Wildfire and carbon neutralize Novel Weapons and harms reproductive potential of an allelopathic invasive plant

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Abstract

Invasive species are a leading cause of global biodiversity loss, and in Southern California scrublands, black mustard (*Rhamphospermum nigrum*) is a prominent, harmful invasive plant. *Rhamphospermum nigrum* employs allelopathic *Novel Weapons* to directly harm heterospecifics via inhibition of germination and indirectly by killing mycorrhizae, generating near monocultures across hillsides. However, active carbon, often deposited by wildfires, is known to bond to and neutralize plant exudates. This led to a hypothesis that active carbon soil amendments neutralize *R. nigrum Novel Weapons,* and thus, the invasive's superior competitiveness. In the greenhouse and field, carbon treatments reduced or completely inhibited *R. nigrum* reproductive potential (Inflorescence) and mitigated some of the invasive's negative effects on the growth of deerweed (*Acmispon glaber*), a common Southern California native "fire-follower". Carbon soil amendments, whether administered by hand or by fire, may thus reduce the fitness, abundance, and negative effects of *R. nigrum* on the native plant community.

Introduction

After habitat restoration volunteers spend hundreds of hours digging up non-native black mustard (*Rhamphospermum nigrum;* formerly, *Brassica nigra*) from California parks, the harmful invasive readily resprouts from the seedbank following winter rains (Moyes et al., 2005). Indeed, water utilities attempting to restore watersheds with native plants pay restoration assistants to dig up *R. nigrum,* only to see the aggressive weed come back with those same rains (LA DWP 2015). Fire prevention budgets are strained with the amount of labor hours needed to remove the semi-woody and thus easily ignitable plants, including *R. nigrum,* from roadsides, but must do so every year if fuel loads and thus fire risk are to be reduced (Keeley, 2006). In all,

California alone spends over $82.2 million a year on efforts to control invasive plants with minimal success (CAL-IPC 2022).

Invasive species are the fifth leading cause of species loss (Mollot et al., 2017). Attempts to manage their spread are more often than not met with minimal success at best (see Moyes et al., 2005; LA DWP 2015 above). Invasive plants pose especially challenging problems to conservation efforts (see again Moyes et al., 2005; LA DWP 2015 above), as they are often fast-growing annuals that dedicate a majority of their acquired resources to reproduction, instead of long-term growth and survival (see Review by Gioria et al., 2023). Non-native plant species are largely understood to become invasive and degrade native biodiversity through *Novel Weapons* to which native species have yet to co-evolve counter adaptations (Callaway & Ridenour, 2004). Further, invasive plants degrade entire ecosystems (e.g. Bowler 2000; Linders et al. 2019). Non-native annuals often grow faster than natives, outcompeting native grasses and forbs for water, sunlight, and space (D'Antonio & Vitousek, 1992). As invasives displace native plant diversity, they homogenize the landscape, which can reduce the variety of energy sources available to herbivores (Bowler 2000) and pollinators (Stout & Tiedeken, 2016). The reduction of herbivores and pollinators, in turn, can reduce the diversity of predatorial species, potentially affecting ecosystem scale services (Linders et al., 2019). In terms of plant species interactions, these novel weapons can come in the form of allelopathic exudates, which directly or indirectly harm native heterospecifics (Zhang et al., 2020). However, active carbon, commonly deposited by wildfires fueled by invasives, has the potential to bond to and neutralize active sites of allelopathic secondary metabolites (Kulmatiski & Beard, 2006).

One particularly harmful, and hence particularly abundant, invasive annual in Southern California and much of the U.S. Southwest is black mustard (*Rhamphospermum nigrum*). In addition to outcompeting native plants for water, nutrients, and space with rapid growth behavior, much of *R. nigrum's* success in its novel habitats has been attributed to *Novel Weapons,* in the form of isothiocyanate and a suite of other herbicidal and fungicidal compounds (Turk and Tawha, 2003; Tawaha and Turk, 2003; Oduor et al., 2020). *Rhamphospermum nigrum* secondary metabolites inhibit herbivore consumption (Traw & Dawson, 2002), providing *Enemy Release.* However, the invasive's ability to directly and indirectly harm heterospecific flora make *R. nigrum* of particular concern for biodiversity. *Rhamphospermum nigrum* exudates directly harm heterospecifics by inhibiting germination (*again Turk and Tawha 2003; Tawaha and Turk 2003; Oduor et al. 2020*) and indirectly reduce the fitness of other species with fungicidal compounds, killing mycorrhizae mutualists, with which *R. nigrum* does not associate (Schreiner and Koide, 1993; also see Fig. 1). The loss of fungi from the rhizosphere reduces host plants ability to acquire water and nutrients through increased surface area within the rhizosphere while reducing mycorrhizae's external digestive enzymes that mobilizes nutrients otherwise bound to undecomposed organic matter (Bowles et al., 2017). Like many other invasive plants, the fast-growing annual senesces tissues early in the growing season, leaving behind semi-woody, easily ignitable shoot tissues in dense patches that often take over entire hillsides (Minnich & Dezzani, 1998).

Wildfires not only clear out vegetation, opening soil niches for fast-growing, opportunistic invasive annuals, they alter soil chemistry (see Review by Certini 2005). Increasing the frequency of fires has been observed to increase the abundance of invasives at the expense of

native plants, even in fire-prone ecosystems, such as chapparal and California coastal sage scrub (*e.g. Westman 1981, see review by Rundel et al. 2018*), in which many fire-adapted natives have evolved. Fires deposit carbon, mobilizes nutrients, and either increase or decrease soil pH, depending on pre-existing soil characteristics (again see Review by Certini 2005). Previous research (Schlau 2022) showed that high severity wildfire disturbance increased the abundance of invasive *R. nigrum* in the first year of post-fire vegetative recovery. However, the second year of vegetative recovery significantly reduced the abundance and ability of *R. nigrum* to replace itself throughout coastal sage scrub, chaparral, and transitional ecosystems in the Santa Monica Mountains following the high severity Woolsey Fire. Surprisingly, in the second year of vegetative recovery, native plants were more likely to replace *R. nigrum* than other invasives. One of the of the most abundant native species observed to retake territory for the native plant community was the "fire-follower" deerweed (*Acmispon glaber*) (Schlau 2022).

The success of *R. nigrum* may be the key to its demise. The previous study (Schlau 2022) failed to identify which fire-induced alterations of soil chemistry drove the loss of reproduction in *R. nigrum.* This study attempts to tease apart the effects of changes to soil chemistry - from fires fueled partially by *R. nigrum* - on multiple aspects of the invasive plant's growth and reproductive potential in the greenhouse. Field experiments test a hypothesis that active carbon neutralizes *R. nigrum* exudates and drives the invasive's reduced reproductive potential, possibly by forcing *R. nigrum* to shunt resources away from flowers and toward competition for nutrients and other necessary resources once the plant's ability to reduce heterospecifics' competitiveness with fungicides has been neutralized. If active carbon can significantly thwart the spread of *R.*

nigrum, then this research may provide insight into a potentially cost effective and less labor-intensive means of managing this invasive species without actually setting fires.

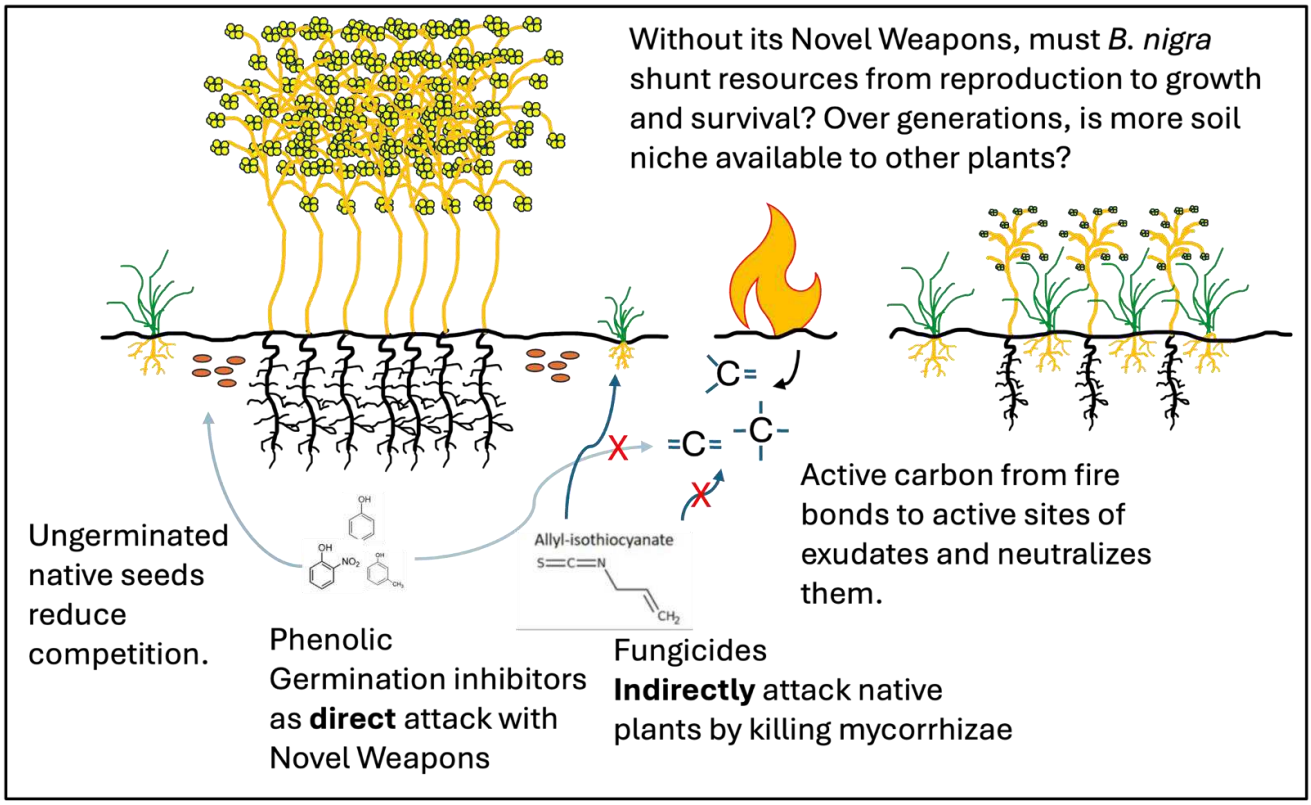


Fig 1. Conceptual model of fire-deposited active carbon neutralizing the Novel Weapons of the invasive *R. nigrum*, and allowing an increase in native fire-followers to establish.

Methods

Target Species

Rhamphospermum nigrum is one of the most abundant fast-growing invasive annuals through Southern California and many other parts of the world (Bell & Muller, 1973). Like other short-lived invasives, *R. nigrum* quickly uptakes nutrients, water, and sunlight before native plants can

reestablish (CNPS report, 2002). Unlike nearly all other vascular plants, *R. nigrum* does not associate with mycorrhizae (Turk & Tawha, 2003). Thus, the invasive is deprived of mycorrhizal mutualism benefits. Instead, *R. nigrum* reduces the competitiveness of other plant species by killing their fungi mutualist partners through allelopathy (again Turk & Tawha, 2003; Tawaha & Turk, 2003; Oduor et al., 2020). Theoretically, the death of competitors' root fungi allows *R. nigrum* to outperform competitors for the acquisition of nutrients and water. Inversely, the loss of *R. nigrum Novel Weapons* exudates due to neutralization via active carbon soil amendment potentially allows native species to keep their mycorrhizae and force *R. nigrum* to alter its growth behavior away from reproduction and toward root and shoot growth in order to compete with natives and away from reproduction.

Acsimpson glaber is a perennial shrub native to Southern California's CCS and inland chapparal habitats (Moyes et al., 2005). The shrub has earned the unofficial caterogization as a "fire-follower" because *A. glaber* tends to germinate and grow well in the 3 years following fire disturbance (Litle et al., 2019). However, this native fire-follower can lose territory to *R. nigrum* in the first year of post-fire vegetative recovery (Schlau 2022). Observations of second year post-fire vegetative recovery saw *A. glaber* as one of the most abundant native plants replacing the first year's *R. nigrum* (Schlau, 2022), which is the reason *A. glaber* was chosen as a native competitor in the greenhouse experiment here.

Greenhouse Experiment

In order to tease apart fire-induced changes to soil chemistry, *R. nigrum* and *A. glaber* seedlings were grown in the greenhouse in mono- and polycultures in 0.5 L pots with two plants in each

pot in the Pitzer College greenhouse utilizing a full factorial design (n = 10 ea. treatment). All pots consisted of live field soil gathered from the coastal sage scrub habitat at the Bernard Field Station at the Claremont Colleges homogenized with commercial potting soil in a 5:1 ratio in order to improve soil moisture retention in 0.5 L pots. Fires deposit active carbon into soils, often lower pH in alkaline soils, and mobilize nutrients. To reproduce analogous post-fire conditions in the greenhouse, ten replicates of each plant culture were treated with 5g active carbon (Thermo-fischer), 3g elemental sulfur (Thermo-fisher), and 3g of soluable fertilizer (Mircale Grow^{TM}) in a full factorial design. Plants were started in germination trays under misting benches, then replanted in 0.5 L pots following emergence of first true leaves.

Rhamphospermum seeds were gathered from Chino Hills State Park, California. Acmispon glaber seeds ordered from the California Native Plant Society. Soil pH was recorded for all pots using a 1:1 slurry method with a HANA field pH meter prior to planting but after conditioning soil with local "hard" tap water (pH 7.8-8.2) for two weeks. All pots were continuously watered with tap water through misting benches for 15 minutes three times a day. All pots were rerandomized every two weeks. Soil pH was remeasured prior to destructive harvest.

To test how pairwise species interactions affected overall growth under different soil conditions after 180 days in the greenhouse, statistical analysis of root DW, shoot DW, and root:shoot ratios were analyzed following destructive harvest. To test implications for reproductive potential of the invasive *R. nigrum,* inflorescence counts were recorded prior to destructive harvest.

In order to test which alterations of soil chemistry may reduce the competitiveness of *R. nigrum* on the common "fire-follower" *A. glaber,* mixed model Two-way ANOVAs were used to test for interactive effects between monocultures and polycultures and soil treatments. No treatments drove a significant reduction in harm to *A. glaber.* Two-way ANOVA analysis of *A. glaber* root DW was statistically insignificant (see more below), but a general pattern of soil amendments reducing the reduction in *A. glaber* root DW emerged. Therefore, Effect Size with Log Ratios was performed to see if the reduction in *A. glaber* root DW and root:shoot ratios in polycultuures (competition with *R. nigrum*) was significantly reduced in any of the soil treatments compared to monoculture controls.

While taking pH readings, carbon alone appeared to lower pH as much as treatments with sulfur amendments, and within carbon treatments, soil pH ranged from 3.8-7.2. To test this additional hypothesis that carbon-reduced soil pH as a mechanism of carbon's inhibition of *R. nigrum* flowering, an ANCOVA and subsequent Test for Homogeneity of Slopes and linear regression analysis were run on greenhouse treatments. Soil treatments were the independent categorical variable. Soil pH was the covariate. Mean inflorescence count was the dependent variable. Linear regression analysis of *R. nigrum* inflorescence count versus soil pH with observational field data was also tested. 3.8-7.2

Field Experiments

Active Carbon Effects on growth and reproductive potential of R. nigrum.

A primary hypothesis of this study was that active carbon neutralizes *R. nigrum* herbicidal and fungicidal exudates, thereby robbing the invasive of its Novel Weapons – its primary competitive

advantage over native plants. To do this, 300g active carbon was spread over five $1m^2$ plots of randomly chosen *R. nigrum* seedling monoculture patches on March 15, 2024 on private property adjacent to the Chino Hills California State Park in Chino Hills, CA. After 120 days, the means of height, shoot DW, root DW, root:shoot ratios, and inflorescence count were compared to means in adjacent 1 m^2 plots with Student's t-tests.

Legacy effects of wildfire-induced changes to soil chemistry.

Active carbon eventually washes from soil (Ciais et al., 2008), soils are re-hardened by CaCO3 deposits, and nutrients cycle. In order to test the length and strength of fire-induced changes to soil chemistry, mean height, shoot DW, root DW, root:shoot ratios, and inflorescence counts were compared between two areas of the Chino Hills California State Park in two separate regions of the park, one which burned in 2020 (centered randomly around 33.946435, -117.703711) and the other in 2008 (centered randomly around 33.913420, -117.814349). Metrics of plant growth and reproductive potential were compared with a series of independent Student t-tests.

All stat analyses were performed in RStudio v. 2023.09.0+463. Graphs built in MS Excel and PowerPoint v.16.82.

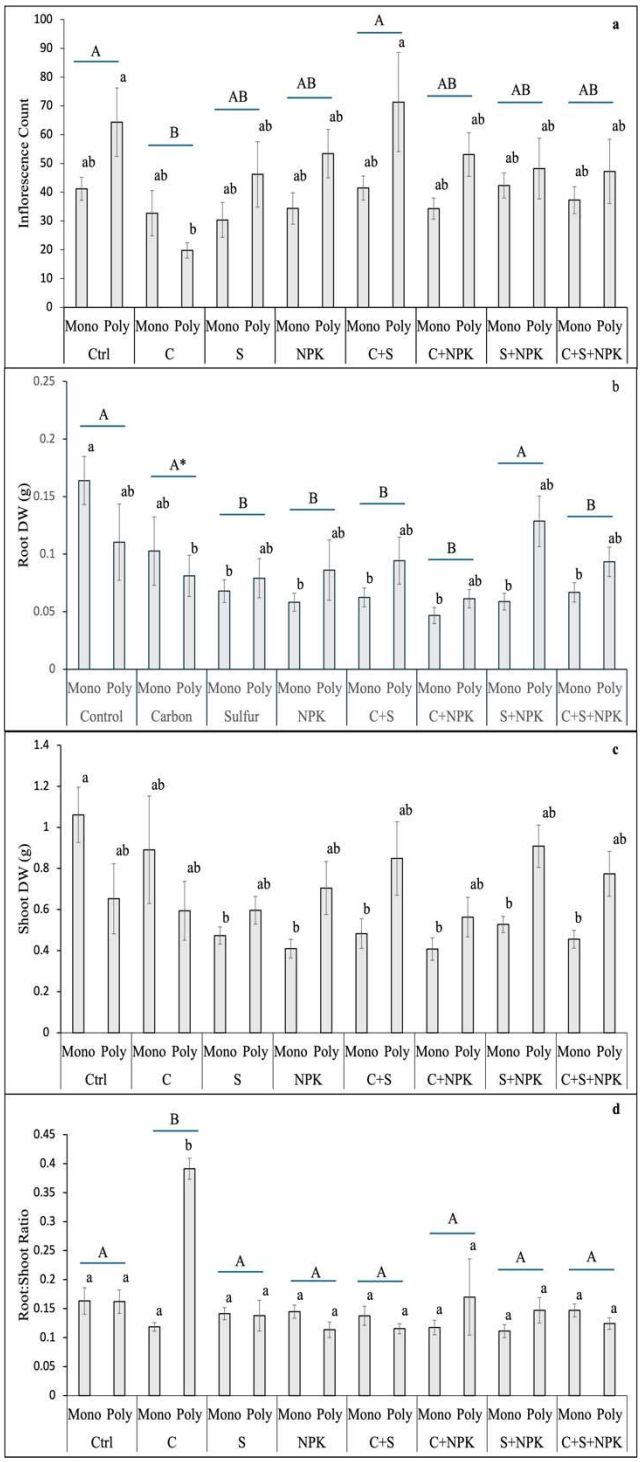
Results

Greenhouse

In order to test the effects of wildfire-induced alterations of soil chemistry on *R. nigrum*, *R. nigrum* was grown in monocultures and polycultures (with *A. glaber*) under various soil

196 treatments. Compared to greenhouse controls, carbon harmed *R. nigrum,* most notably in terms
197 of inflorescence count and root:shoot ratio, while all other treatments, except carbon significantly
198 reduced shoot and root DW in monocultures (Fig. 2a-d). Combinations of carbon with sulfur and
199 fertilizers reversed the negative effects on inflorescence and root:shoot ratios. While there was
200 no interactive effect of soil treatments and competition with *A. glaber* on *R. nigrum* inflorescence
201 count ($Two-way\ ANOVA\ n=\ F_{7,143}=1.024, p=0.417$), soil treatments significantly
202 affected inflorescence count ($One-way\ ANOVA\ n=, F_{7,143}=2.147, p=0.0425; Fig.2a$).
203 In particular, carbon in polycultures significantly reduced *R. nigrum* inflorescence count 69.2%
204 compared to polyculture controls ($Tukey\ HSD\ post\ hoc\ p=0.0426; Fig.2a$), but carbon plus
205 other soil treatment reversed these effects in both mono- and polycultures.
206
207 Interactive effects of soil treatments drove a significant reduction in *R. nigrum* shoot DW
208 compared to controls, except for carbon alone ($Two-way\ ANOVA\ n=, F_{7,106}=2.88, p=$
209 $0.00856, Fig\ 2b$).
210
211 Carbon and competition in polycultures with *A. glaber* significantly reduced *R. nigrum* root DW
212 50.6% compared to monoculture controls ($Tukey\ HSD\ post\ hoc: p=0.0405;\ Fig.2b$).
213 However, no interactive effect on *R. nigrum* root DW was observed ($Two-way\ ANOVA\ n=$
214 $, F_{7,106}=2.081, p=0.0518; Fig.2b$), but all monoculture soil treatments, except carbon and
215 S+NPK exhibited a negative effect on root DW compared to monoculture controls ($F_{7,106}=$
216 $4.28, p<0.001; Fig.2b$).
217

Soil treatments and competition elicited a significant interactive effect on root:shoot ratios (Two-way ANOVA: $n = , F_{7,106} = 3.94, p = 0.000735; Fig. 2d$), driven almost entirely by an interactive effect between carbon and competition. When in competition with *A. glaber,* carbon induced a 141.4% increase in *R. nigrum* root:shoot ratios compared to polyculture controls ($Tukey\ p < 0.0001 \ldots . Fig. 2d$) as well as all other treatments ($p =< 0.0001 - 0.01$).



224

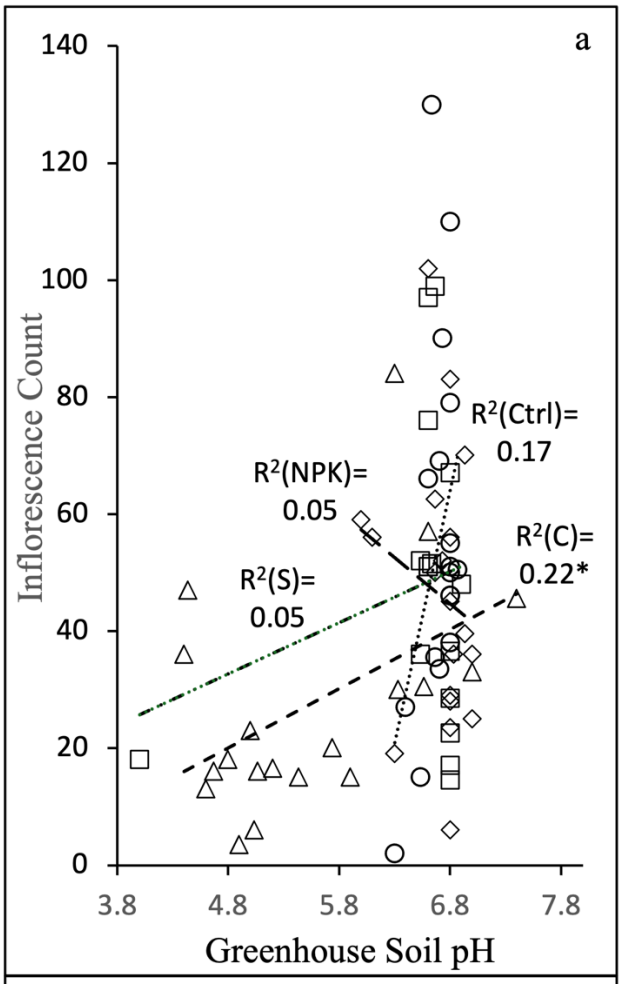
225

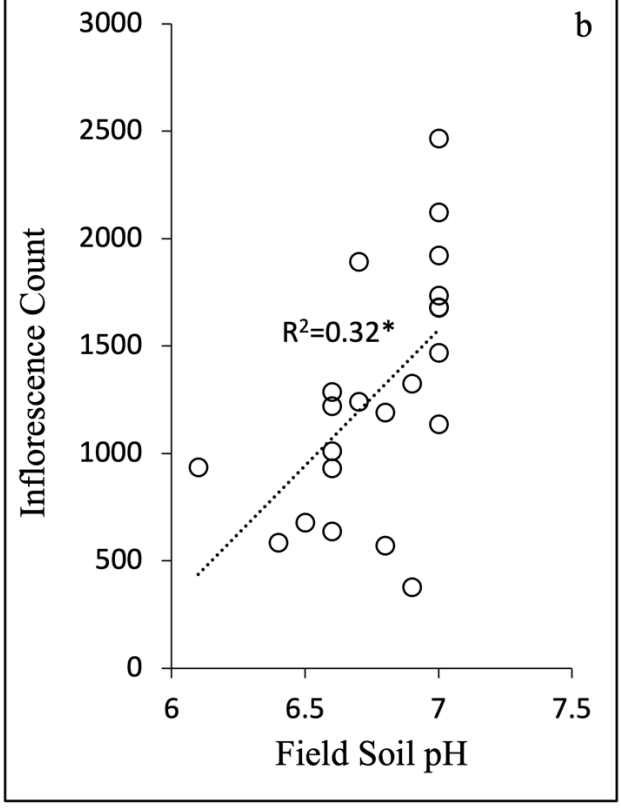
226 **Fig. 2** Mean $(+/-\ SE)$ (a) Inflorescence Count, (b) Root DW, (c) Shoot DW, and (d) Root:Shoot

227 Ratio for *R. nigrum* in greenhouse Monocultures and Polycultures. Soil treatment codes: (Ctrl)

Controls, (C) Active Carbon, (S) Elemental Sulfur, (NPK) Soluble Fertilizer, and combinations. Lowercase letters above bars represent significant differences ($p<0.05$) from Tukey HSD post hoc test on interactive effects. Uppercase letters above pairs of bars represent significant difference ($p<0.05$) from one way effects of soil treatments from Tukey HSD post hoc test. Astrex above letter indicates moderate significance ($p<0.10$) from control.

In order to tease apart which soil amendments drove the reduction in inflorescence count and whether soil pH was a partial mechanism of any reduction in inflorescence, an ANCOVA was run among soil treatments with soil pH as the covariate. Again, carbon significantly reduced mean inflorescence count compared to controls ($ANCOVA\ p\ =\ 0.0187$) while all other soil treatment effects were insignificant ($ANCOVA\ p > 0.05$). Multiple soil amendment resulted in significantly different slopes compared to the control (see Table 1). However, subsequent linear regression analysis showed only carbon treatments had a significant relationship between mean inflorescence count and soil pH (Linear regression $R^2=0.22$, $p=0.0418$; Fig. 3).


a
140
120
100
80
60
40
20
0
Inflorescence Count
R²(Ctrl)= 0.17
R²(NPK)= 0.05
R²(C)= 0.22*
R²(S)= 0.05
3.8
4.8
5.8
6.8
7.8
Greenhouse Soil pH


b
3000
2500
2000
1500
1000
500
0
Inflorescence Count
R²=0.32*
6
6.5
7
7.5
Field Soil pH

243

Fig. 3 Mean Inflorescence Count relationship to (a) Greenhouse Soil pH and (b) Field Soil pH. Greenhouse treatments included are (Ctrl) Controls, (C) Active Carbon, (S) Elemental Sulfure, and (NPK) Soluble Fertilizer. R^2 values with astrex above indicate significant ($p < 0.05$) linear relationships from regression analysis. Greenhouse combination treatments in Greenhouse were not included because relationships were not significant and maintain clarity in the graph. Field observations were across both burn sites.

When analyzed with Two-way ANOVAs, soil treatments failed to reverse the negative effects on *A. glaber* shoot DW, root DW, root:shoot ratio, height, or leaf count (Two-way ANOVAs: p>0.05 for all dependent variables by treatment; see supplemental materials for more detail). However, all soil treatments appeared to reduce the negative effects of *R. nigrum* competition on *A. glaber* root DW. So the Effect Size (Log Response Ratios) were calculated with 95% CI, revealing all soil treatments significantly mitigated reduction in *A. glaber* root DW when competing with *R. nigrum* (Fig. 4a). Soil treatments had no effect on *A. glaber* shoot DW in Two-way ANOVA (n=121, $F_{7,109} = 1.521, p = 0.168$); $data\ not\ shown$) or Effect Size Log Response Ratios ($data\ not\ shown$) and root:shoot ratios were unaffected by competition and soil treatments, except that competition and sulfur combined increased root:shoot ratios 360.0% compared to polyculture controls ($Tukey\ HSD\ post\ hoc: p = 0.0253;\ data\ not\ shown$). Effect size analysis of competition effects on root:shoot ratios show treatments with carbon or sulfur neutralized significant changes to root:shoot ratios and that fertilizer alone increase root:shoot ratios (Fig. 4b).

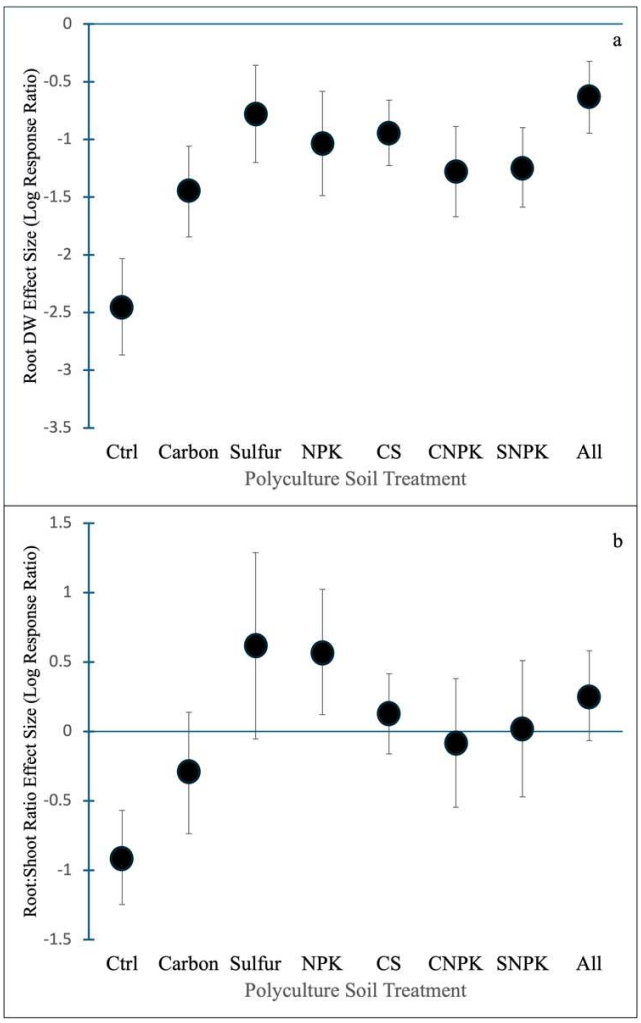


Fig. 4 (a) Root DW and (b) Root:Shoot Ratio Effect Size (Log Response Ratio) of *R. nigrum* competition on *A. glaber* polycultures compared to Monoculture Controls. Filled circles represent means of greenhouse polyculture soil treatments: (Ctrl) Controls, (Carbon) Active Carbon, (Sulfur) Elemental Sulfur, (NPK) Soluble Fertilizer, (CS) Carbon plus Sulfur, (CNPK) Carbon plus Soluble Fertilizer, (SNPK) Sulfur plus Soluble Fertilizer, and (All) Carbon plus Sulfur and Soluble Fertilizer. Error bars calculated from pooled standard deviation.

Field Observation

Rhamphospermum nigrum were collected across two nearby sites that burned in 2020 and 2008 to test fire legacy effects. More recently burned sites saw a 49.0% decrease in inflorescence ($Student's\ t-test: n = 22, p < 0.0001;\ Fig. 5a$) and 21.9% decrease in height ($p = 0.0198;\ Fig. 5b$) Similar to *R. nigrum* pots treated with carbon in the greenhouse, decreasing soil pH drove a significant reduction in *R. nigrum* mean inflorescence count ($n = 22, R^2 = 0.32, p = 0.006; Fig. 3b$).

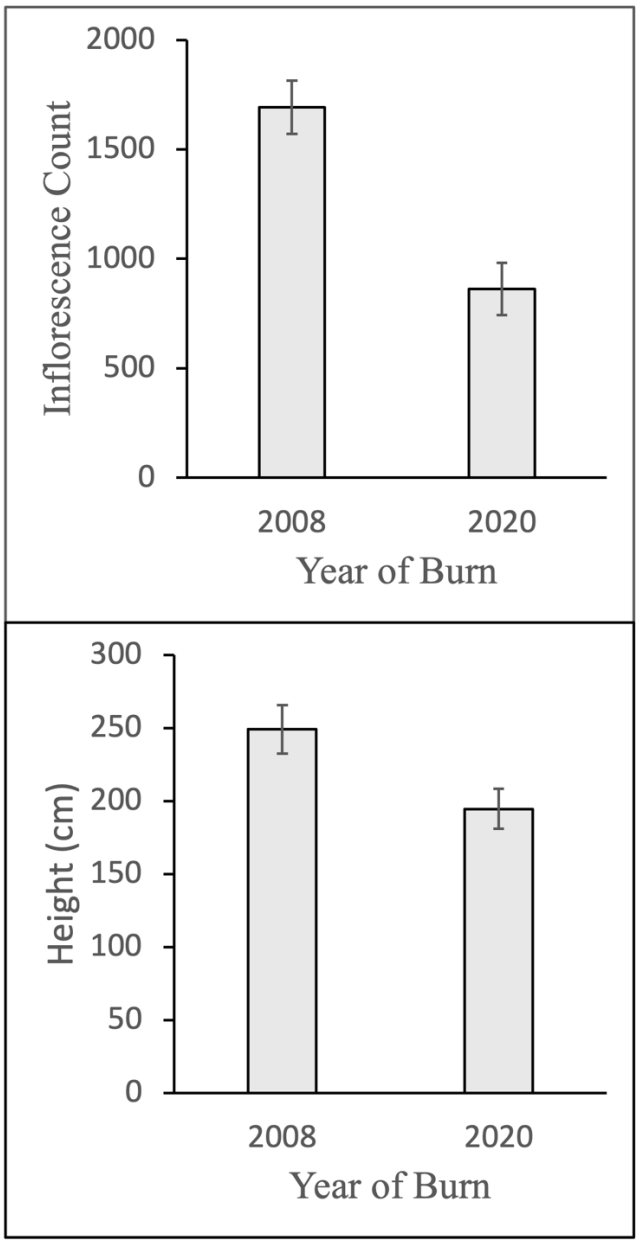


Fig.5 Mean $(+/-\ SE)$ (a) Inflorescence Count and (b) Height (cm) for *R. nigrum* in sites that burned in 2008 compared to sites that burned in 2020 in Chino Hills State Park, California. Both mean inflorescence count ($Student's\ t-test$: $n = 22, p < 0.0001$) and height ($p = 0.0198$) were significantly reduced in the more recent burn scars.

Field Manipulation

To test the hypothesis that active carbon can bond to and neutralize *R. nigrum* harmful herbicides and fungicides, carbon was added to plots under field conditions. Active carbon soil amendments reduced *R. nigrum* shoot DW 80.9% ($Student's\ t-test\ n=5, p=0.0238; Table\ 1$) and completely inhibited the growth of inflorescence ($Fig. 6$), but did not affect root DW ($p = 0.0613; Table\ 1$) nor root:shoot ratios ($Student's\ t-test: n=5, p=0.559; Table\ 1$).

Table 1: Field Manipulation means (Std Error) and p-values from student's t-tests.

	Mean (Std Error)	p-value
Shoot (g)		
Control	54.61 (15.74)	**0.0238***
Carbon	10.41 (2.09)	
Root (g)		
Control	4.064 (1.58)	0.0613
Carbon	0.618 (0.098)	
Total (g)		
Control	58.674 (17.18)	**0.025***
Carbon	11.028 (2.18)	

Root:Shoot		
Control	0.0689 (0.01)	0.559
Carbon	0.062 (0.0052)	

Fig.6 Photograph of example $1m^2$ field plots treated with 300g active carbon (foreground) and adjacent untreated plot (background). All *R. nigrum* in treated plots did not initiate bud emergence while all *R. nigrum* in adjacent untreated plots did.

Discussion

This study sought to tease apart the effects of fire-induced chemical changes to soils on interactions between the invasive *Rhamphospermum nigrum* and native *Acmispon glaber* in the greenhouse and *R. nigrum* in the greenhouse and the effects of fire and active carbon deposition on growth behavior of *R. nigrum* in the field (Fig. 1). Carbon reduced reproductive potential, as evidenced by reduced or completely inhibited inflorescence in the greenhouse (Fig. 2a) and

complete inhibition of inflorescence in the field (Fig. 5a), suggesting a potential for transgenerational reduction in *R. nigrum* negative effects. In the greenhouse, all soil treatments mitigated reduction of *A. glaber* root DW in polycultures (Fig. 4a) and neutralized *R. nigrum* increase of *A. glaber* root:shoot ratios (Fig. 4b) compared to controls. In all, data presented here suggest the potential to use active carbon soil amendments – as opposed to traditional, labor-intensive manual removal - to reduce the abundance of *R. nigrum,* and thus, the invasives negative effects on biodiversity and increased fire risk.

Westoby's Leaf-Height-Seed Hypothesis (1998) explains that plants have limited resources to divide among the growth of roots, shoots, leaves, and flowers. In the greenhouse, active carbon amendments reduced inflorescence count (Fig. 2a) and increased *R. nigrum* root:shoot ratios (Fig. 2d). Taken together, the increase in root:shoot ratio in active carbon treatments suggests *R. nigrum* was under more competitive stress. The loss in inflorescent count in the same treatments (Fig. 2a-d) suggests *R. nigrum* may have redirected resources from reproduction toward growth and survival. The reallocation of resources from reproduction to competition reduced the harmful effects on *A. glaber* root DW (Fig. 4a) while neutralizing a significant increase in *A. glaber* root:shoot DW (Fig. 4b). However, of greater significance regarding *R. nigrum* multigenerational fitness, carbon's reduction in reproductive potential (i.e. inflorescence count) would hypothetically reduce the overall harmful effects of *R. nigrum* on biodiversity, and perhaps even fire risk over multiple generations. In terms of land management, the fact that carbon deposits eventually wash out of soils needs to be considered into application and budgeting for the utilization of active carbon amendments for the purposes of management of *R. nigrum* populations.

In the greenhouse, multiple treatments of fertilizer and elemental sulfur mitigated or eliminated the effects of carbon when *R. nigrum* was in competition with *A. glaber* in polycultures, often resulting in intermediate values for growth metrics (Fig. 4a-b). Carbon's reactivity, stemming from the element's ability to form single, double, and triple covalent bonds, may have resulted in carbon bonding to sulfur and fertilizer ions, reducing the concentration of carbon atoms to bond to *R. nigrum* exudates. Fire-induced changes in soil pH and mobilization of nutrients could complicate the application of carbon as a means of invasive plant management. However, carbon treatments alone resulted in the greatest reduction in pH ($ANOVA\ n = 70, p < 0.0001$; *data not shown*), suggesting that fire-induced carbon deposition drives fire-induced changes in soil pH. Thus, carbon's effects may not be reduced in the field, unless elemental sulfur or other abundant reactive ions are somehow also present. Indeed, in the field carbon soil amendments and observations suggest that carbon can and does reduce *R. nigrum* reproductive potential under field conditions in which the invasive is prevalent.

Interestingly, sulfur, fertilizer, and all other treatment combinations, except carbon alone, reduced *R. nigrum* shoot DW in monocultures, but polyculture effects were intermediate on *R. nigrum* shoot DW (Fig. 3c). A similar effect was seen for *R. nigrum* root DW, except for carbon with fertilizer and all treatments (Fig. 3b). Sulfur, additional nutrients, and combinations of the treatments may increase intraspecific competition. While these treatments did not significantly reduce inflorescence count, the observed means were uniformly reduced, suggesting increased dosages of these treatments could potentially reduce the reproductive potential of *R. nigrum.* The intermediate effects on *R. nigrum* shoot and root DW is not likely driven by carbon's affinity to

bond with sulfur and plant macronutrient ions, reducing the carbon available to neutralize allelopathic exudates, since the intermediate observed means in monocultures occurred in treatments without carbon were similar to combination treatments with carbon (Fig.3a-d). Instead, sulfur and fertilizer results in increased intraspecific competition by other means. Studies of other intraspecific competition have shown that increased nutrient availability or soil pH stress can also drive intraspecific competition (*e.g.* Goldberg 1985). However, in terms of managing the spread of *R. nigrum,* the increased costs of adding sulfur and fertilizer in the field would unnecessarily strain conservation budgets and labor power while producing less effective results. Sulfur and additional nutrients may increase stochastic responses in desired plant species, as well. Thus, land managers may not wish to consider these additional soil amendments, especially since carbon alone produced greater reduction in *R. nigrum* inflorescence count. It should be noted that carbon and other soil treatments did not harm *A. glaber* in the greenhouse in terms of increasing root:shoot ratios, or decreasing shoot DW and root DW. However, other species may respond in different ways.

None of the soil treatments affected *A. glaber* root DW, shoot DW, or root:shoot ratios in intraspecific competition monocultures, and all soil treatments reduced or eliminated the reduction in root DW (Fig. 4a-b). Concurrently, competition with *R. nigrum* in untreated control pots had the highest reduction in root:shoot ratios, while other soil treatments other than fertilizer (NPK) eliminated the effect. Considering *A. glaber* relies on mycorrhizae to increase root surface area and thus root DW and *R. nigrum* is known to kill soil fungi, the neutralization of an effect of *R. nigrum* competition on *A. glaber* root DW and decrease in root:shoot ratios, suggests that treatments with carbon or sulfur may neutralize *R. nigrum* fungicidal exudates. The immediate

effects did not significantly change the means for root DW or root:shoot ratios, but if carbon or sulfur can neutralize the harmful effects of *R. nigrum* on *A. glaber* as carbon reduces *R. nigrum* reproductive potential, over generations, the longer term effects on *A. glaber* may continue to be reduced. In other words, if there are subsequently fewer *R. nigrum* to compete with after every reproductive event, *A. glaber* could potentially increase its fitness, but further study of multigenerational effects of carbon (or sulfur) are needed to state this more conclusively.

The field manipulation and observational experiments mirrored effects of carbon treatments in the greenhouse on *R. nigrum*. Active carbon treatments in the field, while small in sample size, showed a uniform total inhibition of flowering on *R. nigrum* while adjacent control plots showed an abundance of bright yellow flowers (Fig. 6). Years since fire in the observational field experiment suggest that negative effects of fire on *R. nigrum* may be reduced over generations (Fig. 5a-b) as carbon washes out of field soils (Ciais et al., 2008). Reapplication of active carbon for the purposes of managing the invasive should, therefore, be considered in budgets and allocation of labor resources. Further, carbon's ability to reduce soil pH in alkaline soils tested here in both the greenhouse (Fig. 3a) and the field (Fig. 3b), suggest reducing soil pH is a partial mechanism for carbon's reduction in *R. nigrum* reproductive potential. However, it is only partial, as sulfur amendments also reduced soil pH, but there was no evidence in sulfur treatments that a reduction in soil pH alone reduces *R. nigrum* inflorescence count (Fig. 3a).

Conclusion

Analysis of growth metrics for *A. glaber* suggest that there may be only moderate relief from the immediate effects of competition with *R. nigrum*. However, if carbon reduces the reproductive

potential of *R. nigrum* – as suggested by inflorescence count here and by diminished dispersal in the second year of post-fire vegetative recovery (Schlau 2022), over generations the *R. nigrum* Novel Weapons may have less effect, which may be sustained for years after burns. The field manipulation suggests a relatively cost-effective means of reducing the reproductive potential of the harmful invasive *R. nigrum* with minimal environmental impact and less than disturbing soils with manual removal of *R. nigrum*. Field observations suggest the possibility that treatments need not be executed every year. Active carbon soil amendments may be especially useful in urban settings, in which disturbance of native vegetation is often caused by activities other than fire and prescribed fires meet public resistance.

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