

Landscape variation in plant leaf flammability is driven by leaf traits responding to environmental gradients

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Citation: Krix, D. W., and B. R. Murray. 2018. Landscape variation in plant leaf flammability is driven by leaf traits responding to environmental gradients. *Ecosphere* 9(2):e02093. 10.1002/ecs2.2093

Abstract. Landscape differences in environmental conditions select for divergences among plant species in strategically important leaf traits such as leaf mass per area (LMA) and leaf area (LA). Interspecific variation in some of these same leaf traits has been associated to varying degrees with differences among species in leaf flammability, including the attributes ignitability, sustainability, and combustibility. Yet, how environmentally selected variation in leaf traits drives variation in leaf flammability at landscape scales remains largely unknown. Here, we compared leaf traits and flammability attributes between species of sheltered forest vegetation (low light, moist habitat) and plant species of exposed woodland vegetation (high light, dry habitat) in a fire-prone landscape of south-eastern Australia. We found that leaves of sheltered forest species were significantly more flammable via both higher ignitability and combustibility compared with exposed woodland species. These significant differences in leaf ignitability and combustibility were underpinned by sheltered forest species having leaves with significantly larger LA and lower LMA compared with exposed woodland species. Further, multiple regression analyses revealed that both LA and LMA were significantly and uniquely related to faster time to ignition (TTI; ignitability) and higher mean mass loss rate (combustibility). Most notably, although significantly higher fuel moisture content (FMC) of leaves of sheltered forest species significantly lengthened TTI, the lower LMA of these species played a more critical role in reducing TTI, with low LMA explaining more unique variation (partial $r^2 = 0.78$) in high leaf ignitability than low FMC (partial $r^2 = 0.49$). Our findings provide the first evidence that landscape-scale variation in leaf flammability is tightly coordinated with the primary strategic response of the leaf traits LMA and LA to an environmental gradient. Furthermore, projections for increasing wildfire frequency and intensity in the region will likely allow wildfires to overcome the once protective nature provided by topography to sheltered forest vegetation, which means that higher leaf flammability in sheltered forest species has the potential to exacerbate the effects of changing weather conditions to place sheltered forest habitat, their plants, and their animals, at even higher risk of catastrophic wildfire.

Key words: climate change; leaf flammability; plant traits; trait-based ecology; wildfire.

Received 25 September 2017; revised 15 December 2017; accepted 18 December 2017. Corresponding Editor: Debra P. C. Peters.

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INTRODUCTION

Research in trait-based ecology has identified fundamental patterns of variation in plant traits along environmental gradients (Reich et al. 1992, 2003, Wright et al. 2004, 2010, McGill et al. 2006, Poorter et al. 2009). Plant leaves, given their critical

role in plant metabolism, have been a key focus of much of this research. Landscape differences in abiotic conditions such as available light, ambient temperature, vapor pressure deficit, and rainfall have selected for physical adaptations in leaves to intercept sufficient light and maintain transpiration at acceptable levels, while maintaining a low

carbon cost in construction (Wright et al. 2004). Low light conditions favor larger leaves that are better at intercepting scarcer available light, often at a lower carbon investment per unit area (i.e., leaf mass per area, LMA; Bragg and Westoby 2002, Cornwell and Ackerly 2009). In brighter environments, which are also often drier and hotter, smaller leaves with higher LMA provide a better fit to abiotic conditions, given that light availability is not a strong limiting factor and that higher LMA allows lower transpirational water loss and greater resistance to wilting (Castro-Díez et al. 1997, Wright et al. 2001).

While landscape variation among species in traits such as LMA is driven largely by environmental gradients, laboratory studies have also found to varying degrees that interspecific variation in leaf traits can often be a predictor of variation in leaf flammability (Gill and Moore 1996, Ganteaume et al. 2013, Murray et al. 2013, Grootemaat et al. 2015). For example, lower leaf water content and lower LMA are associated with faster leaf ignition (Bianchi and Defossé 2015, Grootemaat et al. 2015), while higher LMA and larger leaf area (LA) are related to longer duration of leaf burn (Grootemaat et al. 2015). What has yet to be considered, however, is the possibility that if leaf traits are found to vary among species as a function of environmental conditions across a landscape, with these traits in turn driving differences among species in leaf flammability, then landscape variation in leaf level flammability might be largely predictable on the basis of variation in a few key leaf traits responding to environmental gradients. Uncovering such a predictive relationship, underpinned by fundamental environmental filtering of key leaf traits, is critical as it has the potential to provide an important initial link between flammability research at the leaf scale and research examining flammability at the scale of vegetation communities (Gill and Zylstra 2005). Identifying such links between leaf and community scales is crucial considering that leaves are considered to be one of the most important flammable plant structures (Gill and Moore 1996, Etlinger and Beall 2004). Leaves are frequently the first structures to ignite during bushfire (Pickett et al. 2009), promulgating fire to other plant structures and fuel sources. Furthermore, leaves contribute a large proportion of the living biomass available to a fire and recent modeling has shown

that the properties of living fuel, in particular plant leaves, are an important factor in landscape fire (Zylstra et al. 2016).

Leaf flammability is a multidimensional plant trait (Pérez-Harguindeguy et al. 2013) with implications for fire behavior based on ignitability, sustainability, and combustibility (Anderson 1970). Ignitability is a measure of how quickly combustion begins when a leaf is exposed to a heat source and how soon it can contribute heat from its own combustion to a larger fire. Sustainability represents how long combustion continues once a leaf has ignited and the length of the period it is generating heat from combustion. Combustibility is how much heat is given off while the leaf is burning. Taken together, a highly flammable leaf could be considered as having high ignitability (easily ignited), high sustainability (burning for a long period), and high combustibility (giving off a large amount of heat in a given amount of time). Several studies across the world have found that these three leaf flammability attributes vary substantially among species. For instance, leaves of some plant species ignite within a few seconds while others take much longer to ignite, if at all (Murray et al. 2013, Grootemaat et al. 2015).

In the present study, we first compare three key leaf traits including LMA, LA, and fuel moisture content (FMC) between plant species of sheltered forest vegetation along gullies (low light, moist habitat; Fig. 1a) and plant species of exposed woodland vegetation on ridgetops (high light, dry habitat; Fig. 1b), in a rugged area prone to intense wildfires in the Blue Mountains of south-eastern Australia. We test predictions that, relative to species from exposed woodland habitat, leaves of species from sheltered forest habitat have larger area to maximize light interception, lower LMA as transpiration is relatively less costly and water stress less likely, and higher FMC as water availability is greater in sheltered gullies with watercourses. We then test the hypothesis that leaf flammability attributes differ across the landscape in a way that is tightly coordinated with the primary strategic response of leaf traits to the environmental gradient between the habitats. To do this, we first compare three major leaf flammability attributes representing ignitability (time to ignition [TTI]), sustainability (burn duration [BD]), and combustibility (mean mass loss rate [MLR], a proxy for the mean rate heat is generated by

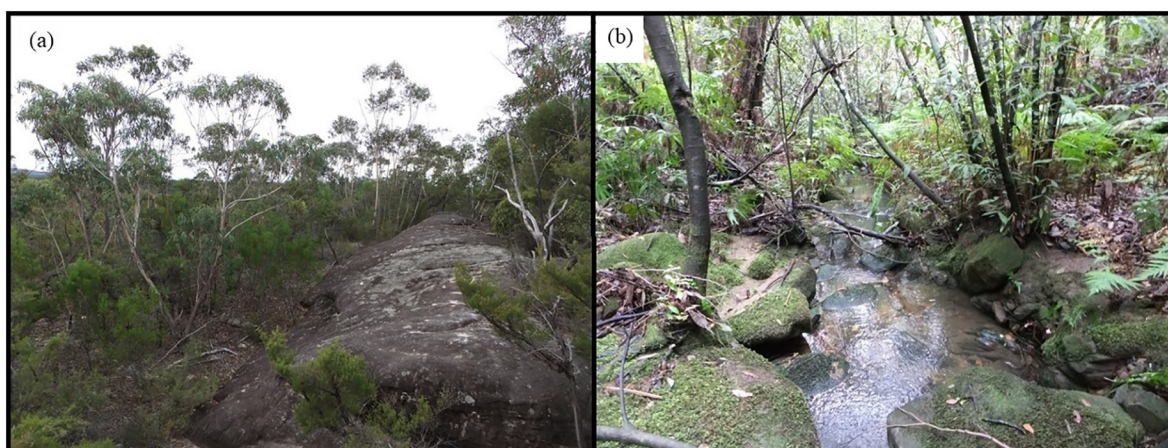


Fig. 1. Typical exposed woodland habitat on ridge tops (a) and sheltered forest habitat (b), in the study region. Despite large differences in abiotic conditions, these habitats can often be found within short distances of each other (<500 m).

combustion; Grootemaat et al. 2017, Prior et al. 2017) between sheltered forest and exposed woodland species to determine habitat differences in leaf flammability. We then examine interspecific relationships between the three leaf traits and the three flammability attributes to test the predictions that high ignitability is related to large LA and low LMA found in gully species, and that overall these two leaf traits counteract the buffering effect of high FMC in gully species; and that high sustainability and high combustibility are related to large LA and high LMA in gully species. These predictions are based on high ignitability being driven by large LA which increases the surface area available to intercept thermal energy and by low LMA which decreases thermal mass; on high sustainability being driven by large LA and high LMA which represent an increase in both the area and amount of material over which combustion can spread; and on high combustibility being driven by long BDs generating higher temperatures at the leaf surface through preheating.

METHODS

Study region

The study was undertaken in the Blue Mountains of south-eastern Australia, 75 km west of Sydney ($-33^{\circ}39'31''$ N, $150^{\circ}32'45''$ E). The area experiences a mild climate (mean temperature 17.2°C), with warm summers (mean temperature 21.9°C) and cool winters (mean

temperature 11.9°C), and a mean annual rainfall of 1086 mm with the majority of this falling in the summer (Australian Bureau of Meteorology 2014). The region is a deeply dissected sandstone plateau, with a system of ridges exposed to high solar insolation and deep, steep-sided gullies with lower light availability and permanent or ephemeral waterways at their base. The vegetation of the region varies from dry sclerophyll woodland on exposed ridgetop areas to temperate closed forest in sheltered gullies.

Exposed woodland vegetation is characterized by an open canopy consisting mostly of species in the family Myrtaceae such as *Corymbia gummifera*, *Eucalyptus piperita*, and *Eucalyptus sparsifolia*, and a high diversity of shrub species, for example, *Acacia suaveolens*, *Banksia spinulosa*, and *Petrophile pulchella*. Sheltered forest vegetation is dominated by canopy species such as *Backhousia myrtifolia*, *Ceratopetalum apetalum*, and *Tristaniopsis collina*, and understory species including *Acrotriche divaricata*, *Austromyrtus tenuifolia*, and *Hibbertia saligna*, along with fern (e.g., *Calochlaena dubia*, *Blechnum cartilagineum*) and climber species (e.g., *Cissus hypoglauca*, *Smilax australis*). These two vegetation associations are representative of large areas of the Blue Mountains and the Great Dividing Range, a World Heritage Listed Area that is prone to wildfire. Wildfire frequency can be variable; however, most areas in the region experience high-intensity fire every ten years (Hammill and Tasker 2010). Fire events may last a single day in

localized cases or continue for weeks and burn hundreds of hectares as fire moves over the landscape. Sheltered forests in gullies tend to burn less frequently than exposed woodlands on ridgetops (Penman et al. 2007, Leonard et al. 2014), due largely to gully forests being sheltered from high winds by topography. Sheltered areas also tend to experience relatively lower intensity fires which leaves the tree canopy intact in contrast to exposed areas where canopy (or crown) fires more often occur (Bradstock et al. 2010). Plant species in both sheltered and exposed habitats have adaptations to persist through fire, either as an individual by re-sprouting after fire events, or through offspring via canopy-held seed or soil seed banks.

Study sites and species

We performed plant species surveys in contiguous unmodified vegetation in the study region to generate a list of common species of exposed woodland habitat on ridgetops and common species of sheltered forest habitat in gullies adjacent to creeks (Appendix S1). We recorded all plant species present in a total of 50 sites across a 6 km² area to ensure a representative sample of commonly occurring species in the habitats was obtained, with 25 sites located in each of the two habitats. Each site measured 25 m² (5 × 5 m), and all sites were located at least 100 m from each other. Species identified in our surveys were assigned to either the understory stratum (<3 m mature height) or the overstory stratum (>3 m mature height). A total of 92 plant species were included in the study, comprising 57 species from exposed woodland habitat (10 overstory species, 47 understory species) and 35 species from sheltered gully habitat (12 overstory species, 23 understory species). Six species were found to occur in both habitats (*Lepidosperma laterale*, *Lomatia silaifolia*, *Phyllanthus hirtellus*, *Pteridium esculentum*, *Stylidium graminifolium*, *Xanthorrhoea media*). We excluded this small number of species from the study as our focus was on the distinctive flora of each habitat and we wanted to avoid the potentially confounding issue of the same species being represented in both habitat groups in our statistical analyses. One further unidentified species was excluded as only one individual was recorded. Taxonomic nomenclature for the study taxa followed Fairley and Moore (2010).

Leaf collection

Fully expanded, non-senescent leaves were collected from the outer exposed plant canopy from five mature individual plants representative of each species within the study region (Pérez-Harguindeguy et al. 2013). Each of the five sampled plants of each species occurred at five different sites from among the 50 study sites, with collection sites for each species dependent on the presence of the species in abundance. For woody species, short sections of a branch with several adult leaves attached were cut from the plant. For fern species, a section of rachis with several pinnae attached was cut from the frond. For climbers, sections of stem with several leaves attached were cut and removed. No more than two plant sections with leaves or pinnae of a given species were collected on a single day, with collections spread across June to August 2016. After removal from the plant, plant sections were wrapped in wet cloth, placed inside airtight plastic bags, and refrigerated overnight, consistent with the procedure outlined in Pérez-Harguindeguy et al. (2013), before measurement of leaf traits and leaf flammability the following day. The potential for this method of leaf storage to allow rehydration of leaf samples (Breshears et al. 2008) was minimized by ensuring quick turnaround between leaf collection and leaf measurements.

Leaf trait and flammability measurements

To sample leaves for measurements of the three leaf traits and three flammability attributes, leaves or phyllodes were detached at natural abscission points from the five collected plant sections of each species. In the case of ferns, pinnae were cut at the junction of the pinna and rachis. Only one compound-leafed species was included in the study, the bipinnate species *Acacia terminalis*, which was processed by detaching full lengths of a secondary rachis where it joined a primary rachis. Phyllodes, pinnae, and rachises are all hereafter referred to as leaves.

Measurements of LMA and FMC require total dehydration of samples. As our focus was on the flammability of fresh leaves and not dried leaves, it was not feasible to use the same leaves for trait and flammability measurements. Thus, measurements of LMA and FMC involved the use of leaves directly adjacent to the leaf that was used to measure flammability on each of the five replicate

plant sections. First, five trait leaves adjacent to a flammability leaf were detached from each of the five plant sections and scanned to calculate LA using ImageJ (Schneider et al. 2012). Following this, the five leaves were weighed on a Sartorius R200D balance to give their fresh mass, and then dried in an oven for three days at 80°C (Pérez-Harguindeguy et al. 2013), before reweighing to determine dry leaf mass. Fuel moisture content was then calculated as $([\text{fresh leaf mass}:\text{dry leaf mass}] \times 100)$, with low values representing leaves with low water content. Values of FMC ranged from 70 (*Leucopogon setiger*, exposed habitat) to 330 (*H. saligna*, sheltered habitat). We calculated LMA as $(\text{leaf dry mass in g/LA in m}^2)$ to give LMA in units of g/m^2 . Values of LMA ranged from 15 g/m^2 (*Adiantum aethiopicum*, sheltered habitat) to 640 g/m^2 (*Lomandra multiflora*, exposed habitat). For each flammability leaf, LA was measured directly, by scanning the leaf immediately prior to the leaf being burnt and later analyzing the scan in ImageJ (LA of the five adjacent leaves was used only to calculate LMA). Values of LA ranged from 13 mm^2 (*Leptospermum trinervium*, exposed habitat) to 5900 mm^2 (*C. dubia*, sheltered habitat).

Experimental burns of leaves were performed in a fan-forced muffle furnace set to 700°C following established procedures (Gill and Moore 1996, Murray et al. 2013, Grootemaat et al. 2015). This temperature permitted clear differentiation among species in leaf flammability and represents fire front temperatures during fire in eucalypt forest (300–1100°C, Wotton et al. 2012). Our study focused on leaf flammability, and while this is only one of several important components contributing to fire behavior in fire-prone systems, it is a critical component given that leaves are frequently the first structures to ignite during bushfire (Gill and Moore 1996, Pickett et al. 2009) and will likely dictate much of the behavior of fire spread through vegetation (Zylstra et al. 2016). Once the furnace temperature was stable at 700°C, the furnace door was opened and the leaf sample placed in the center of the furnace, so that there was no contact between the sample and the furnace walls. Samples were clamped by their petiole to the arm of a wheeled stand, horizontally orientated with their adaxial surface parallel to the uppermost surface of the furnace. This allowed us to place samples of all species in the furnace interior at a consistent height and orientation. Species with long leaves

too large to fit in the interior of the furnace (e.g., *Lomandra* and *Lepidosperma* spp.) were cut into 15 cm long samples. Trifoliate species (e.g., *Gompholobium grandiflorum*) were burned as three complete leaflets. Experimental burns of leaves were recorded and analyzed using a digital video recorder. Ignitability was measured as the time taken to the first appearance of glowing combustion (TTI in s), sustainability was measured as the time duration between the start and finish of combustion (BD in s), and combustibility was calculated as $(\text{leaf dry mass in mg/BD in s})$ for each leaf to give the mean rate of combustion (mean MLR in mg/s). Our measure of MLR should not be interpreted as a measure of intrinsic MLR (see Appendix S2). Initial leaf dry mass corresponded approximately with the total mass of leaf that burnt in the muffle furnace (Grootemaat et al. 2017). Values of leaf dry mass were calculated using LA values of flammability leaves and LMA values of the five adjacent trait leaves: $(\text{LA} [\text{flammability leaf}] \times \text{LMA} [\text{adjacent trait leaves}])$.

Statistical analyses

Mean values for traits (LA, LMA, and FMC) and flammability attributes (TTI, BD, and MLR) were calculated for each species (five replicate measurements for each trait/attribute per species) for use in statistical analysis. We employed traditional general linear models (GLMs) matched with complementary phylogenetic generalized least squares (PGLS) models to examine interspecific patterns in leaf traits (LA, LMA, and FMC) and leaf flammability attributes (TTI, BD, and MLR). We used a two-way ANOVA design in GLMs and PGLS models to determine whether our response variables (the three leaf traits and the three flammability attributes in separate models) varied significantly as a function of habitat (fixed explanatory variable with two levels, exposed vs. sheltered), vegetation stratum (fixed explanatory variable with two levels, understory vs. overstory), or the habitat \times stratum interaction.

We used a multiple regression design in GLMs and PGLS models to determine relationships between each of the three flammability attributes (response variables) and the three leaf traits (continuous explanatory variables). These models included the three categorical variables habitat (sheltered or exposed), stratum (understory or overstory), and the habitat \times stratum interaction

term as control variables. Thus, if any of these categorical variables were statistically significant in a model for one of the flammability attributes, then other unmeasured variables differentiating the habitats or strata would explain significant variation in that flammability attribute, in addition to any variation significantly explained by our measured leaf traits. However, if habitat, stratum, or the habitat \times stratum interaction did not have a significant effect on a flammability attribute, then any significant differences in leaf flammability attributes between the habitats could be principally explained by the differences in leaf traits between habitats. This final test was important to provide support for our overarching hypothesis that contrasting environmental conditions between the two habitats first and foremost drive habitat differences in leaf traits of species which are then responsible for differences in leaf flammability between habitats.

In multiple regression models, we employed a partial regression technique to determine whether each leaf trait could uniquely (i.e., independently of the other two leaf traits) explain variation in each flammability attribute. First, we calculated residuals from a series of separate multiple regression models of each flammability attribute (response variable) regressed against two of the leaf traits (the non-target predictor variables) and excluding the leaf trait (the target predictor variable) of interest. These non-target residuals represented the unexplained variation in a flammability attribute after accounting for the non-target predictors. We then removed collinearity effects among target and non-target predictors by calculating model residuals of the target predictor regressed against the non-target predictors. These target residuals represented variation in the target predictor not correlated with variation in the two non-target variables. Finally, the target residuals were regressed against the non-target residuals to give the variation in a flammability attribute uniquely explained by a target leaf trait predictor and independent of the two non-target leaf trait predictors.

A phylogenetic tree with branch lengths for the PGLS models was created for our species using the phylogeny of Zanne et al. (2014) generated in Phylomatic (Webb and Donoghue 2005). We built separate PGLS models assuming either Brownian motion (BM) or Ornstein-Uhlenbeck

(OU) processes (Appendix S2). The models were compared to each other using Akaike's information criterion (AIC). The best-fitting PGLS model (BM or OU) was then selected on the basis of the lowest AIC value (Appendix S3). To account for unequal species numbers in models containing categorical terms, marginal sums of squares (type II sums of squares) were used for significance testing. In the event, across all analyses in the study, the role of phylogeny was minimal, with results from phylogenetically informed analyses differing little, if at all, from traditional modeling.

All responses and continuous predictors were log transformed prior to analysis. Analyses and plotting were performed in R 3.3.1 (R Core Team 2016), using the packages *ape* (Paradis et al. 2004), *beanplot* (Kampstra 2008), *car* (Fox and Weisberg 2011), *nlme* (Pinheiro et al. 2017), and *phytools* (Revell 2012).

RESULTS

Leaf traits in relation to habitat and stratum

We found that LA was significantly larger in sheltered forest species compared with exposed woodland species (GLM: $F_{1,88} = 11.6$, $P = 0.0009$; PGLS [BM]: $F_{1,88} = 46.7$, $P < 0.0001$) and in the overstory compared with the understory stratum in both habitats (GLM: $F_{1,88} = 15.2$, $P = 0.0002$; PGLS [BM]: $F_{1,88} = 65.7$, $P < 0.0001$; Fig. 2a), with no significant habitat \times stratum interaction (GLM: $F_{1,88} = 2.8$, $P = 0.1$; PGLS [BM]: $F_{1,88} = 3.1$, $P = 0.08$). Values of LMA in sheltered forest species were significantly lower than for exposed woodland species (GLM: $F_{1,88} = 73.0$, $P < 0.0001$; PGLS [OU]: $F_{1,88} = 73.0$, $P < 0.0001$; Fig. 2b) and significantly lower in the understory compared with the overstory stratum in both habitats (GLM: $F_{1,88} = 14.0$, $P = 0.0003$; PGLS [OU]: $F_{1,88} = 14.0$, $P = 0.0003$; Fig. 2b), with no significant habitat \times stratum interaction (GLM: $F_{1,88} = 0.8$, $P = 0.4$; PGLS [OU]: $F_{1,88} = 0.8$, $P = 0.4$). Significantly higher FMC was found in sheltered forest species compared with exposed woodland species (GLM: $F_{1,88} = 38.3$, $P < 0.0001$; PGLS [OU]: $F_{1,88} = 38.3$, $P < 0.0001$; Fig. 2c) and in the understory of both habitats (GLM: $F_{1,88} = 17.5$, $P < 0.0001$; PGLS [OU]: $F_{1,88} = 17.5$, $P < 0.0001$; Fig. 2c), with no significant habitat \times stratum interaction emerging for FMC (GLM: $F_{1,88} = 0.2$, $P = 0.7$; PGLS [OU]: $F_{1,88} = 0.2$, $P = 0.7$).

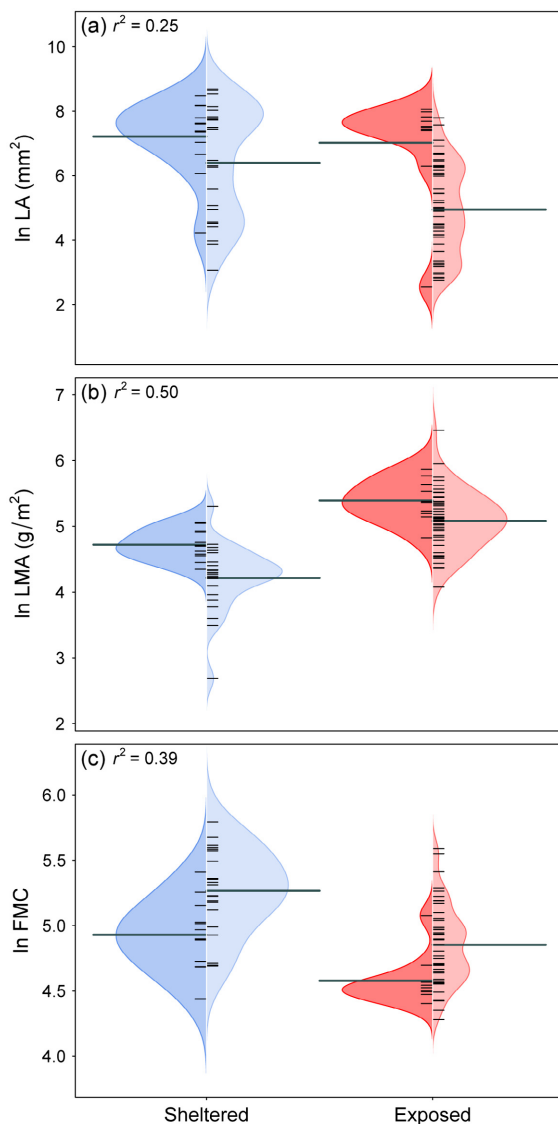


Fig. 2. Bean plots of leaf traits (a) leaf area (LA), (b) leaf mass per area (LMA) and (c) fuel moisture content (FMC) by habitat and stratum. Blue beans are sheltered habitat, and red beans are exposed habitat. Darker beans represent overstory species and lighter beans represent understory species. The shaded areas of the beans represent the estimated density of the distribution with short black lines in the shaded areas of the beans showing the observations. Long black lines are means of observations for a given group.

Leaf flammability attributes in relation to habitat and stratum

We found that TTI (ignitibility) was significantly faster in sheltered forest species compared with

exposed woodland species (GLM: $F_{1,88} = 34.1$, $P < 0.0001$; PGLS [OU]: $F_{1,88} = 34.2$, $P < 0.0001$; Fig. 3a). There was no significant difference in TTI between the overstory and understory strata in either of the habitats (GLM: $F_{1,88} = 0.2$, $P = 0.7$; PGLS [OU]: $F_{1,88} = 0.1$, $P = 0.7$; Fig. 3a), and no significant habitat \times stratum interaction (GLM: $F_{1,88} = 3.1$, $P = 0.08$; PGLS [OU]: $F_{1,88} = 3.0$, $P = 0.09$). It emerged that BD (sustainability) did not differ significantly between sheltered and exposed habitats (GLM: $F_{1,88} = 3.2$, $P = 0.08$; PGLS [OU]: $F_{1,88} = 2.9$, $P = 0.09$; Fig. 3b), and there was no significant habitat \times stratum interaction (GLM: $F_{1,88} = 0.3$, $P = 0.6$; PGLS [OU]: $F_{1,88} = 0.3$, $P = 0.6$). We found that BD was, however, significantly longer in the overstory compared with the understory in both habitats (GLM: $F_{1,88} = 19.4$, $P < 0.0001$; PGLS [OU]: $F_{1,88} = 19.4$, $P < 0.0001$; Fig. 3b). Values of MLR were significantly higher in sheltered forest species than in exposed woodland species (GLM: $F_{1,88} = 4.6$, $P = 0.04$; PGLS [BM]: $F_{1,88} = 33.5$, $P < 0.0001$) and in the overstory compared with the understory in both habitats (GLM: $F_{1,88} = 14.8$, $P = 0.0002$; PGLS [BM]: $F_{1,88} = 38.7$, $P < 0.0001$; Fig. 3c), with no significant habitat \times stratum interaction (GLM: $F_{1,88} = 2.1$, $P = 0.1$; PGLS [BM]: $F_{1,88} = 3.0$, $P = 0.08$).

Relationships between leaf flammability attributes and leaf traits

In all models, habitat, stratum, and the habitat \times stratum interaction were non-significant terms (Table 1), which meant that the three leaf traits LA, LMA, and FMC on their own were the most important features of habitats and strata driving observed differences in leaf flammability between the two habitats and the two strata. Faster TTI was significantly and uniquely related to larger LA ($t_{1,90} = -4.7$, $P < 0.0001$, partial $r^2 = 0.20$; Fig. 4a), lower LMA ($t_{1,90} = 18$, $P < 0.0001$, partial $r^2 = 0.78$; Fig. 4b), and lower FMC ($t_{1,90} = 9.2$, $P < 0.0001$, partial $r^2 = 0.49$; Fig. 4c). Longer BD was significantly and uniquely related to larger LA ($t_{1,90} = 17.1$, $P < 0.0001$, partial $r^2 = 0.76$; Fig. 4d) and higher LMA ($t_{1,90} = 9.7$, $P < 0.0001$, partial $r^2 = 0.51$; Fig. 4e), but not to FMC ($t_{1,90} = -1.0$, $P = 0.3$, partial $r^2 = 0.01$; Fig. 4f). Higher MLR was significantly related to larger LA ($t_{1,90} = 43.5$, $P < 0.0001$, partial $r^2 = 0.95$; Fig. 4g) and higher LMA ($t_{1,90} = 7.2$, $P < 0.0001$, partial $r^2 = 0.36$; Fig. 4h), but not to FMC ($t_{1,90} = 0.8$, $P = 0.4$, partial $r^2 = 0.007$; Fig. 4i).

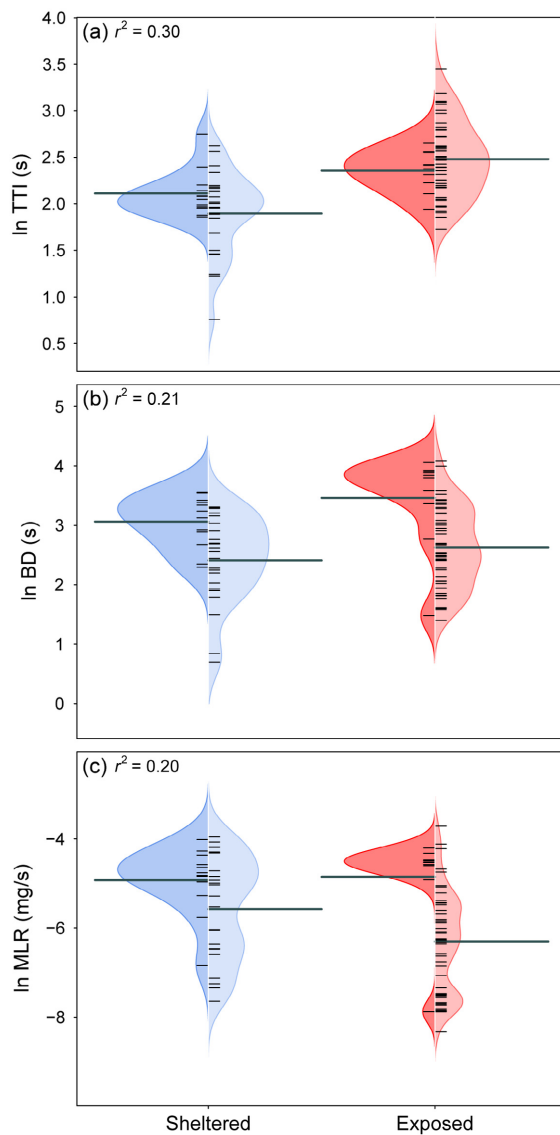


Fig. 3. Bean plots of flammability attributes (a) ignitibility, (b) sustainability and (c) combustibility by habitat and stratum. Blue beans are sheltered habitat, and red beans are exposed habitat. Darker beans represent overstory species and lighter beans represent understory species. The shaded areas of the beans represent the estimated density of the distribution with short black lines in the shaded areas of the beans showing the observations. Long black lines are means of observations for a given group.

DISCUSSION

Our study has provided evidence supporting the hypothesis that leaf flammability attributes

vary across the landscape in a way that is tightly coordinated with the primary strategic response of leaf traits to an environmental gradient. The hypothesis is centered on the notion that variation in the leaf traits LA, LMA, and FMC first and foremost represents adaptations to the environmental gradient between the two habitats. These trait responses then secondarily drive variation in leaf flammability, rather than the leaf traits themselves having evolved to increase (or decrease) flammability at the leaf level. Thus, the differences we observed in leaf flammability between the two habitats are driven principally by leaf trait responses to the environmental conditions in each of the habitats. While our analyses are correlative, we believe that this description of the nature of trait and flammability patterns is the most parsimonious explanation. We first showed that all three leaf traits LA, LMA, and FMC differed significantly and as predicted between sheltered forest species and exposed woodland species. Sheltered forest species had leaves with larger LA, higher LMA, and higher FMC than exposed woodland species. We then showed that leaf ignitibility (TTI) and combustibility (mean MLR) were significantly higher in sheltered forest species than in exposed woodland species, which matches two of our predictions that high ignitibility is related to large LA and low LMA found in gully species, and that overall these two leaf traits counteract the buffering effect of high FMC in gully species; and that high combustibility is related to large LA and high LMA in gully species. Our analyses of interspecific relationships between the leaf traits and the flammability attributes quantitatively confirmed the predictions. Importantly, in all of these models relating the leaf traits to the leaf flammability attributes, the habitat and stratum terms did not explain significant variation in leaf flammability. Thus, significant differences in leaf flammability attributes between the habitats were principally explained by the differences in leaf traits between habitats. Furthermore, all three leaf traits explained unique variation in the three leaf flammability attributes to varying degrees. Notably, LMA emerged as a consistently important trait driving variation in leaf flammability, even playing a more important unique role than FMC in lengthening TTI.

Ignitibility was best predicted (i.e., largest partial r^2) by LMA, followed by FMC, with higher

Table 1. Results from GLMs and PGLS models (with PGLS OU models in parentheses) of flammability attributes as a function of habitat, stratum, habitat and stratum interaction, and leaf traits (LA, LMA, and FMC).

Response	Term	$F_{6,85}$	P
Ignitibility	Habitat	1.3 (1.3)	0.3 (0.3)
	Stratum	0.2 (0.2)	0.6 (0.6)
	Habitat \times stratum	2.4 (2.4)	0.1 (0.1)
	LA	10.3 (12.9)	<0.0001 (0.0006)
	LMA	188.4 (198.3)	<0.0001 (<0.0001)
	FMC	73.6 (75.1)	<0.0001 (<0.0001)
Sustainability	Habitat	1.5 (1.5)	0.2 (0.2)
	Stratum	0.01 (0.01)	0.9 (0.9)
	Habitat \times stratum	0.6 (0.6)	0.4 (0.4)
	LA	204.7 (208.8)	<0.0001 (<0.0001)
	LMA	48.2 (50.7)	<0.0001 (<0.0001)
	FMC	0.8 (0.02)	0.4 (0.4)
Combustibility	Habitat	1.8 (1.8)	0.2 (0.2)
	Stratum	1.9 (1.9)	0.2 (0.2)
	Habitat \times stratum	0.08 (0.08)	0.8 (0.8)
	LA	1211.3 (1267.3)	<0.0001 (<0.0001)
	LMA	42.5 (42.8)	<0.0001 (<0.0001)
	FMC	0.02 (0.02)	0.9 (0.9)

Note: FMC, fuel moisture content; GLM, general linear models; LA, leaf area; LMA, leaf mass per area; OU, Ornstein-Uhlenbeck; PGLS, phylogenetic generalized least squares.

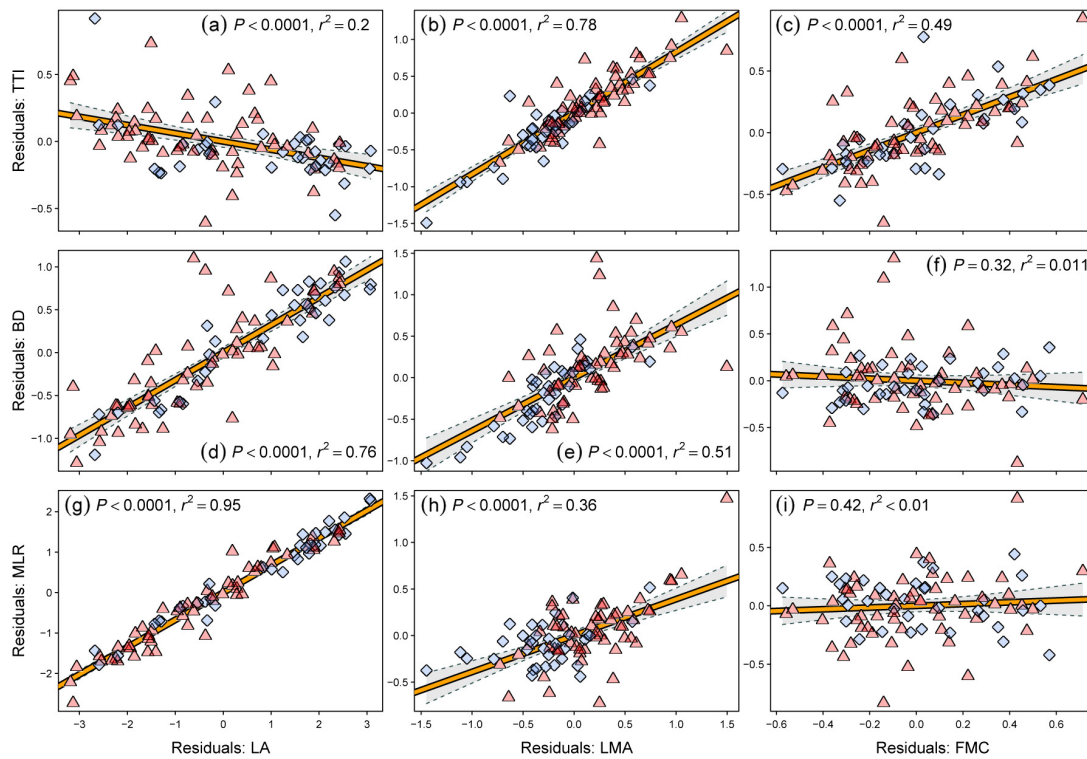


Fig. 4. Scatter plots of partial regressions of flammability attributes (y axes) against leaf traits (x axes). Blue rhombuses represent sheltered species, red triangles represent exposed species. Line of best fit from general linear models model shown as an orange line (identical to best fitting phylogenetic generalized least squares model). The grey shaded area represents the 95% confidence interval of the mean.

values of both being associated with lower ignitibility, and then by LA, with higher LA being related to higher ignitibility. Interestingly, the effect of FMC on lengthening TTI was weaker than that of LMA, so that while sheltered habitat species generally exhibited higher FMC, their much lower LMA and larger LA resulted in their overall higher ignitibility than exposed woodland species. These findings are consistent with those of some previous studies (Gill and Moore 1996, Grootemaat et al. 2015, Mason et al. 2016). The differences in all leaf traits between overstory and understory did not result in significant canopy stratum differences in TTI. Relative to overstory species, understory species had smaller leaves and lower LMA, both related to quicker TTI; however, FMC was also higher, which contributed to slowing leaf ignition. Sustainability, which did not differ between habitats, was best predicted by LA and then LMA, with larger leaved and higher LMA species burning for a longer period, with no significant effect of FMC on sustainability. The lack of a significant difference in sustainability between habitats can be explained by the combination of larger LA and lower LMA in sheltered forest species and the combination of smaller LA and higher LMA leaves in exposed woodland species both providing within habitat combination trade-offs precluding either faster or longer burning times for leaves. While large area leaves will burn for longer than small area leaves, the lower LMA of large area leaves shortens BD times in sheltered forest species. And while leaves with higher LMA will burn for longer than leaves with lower LMA, the smaller area of high LMA leaves shortens BD times in exposed woodland species. This also explains the significantly longer BD of overstory species in both habitats, as overstory species tend to have larger, higher LMA leaves, relative to understory species. Combustibility was best predicted by LA and then LMA, with FMC having no significant effect. Higher LA and LMA were both associated with greater combustibility, but unlike sustainability, these two traits worked with each other in this case to increase flammability (i.e., MLR) in sheltered habitat species. Overstory species showed significantly higher combustibility compared to understory species; similarly to the effect on BD, this is likely due to their larger, higher LMA leaves. Again, FMC had no

significant effect on combustibility. With respect to FMC, we suggest that the lack of significant effect of FMC on both sustainability and combustibility is likely due to leaf water being largely evaporated by the time the leaf has ignited.

Mutch (1970) hypothesized that fire-prone plant communities have evolved to promote fire, with subsequent research and argument focused on the possibility that plant flammability is an evolved trait (Bond and Midgley 1995, Gagnon et al. 2010). Given that fire-prone plant communities are often found in hot, dry regions, maximizing flammability would require evolution toward traits unlike those that are actually observed, and increasing flammability in one dimension may mean another dimension is decreased (e.g., increased ignitibility with large area, low LMA leaves, and decreased combustibility). This does leave open the possibility that evolutionary fire strategies exist, with only one or few dimensions of flammability being selected for, although how such strategies would be defined given the large number of possible measures of flammability is unclear. With our findings, evolution toward increased flammability at leaf level would seem unlikely, as it would require modification of important, interrelated leaf traits that allow plant species to persist in their environment. It is more parsimonious to interpret leaf flammability as arising from what is ultimately physical and physiological adaptation to the abiotic environment.

With predicted increases in the number of fire weather (hot, dry, and windy) days each year likely to be experienced in the region as a result of climate change (Pitman et al. 2007, Clarke et al. 2011), sheltered forest habitat in south-eastern Australia in particular will experience more frequent and intense wildfires (Clarke 2015). Currently, sheltered forest tends to burn less frequently and less intensely than exposed woodland (Penman et al. 2007, Bradstock et al. 2010, Leonard et al. 2014), due largely to such forests being sheltered from high winds by topography and being overall wetter environments with less direct insolation. However, the projected changes to climate will allow wildfires to overcome the once protective nature provided by topography to sheltered forest vegetation. Worryingly, our results indicate that a critical fuel component essential to wildfires—plant leaves—is significantly more flammable in sheltered forest species than in exposed woodland species. This

would have the potential to exacerbate the effects of changing weather conditions and would place sheltered forest habitat, their plants, and their animals, at even higher risk of catastrophic wildfire. This has serious implications for native biodiversity, as during intense wildfires, sheltered forests can be the only remaining vegetation in an otherwise completely burnt landscape, providing critical refuge habitat and food resources for animal species during fire after it has passed (Collins et al. 2012, Robinson et al. 2014, Chia et al. 2015, Swan et al. 2016). In burnt areas, abundances of small mammal species (Banks et al. 2011, Roberts et al. 2015) and bird species are reduced (Robinson et al. 2014), with sheltered forests providing refugia until surrounding burnt areas have recovered.

Our study has focussed on intrinsic leaf flammability, which is just one important part of a larger story that must be considered in order to reach a comprehensive understanding of how plants contribute to wildfire behavior (Gill and Moore 1996, Grootemaat et al. 2015, Zylstra et al. 2016). Future work in this area needs to explore how plant leaf flammability scales up to larger plant sections (e.g., branches, Wyse et al. 2016) and to whole plants, which will be facilitated through understanding how leaves are arranged spatially and how leafing intensity varies among species. Such work will help us to gain a better understanding of what traits might influence plant flammability at these larger scales.

ACKNOWLEDGMENTS

We thank Megan Phillips, Leigh Martin, Gemma Armstrong, Colin Brown of the Rural Fire Service, and Charlotte Krix for helpful discussions and logistical support. We are grateful for comments from two anonymous reviewers that improved the manuscript. We thank the Nature Conservation Council of NSW for giving us the opportunity to present our research at their 2017 Bushfire Conference. This research was supported by an Australian Government Research Training Program Scholarship to DK.

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