thy neighbour : an individualistic argument for the evolution of flammability kill thy neighbour. Oikos 73:79–85.

Bouchenak-Khelladi, Y., G. A. Verboom, V. Savolainen, and T. R. Hodkinson. 2010. Biogeography of the grasses (Poaceae): A phylogenetic approach to reveal evolutionary history in geographical space and geological time. Botanical Journal of the Linnean Society 162:543–557.

Bowman, D. M. J. S., C. Haverkamp, K. D. Rann, and L. D. Prior. 2017. Differential demographic filtering by surface fires : How fuel type and fuel load affect sapling mortality of an obligate seeder savanna tree. Journal of Ecology:1–13.

Bowman, D. M. J. S., H. J. MacDermott, S. C. Nichols, and B. P. Murphy. 2014. A grass-fire cycle eliminates an obligate-seeding tree in a tropical savanna. Ecology and Evolution 4:4185–4194.

Cardoso, A. W., I. Oliveras, K. A. Abernethy, K. J. Jeffery, D. Lehmann, J. E. Ndong, I. Mcgregor, C. M. Belcher, W. J. Bond, and Y. S. Malhi. 2018. Grass Species Flammability , Not Biomass , Drives Changes in Fire Behavior at Tropical Forest-Savanna Transitions. Frontiers in Forest and Global Change 1.

Catry, F. X., F. Rego, F. Moreira, P. M. Fernandes, and J. G. Pausas. 2010. Post-fire tree mortality in mixed forests of central Portugal. Forest Ecology and Management 260:1184–1192.

Charles-Dominique, T., G. F. Midgley, K. W. Tomlinson, and W. J. Bond. 2018. Steal the light: shade vs fire adapted vegetation in forest–savanna mosaics. New Phytologist 218:1419–1429.

Choczynska, J., and E. A. Johnson. 2009. A soil heat and water transfer model to predict belowground grass rhizome bud death in a grass fire. Journal of Vegetation Science 20:277–287.

Cleland, E. E., I. Chuine, A. Menzel, H. A. Mooney, and M. D. Schwartz. 2007. Shifting plant phenology in response to global change. Trends in Ecology and Evolution 22:357–365.

D’Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/gire cycle, and global change. Annual Review of Ecology and Systematics 23:63–87.

Dickinson, M. B. 2016. Heat transfer and vascular cambium necrosis in the boles of trees during surface fires. Forest Fire Research & Wildland Fire Safety.

Dimitrakopoulos, A. P., and A. M. Bemmerzouk. 2003. Predicting live herbaceous moisturecontent from a seasonal drought index. International Journal of Biometeorology 47:73–79.

Edwards, E. J., and S. A. Smith. 2010. Phylogenetic analyses reveal the shady history of C4 grasses. Proceedings of the National Academy of Sciences of the United States of America 107:2532–2537.

Ehleringer, J. R., and R. K. Monson. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. Annual Review of Ecology and Systematics 24:411–439.

Emery, N., K. Roth, and A. L. Pivovaroff. 2020. Flowering phenology indicates plant flammability in a dominant shrub species. Ecological Indicators 109:105745.

Engber, E. a., and J. M. Varner. 2012. Patterns of flammability of the California oaks: the role of leaf traits. Canadian Journal of Forest Research 42:1965–1975.

Fill, J. M., B. M. Moule, J. M. Varner, and T. A. Mousseau. 2016. Flammability of the keystone savanna bunchgrass Aristida stricta. Plant Ecology 217:331–342.

Fox, J., M. Friendly, and S. Weisberg. 2013. Hypothesis tests for multivariate linear models using the car package. R Journal 5:39–52.

Gagnon, P. R. 2010. Does pyrogenicity protect burning plants? Ecoloy 91:3481–3486.

Gao, X., and D. W. Schwilk. 2018. Grass Canopy Architecture Influences Temperature Exposure at Soil Surface. Fire 1.

Gonzalez, S. L., L. Ghermandi, and D. V. Peláez. 2015. Fire temperature effects on perennial grasses from northwestern Patagonian grasslands. Ecological Research 30:67–74.

He, T., J. G. Pausas, C. M. Belcher, D. W. Schwilk, and B. B. Lamont. 2012. Fire-adapted traits of Pinus arose in the fiery Cretaceous. New Phytologist:751–759.

Hector, A., S. von Felten, and B. Schmid. 2010. Analysis of variance with unbalanced data: An update for ecology & evolution. Journal of Animal Ecology 79:308–316.

Hendrickson, J. R., and D. D. Briske. 1997. Axillary bud banks of two semiarid perennial grasses: Occurrence, longevity, and contribution to population persistence. Oecologia 110:584–591.

Hoffmann, W. A., S. Y. Jaconis, K. L. Mckinley, E. L. Geiger, S. G. Gotsch, and A. C. Franco. 2012. Fuels or microclimate? Understanding the drivers of fire feedbacks at savanna-forest boundaries. Austral Ecology 37:634–643.

Jin, S., and P. Chen. 2012. Modelling drying processes of fuelbeds of Scots pine needles with initial moisture content above the fibre saturation point by two-phase models. International Journal of Wildland Fire 21:418–427.

Keeley, J. E., J. G. Pausas, P. W. Rundel, W. J. Bond, and R. a. Bradstock. 2011. Fire as an evolutionary pressure shaping plant traits. Trends in Plant Science 16:406–411.

Keeley, J. E., and P. W. Rundel. 2005. Fire and the Miocene expansion of C4 grasslands. Ecology Letters 8:683–690.

Keyser, T. L., F. W. Smith, L. B. Lentile, and W. D. Shepperd. 2006. Modeling postfire mortality of ponderosa pine following a mixed-severity wildfire in the Black Hills: The role of tree morphology and direct fire effects. Forest Science 52:530–539.

Linder, H. P., C. E. R. Lehmann, S. Archibald, C. P. Osborne, and D. M. Richardson. 2018. Global grass ( Poaceae ) success underpinned by traits facilitating colonization , persistence and habitat transformation 41:1125–1144.

Ludwig, F., H. Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. Journal of Vegetation Science 12:579–588.

Luke, S. G. 2017. Evaluating significance in linear mixed-effects models in R. Behavior Research Methods 49:1494–1502.

de Magalhães, R. M. Q., and D. W. Schwilk. 2012. Leaf traits and litter flammability: Evidence for non-additive mixture effects in a temperate forest. Journal of Ecology 100:1153–1163.

Michelaki, C., N. M. Fyllas, A. Galanidis, M. Aloupi, E. Evangelou, M. Arianoutsou, and P. G. Dimitrakopoulos. 2020. Adaptive fl ammability syndromes in thermo-Mediterranean vegetation , captured by alternative resource-use strategies. Science of the Total Environment 718.

Moore, N. A., J. S. Camac, and J. W. Morgan. 2019. Effects of drought and fire on resprouting capacity of 52 temperate Australian perennial native grasses. New Phytologist 221:1424–1433.

Nano, C. E. M., and P. J. Clarke. 2010. Woody-grass ratios in a grassy arid system are limited by multi-causal interactions of abiotic constraint, competition and fire. Oecologia 162:719–732.

Naumburg, E., L. E. DeWald, and T. E. Kolb. 2001. Shade responses of five grasses native to southwestern U.S. Pinus ponderosa forests. Canadian Journal of Botany 79:1001–1009.

Nelson, R. M., and J. K. Hiers. 2008. The influence of fuelbed properties on moisture drying rates and timelags of longleaf pine litter. Canadian Journal of Forest Research 38:2394–2404.

Van Nieuwstadt, M. G. L., and D. Sheil. 2005. Drought, fire and tree survival in a Borneo rain forest, East Kalimantan, Indonesia. Journal of Ecology 93:191–201.

Niinemets, Ü. L. O. 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecol Res:693–714.

Odion, D. C., and F. W. Davis. 2000. Fire , soil heating , and the formation of vegetation patterns in chaparral. Ecological Monographs 70:149–169.

Osborne, C. P., and R. P. Freckleton. 2009. Ecological selection pressures for C4 photosynthesis in the grasses. Proceedings. Biological sciences / The Royal Society 276:1753–1760.

Pausas, J. G., and J. E. Keeley. 2009. A Burning Story: The Role of Fire in the History of Life. BioScience 59:593–601.

Pausas, J. G., J. E. Keeley, and D. W. Schwilk. 2017. Flammability as an ecological and evolutionary driver. Journal of Ecology 105:289–297.

Pearcy, R. W., and J. Ehleringer. 1984. Comparative ecophysiology of C 3 and C 4 plants. Plant, Cell & Environment 7:1–13.

Peláez, D. V., R. J. Andrioli, O. R. Elia, E. E. Bontti, M. A. Tomas, and F. R. Blazquez. 2013. Response of grass species to different fire frequencies in semi-arid rangelands of central Argentina. Rangeland Journal 35:385–392.

Pellizzaro, G., C. Cesaraccio, P. Duce, A. Ventura, and P. Zara. 2007a. Effects of seasonal weather variations and phenology on live fuel moisture content and ignitability of mediterranean species. Sevilla - Espana Wildfire 16:633–641.

Pellizzaro, G., C. Cesaraccio, P. Duce, A. Ventura, and P. Zara. 2007b. Relationships between seasonal patterns of live fuel moisture and meteorological drought indices for Mediterranean shrubland species. International Journal of Wildland Fire 16:232–241.

Peterson, D. L., K. P. Price, and E. A. Martinko. 2002. Discriminating between cool season and warm season grassland cover types in northeastern Kansas. International Journal of Remote Sensing 23:5015–5030.

Poorter, H., Ü. Niinemets, N. Ntagkas, A. Siebenkäs, M. Mäenpää, S. Matsubara, and T. L. Pons. 2019. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. New Phytologist 223:1073–1105.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasband, W. S. (n.d.). ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.

Ripley, B., V. Visser, P.-A. Christin, S. Archibald, T. Martin, and C. Osborne. 2015. Fire ecology of C 3 and C 4 grasses depends on evolutionary history and frequency of burning but not photosynthetic type. Ecology 96:2679–2691.

Roderick, M. L., S. L. Berry, I. R. Noble, and G. D. Farquhar. 1999. A theoretical approach to linking the composition and morphology with the function of leaves. Functional Ecology 13:683–695.

Romanyà, J., P. Casals, and V. R. Vallejo. 2001. Short-term effects of fire on soil nitrogen availability in Mediterranean grasslands and shrublands growing in old fields. Forest Ecology and Management 147:39–53.

Ryan, K. C., and W. H. Frandsen. 1991. Basal injury from smoldering sires in mature Pinus Ponderosa Laws . International Journal of Wildland Fire 1:107–118.

Schwilk, D. W. 2003. Flammability is a niche construction trait: canopy architecture affects fire intensity. The American naturalist 162:725–733.

Schwilk, D. W. 2015. Dimensions of plant flammability. New Phytologist 206:486–488.

Schwilk, D. W., and D. D. Ackerly. 2001. Flammability and serotiny as strategies : correlated evolution in pines. Oikos 2:326–336.

Schwilk, D. W., and B. Kerr. 2002. Genetic niche-hiking: an alternative explanation for the evolution of flammability. Oikos 99:431–442.

Sharrow, S. H., and H. A. Wright. 1977. Effects of fire, ash, and litter on soil Nitrate , temperature, moisture and tobosagrass production in the rolling plains. Society for Range Management 30:266–270.

Shaw, R. G., and T. Mitchell-Olds. 1993. ANOVA for unbalanced data: an overview. Ecology 74:1638–1645.

Simpson, K. J., B. S. Ripley, P. A. Christin, C. M. Belcher, C. E. R. Lehmann, G. H. Thomas, and C. P. Osborne. 2016. Determinants of flammability in savanna grass species. Journal of Ecology 104:138–148.

Smith, A. M. S., A. F. Talhelm, D. M. Johnson, A. M. Sparks, C. A. Kolden, K. M. Yedinak, K. G. Apostol, W. T. Tinkham, J. T. Abatzoglou, J. A. Lutz, A. S. Davis, K. S. Pregitzer, H. D. Adams, and R. L. Kremens. 2017. Effects of fire radiative energy density dose on Pinus contorta and Larix occidentalis seedling physiology and mortality. International Journal of Wildland Fire 26:82–94.

Sorensen, G. E., D. B. Wester, and S. Rideout-Hanzak. 2012. A nondestructive method to estimate standing crop of purple threeawn and blue Grama. Rangeland Ecology and Management 65:538–542.

Stephens, S. L., and M. A. Finney. 2002. Prescribed fire mortality of Sierra Nevada mixed conifer tree species: Effects of crown damage and forest floor combustion. Forest Ecology and Management 162:261–271.

Strömberg, C. A. E. 2011. Evolution of Grasses and Grassland Ecosystems. Annual Review of Earth and Planetary Sciences 39:517–544.

Swezy, D. M., and J. K. Agee. 1991. Prescribed-fire effect on fine root and tree mortality in old-growth Ponderosa pine. Canadian Journal of Forest Research 21:626–634.

Vilà, M., F. Lloret, E. Ogheri, and J. Terradas. 2001. Positive fire-grass feedback in Mediterranean Basin woodlands. Forest Ecology and Management 147:3–14.

Weltzin, J. F., and M. B. Coughenour. 1990. Savanna tree influence on understory vegetation and soil nutrients in Northwestern Kenya. Journal of Vegetation Science 1:325–332.

Whittaker, R. H., L. E. Gilbert, and J. H. Connell. 1979. Analysis of two-phase pattern in a mesquite grassland , Texas. Journal of Ecology 67:935–952.

Wittich, K. P. 2011. Phenological observations of grass curing in Germany. International Journal of Biometeorology 55:313–318.



**Figure 1.** Concept for the correlation between microhabitat preference, ecological strategy, and flammability in grasses. Correlation can arise from traits that can response to light environments which also influence flammability.



**Figure 2.** Relationship between total aboveground biomass, light environments, shade tolerance, and heat release at the soil surface. Lines indicate the best fitted linear model for species mean. Small points in background are individual observations and large points are specie means. Cutoff value used for grouping species by shade tolerance is determined by plot\_model function from “sjPlot” package in R (Lüdecke 2020).



**Figure 3.** Relationship between total aboveground biomass, light environments, shade tolerance, and heat release at 50cm height. Lines indicate the best fitted linear model for species mean of each shade tolerance group. Small points in background are individual observations and large points are specie means. Cutoff value used for grouping species by shade tolerance is determined by plot\_model function from “sjPlot” package in R (Lüdecke 2020).



**Figure 4.** Predicted grass survival rate based on the logistic model built. Prediction here is only based on heat release at the soil surface as to show the fixed effect of soil heating on survival rate.



**Figure 5.** Predicted post-fire percentage biomass recovery based on the linear mixed effect model built. Prediction here is based on pre-fire tiller number and heat release at soil surface to show fixed effects of these two predictors.



**Figure 6.** Relationship between aboveground biomass, live fuel moisture, specific leaf area, and heat release at soil surface. Lines indicate the best fitted linear model for predicted value of heat release at soil surface using the built linear mixed effect model. Points are individual observations. Cutoff values used for grouping observations by live fuel moisture and specific leaf area are determined by plot\_model function from “sjPlot” package in R (Lüdecke 2020).



**Figure 7.** Relationship between aboveground biomass, live fuel moisture, and heat release at 50 cm height. Lines indicate the best fitted linear model for predicted value of heat release at 50 cm using the built linear mixed effect model. Points are individual observations. Cutoff value used for grouping observations by live fuel moisture is determined by plot\_model function from “sjPlot” package in R (Lüdecke 2020).



**Figure 8.** Relationship between live to dead biomass ratio, specific leaf area, light environments, and live fuel moisture. Lines indicate the best fitted linear model for species mean. Points are individual observations. Cutoff value used for grouping observations by specific leaf area is determined by plot\_model function from “sjPlot” package in R (Lüdecke 2020).