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# Fungal pathogens and arbuscular mycorrhizal fungi of abandoned agricultural fields: potential limits to restoration

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## Research Article

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## Abstract

Little is known about impacts of soilborne pathogen legacies on reestablishment of native plant species in abandoned agricultural fields. We tested whether pathogens found in abandoned citrus orchards affect growth of native and invasive plant species in a controlled greenhouse experiment. In previous research, we identified several species of ascomycete (*Fusarium* spp.) and oomycete (*Pythium* spp.) pathogens from field roots and soils. The invasive annual grass, ripgut brome [*Bromus diandrus* (Roth.)], and native forbs, common fiddleneck [*Amsinckia intermedia* Fisch. & C.A. Mey.], coastal tidytips [*Layia platyglossa* (Fisch. & C.A. Mey.) A. Gray], and California goldfields [*Lasthenia californica* (DC. ex Lindl.)], were grown together in four different field soil treatments. Using pesticides on soils collected from abandoned citrus fields, we created four soil treatments that excluded different groups of potential pathogens: (1) untreated control (2) metalaxyl (oomyceticide) (3) fludioxonil (fungicide), and (4) steam-sterilized. Fludioxonil increased aboveground biomass of *L. platyglossa* ($P = 0.005$) and *L. californica* ($P = 0.02$) compared with sterile and metalaxyl-treated soils. *Lasthenia californica* had decreased arbuscular mycorrhizal colonization with metalaxyl, suggesting metalaxyl has non-target effects on mycorrhizae. Fludioxonil decreased potential pathogens in *L. californica* roots while having no effect on mycorrhizal colonization. *Bromus diandrus* had higher biomass in sterile and fludioxonil-treated soils than untreated soils ($P = 0.0001$), suggesting a release from soilborne pathogens. The release from soilborne pathogens with the use of fludioxonil in both native forbs and *B. diandrus*, combined with overall higher biomass across treatments in *B. diandrus*, suggests that pathogen impacts in a field setting are insufficient to reduce success of this invasive grass, and use of a fungicide would not benefit native species in mixed stands with *B. diandrus*.

## Introduction

Conversion of natural habitat to agriculture is one of the dominant forms of land degradation and loss of biodiversity throughout the world. Subsequent abandonment of agricultural lands has led to some of the most dramatic plant invasions (MacDonald et al. 2000). In the United States, there are 68 million hectares of abandoned agricultural fields (Zumkehr and Campbell 2013). The process of returning these abandoned agricultural lands to native vegetation may require intensive management of soils, seedbanks, and vegetation (Cramer et al. 2008; Marushia and Allen 2011; Pywell et al. 2007; Standish et al. 2007; Walker et al. 2004). Previous studies of abandoned agricultural lands increase our understanding of ecosystem recovery and successful restoration practices (Allen et al. 2005a; Bonet 2004; Marushia and Allen 2011; Pywell et al. 2007, 2011). However, the majority of studies focused on aboveground processes, and less is known about how the soil microbial community of abandoned agricultural lands affects restoration success.

Conventional industrial agricultural practices such as tillage, fertilization, pesticides, and monoculture cropping contribute to soil legacies that limit restoration of abandoned fields (Cramer et al. 2008; Filser et al. 1995; Kulmatiski et al. 2006). These traditional practices lead to low mycorrhizal inoculum potential (Miller 2002) as well as higher loads of aggressive soilborne pathogens (Mills and Bever 1998). Arbuscular mycorrhizal fungi (AMF) significantly influence plant growth and diversity (Hilbig and Allen 2015; Pringle et al. 2009; van der Heijden et al. 1998) and the outcome of restoration in old fields (Richter and Stutz 2002). Additionally, AMF increase the ability of plants to withstand pathogen infections (Borowicz 2001; Hilbig and Allen 2015; Newsham et al. 1995). Mycorrhizal fungi inoculations have improved success in restoration of native prairie in abandoned agricultural lands with low mycorrhizal potential (Middleton and Bever 2012; Miller 2002; Richter and Stutz 2002), suggesting low mycorrhizal inoculum limits restoration of abandoned agricultural fields.

### Management Implications

Soil legacies of cultivated lands may affect the establishment of both native and invasive plant species during restoration. We found that root fungal pathogens that occur in abandoned citrus fields decreased biomass of some native species as well as the invasive grass *Bromus diandrus* (ripgut brome). This indicates that applications of fungicides during restoration may increase performance of invasive species while simultaneously benefiting some native species. Therefore, the use of fungicides like fludioxonil may be beneficial in restoration only when invasive species have lower impact from fungal pathogens relative to native species. This was not the case for *B. diandrus*, which responded to fungicide application with increased growth.

Pathogens in agricultural lands may be less diverse than in wildlands, but more aggressive in their impacts on crop plants (Altieri 1999). Many crop pathogens are host-specific (Bullock 1992) and do not impact native plants, while others are generalists that may affect crop and non-crop species (Hersh et al. 2012). Furthermore, pathogens of abandoned agricultural lands differentially affect native and invasive species establishment and growth during restoration. Allen et al. (2005b) showed that the local pathogen *Fusarium semitectum* (Berk. & Ravenel) limited plant species distribution during restoration of simulated slash and burn agriculture in a tropical dry forest. Kulmatiski et al. (2006) used the fungicide benomyl to control soil fungi in an abandoned agricultural site and showed agricultural soil fungi enhance exotic plant cover. Hilbig and Allen (2015) found oomycete pathogenic hyphae infecting the roots of native forbs of coastal sage scrub vegetation, but not the invasive grass ripgut brome [*Bromus diandrus* (Roth.)], when plants were inoculated with abandoned agricultural soils conditioned by *B. diandrus*. However, to date, evidence for the activity of soilborne fungal pathogens comes primarily as inference from plant–soil feedback studies (Bever 1994; Callaway et al. 2004a; Kardol et al. 2007; Mangan et al. 2010; Yelenik and Levine 2011). It remains unclear whether pathogens in abandoned agricultural lands affect the reestablishment of native plant species and the success or failure of restoration in these systems.

Control of soilborne pathogens using different pesticides, a common agricultural practice but seldom done in restoration, can determine whether certain pathogens are affecting target restoration species. Fludioxonil is a nonsystemic, broad-spectrum fungicide with long residual activity (Rosslenbroich and Stuebler 2000) that is commonly used in seed and postharvest treatments of citrus fruits (Zhang 2007). It effectively controls many noxious agricultural pests of the genera *Penicillium*, *Fusarium*, and *Lasiodiplodia* (Zhang 2007). Metalaxyl is an oomyceticide that is used to protect more than 100 agricultural crops through the control of *Pythium* and *Phytophthora* species (Davidse et al. 1988; Fisher and Hayes 1982). *Pythium* and *Phytophthora* are common in citrus roots and cause a number of diseases leading to reduced production and mortality (Cohen and Coffey 1986). In addition to controlling oomycete pests, metalaxyl has been shown to stimulate AMF colonization when applied at low levels effective at controlling oomycetes (Afek et al. 1991; Hetrick and Wilson 1991).

To gain a better understanding of the role of pathogens remaining in an abandoned citrus orchard undergoing restoration as habitat for native species, we manipulated soil fungal communities in controlled greenhouse studies using fludioxonil and metalaxyl. Through the use of different fungicides, we determined whether the suppression of different fungal functional groups present in these fields affects native forb and invasive grass establishment and growth. Here, we used two lines of evidence to show that pathogens were present and active in these soils: (1) plant growth response to fludioxonil and metalaxyl and (2) direct microscopic morphological identification of pathogens and AMF.

## Material and Methods

### Field Site

The study site was at the Lake Mathews Reserve located in western Riverside County, CA (33°36′29.80N, 117°02′00.81W). The area has a Mediterranean-type climate that receives an average of 262 mm precipitation each year, mostly during the winter–spring growing season (November to May). The reserve has approximately 12,000 ha of historically disturbed coastal sage scrub and annual forbland (Minnich 2008). Nearly 500 ha were converted to a citrus orchard beginning in the early 1900s. Citrus agriculture at Lake Mathews Reserve was abandoned in the early 1990s when irrigation ceased due to high cost, but restoration planning and permitting did not begin until 2004. Removal of citrus trees and agricultural infrastructure started in 2006 and was still underway at the time of this research. Since abandonment, the land has been heavily invaded by *B. diandrus* and has transitioned to exotic annual grassland. Previous studies at this site found oomycete hyphae infecting the roots of native forb species (Hilbig and Allen 2015), and the soilborne fungal pathogens *Pythium heterothallicum* (W.A. Campb. & F.F. Hendrix), *Fusarium equiseti* [(Corda) Sacc.], *Fusarium pseudograminearum* (O'Donnell & T. Aoki), *Penicillium brevicompactum* (Dierckx), and *Mortierellales* sp. were isolated from field soils and roots (Hilbig 2015). Field experiments in two sequential winter growing seasons (2011 to 2012 and 2012 to 2013) to test the effects of a fungicide and an oomyceticide on soil organisms failed due to extreme drought (<55% of average precipitation), which caused most native and exotic annuals that germinated to die before maturity in both years (Hilbig 2015). The experiment was therefore conducted in a controlled greenhouse environment.

### Greenhouse Experiment

Field soil was collected in fall 2013 to a 10-cm depth along remnant dead, cut citrus rows under stands of *B. diandrus* from 10 randomly selected locations across a site of ~1 ha and homogenized. The site is representative of lands undergoing restoration of native species but currently dominated by exotic grass. The soil of the abandoned orchard was a Porterville cobbly clay (classified as fine, smectitic, thermic Aridic Haploxererts; Nelson et al. 1919), cut 50% with silica sand to create a sandy clay for better drainage; this is a common practice for inoculum studies in fine-textured soil (e.g., Hilbig and Allen 2015; Johnson et al. 2008). From the field-collected soils four soil treatments were created: untreated, sterile, metalaxyl-treated soils, and fludioxonil-treated soils. Soil for the potting mix was steam sterilized for 24 h, held at room temperature for 24 h, and sterilized for another 24 h. The resulting soil contained total KCl-extractable nitrogen ($NO_3^-$-N plus $NH_4^+$-N) of 17.0 µg g$^{-1}$ soil, and 18.1 µg g$^{-1}$ bicarbonate-extractable phosphorus.

In February 2014, the greenhouse study began. Twenty 15 L rectangular pots (38.7 by 29.2 by 18.4 cm deep) were filled with the sterilized soil described earlier. Sterile pots had no live inoculum added; all other treatments received 4 cm of live inoculum (unsterilized soil) added to the top. This approach was used to minimize differences in soil nutrients among treatments due to potential nutrient release by sterilization techniques. Live inoculum consisted of the original field-collected soil cut 50% with silica sand. Cannonoball®WP (Syngenta, Riverside, CA) was applied immediately to pots with the fludioxonil soil treatment and biweekly throughout the experiment at the manufacturer's recommended rate (3.8 mg ai pot$^{-1}$, total application). Metalaxyl-treated pots received a single application of Subdue® (Judelson H, Riverside, CA) at the beginning of the experiment at the manufacturer's recommended rate (1.9 mg ai pot$^{-1}$). To test for potential non-target effects of these pesticides on plants, previous greenhouse trials included sterile soils with metalaxyl and fludioxonil applications. These trials showed that biomass did not differ significantly between sterile soil and sterile soils treated with either pesticide, demonstrating that metalaxyl and fludioxonil did not have any direct toxicity to plants (Hilbig 2015).

At the same time as soil treatments, three native forb species associated with coastal sage scrub and the invasive grass *B. diandrus* were seeded in the 15 L-pot mixture and grown in a controlled greenhouse condition (~24 C daytime, 18 C night, ambient daylight appropriate to the winter growing season) for 5 wk after seedling emergence. The common native forbs common fiddleneck [*Amsinckia intermedia* Fisch. & C.A. Mey.], California goldfields [*Lasthenia californica* DC. ex Lindl.], and coastal tidytips [*Layia platyglossa* (Fisch. & C.A. Mey.) A. Gray] were chosen based on vegetation surveys conducted at Lake Mathews Reserve in 2010 (EB Allen, unpublished data). Native grasses are very sparse; therefore, only dominant native annual forbs were chosen. Before the start of the experiment, all pots were watered for 2 wk to promote germination of seeds that might have been viable and present in the live inoculum. Seedlings were removed, and seeds of the three native forbs and *B. diandrus* were added. *Amsinckia intermedia* germinates a week after other species, and was therefore seeded a week earlier. Pots contained a constant density of 24 plants across all treatments with six individuals of each species and were arranged in a randomized complete block design with five replicates per soil treatment.

Plants were harvested after 5 wk for aboveground biomass and root biomass. Aboveground biomass was determined after drying at 60 C for 48 h. With the exception of a small subsample of root collected from each species for the assessment of percent fungal colonization, the root biomass of all species per pot was combined. This is due to the fact that it was not possible to untangle the fine roots of individual species within the pots. Roots were washed of soil, and biomass was determined after drying at 60 C for 48 h.

### Fungal Root Colonization

Roots were examined for structures of both AMF and nonmycorrhizal fungi of Ascomycota and Basidiomycota as described by Hilbig and Allen (2015). Dried roots were rehydrated, cleared in 2.5% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue (Kormanik and McGraw 1982; Koske and Gemma 1989). Previous studies confirmed that drying did not change percent colonization of mycorrhizal or potential pathogenic fungi (Hilbig and Allen 2015). Root fragments were then mounted in PVLG on slides, and 80 observations at 400× were made for the root system of each individual per pot (24 individuals pot$^{-1}$).

### Statistical Analyses

Aboveground biomass was analyzed using separate one-way ANOVA for each species with soil treatment as a fixed factor. Root biomass was analyzed across pots using a one-way ANOVA with soil treatment as a fixed factor. Soil treatments were compared using least significant difference ($LSD_{0.05}$). All data were checked for homogeneity of variances using Levene's tests and for normality using the Shapiro-Wilk test. Ratios of percent AMF root colonization to percent ascomycete root colonization were calculated for each species by soil treatment. Percent root colonization data and ratios failed to meet the normality assumption even after a log transformation and were analyzed using Kruskal-Wallis rank-sum test for each species with soil treatment as a fixed factor. Soil treatments were compared using Nemenyi's test with chi-square approximation for independent samples. Box-and-whisker plots were used to detect outliers, and one metalaxyl pot was removed before statistical analyses because of a high biomass >180% of the mean. All statistical analyses were performed using R v. 3.0.2 (R Development Core Team 2013).

## Results and Discussion

Total native forb aboveground biomass varied across soil treatments for each of the species ($P = 0.016$ Figures 1A–4A). Total root biomass per pot was highly variable within treatment and not significantly different across soil treatments (grand mean = 2.83g pot$^{-1}$, SE = 0.46). Roots of native and invasive species were colonized by both AMF and ascomycetes, which included potential pathogen species. No basidiomycetes were observed microscopically from greenhouse-grown roots (no hyphae with clamp connections). Oomycetes were observed in the field soils as described earlier and in previous greenhouse studies (Hilbig and Allen 2015), but were not observed in the roots of plants grown in this greenhouse study.

Pots treated with fludioxonil had greater native forb biomass than those with sterile soil or soil treated with metalaxyl due to increased biomass in two of three forb species. More specifically, the application of fludioxonil increased aboveground biomass of *L. platyglossa* ($P = 0.005$; Figure 1A) and *L. californica* ($P = 0.020$; Figure 2A), but had no effect on *A. intermedia* biomass (Figure 3A). The aboveground biomass is likely related to root fungal colonization of the different fungal functional groups, where greater percent colonization of AMF compared with ascomycetes, which may be primarily pathogens (Hilbig 2015), confers greater aboveground biomass due to the increased nutrient status.

Root colonization data for *L. platyglossa* and *L. californica* demonstrate that individuals treated with fludioxonil had higher percent AMF colonization to percent ascomycete (AMF:ascomycete fungi; Table 1). While AMF:ascomycete ratios varied across treatments and species (Table 1), in all species the highest AMF:ascomycete ratio was found in fludioxonil-treated soils (Table 1). Fludioxonil increases root colonization by AMF (Murillo-Williams and Pedersen 2008) while being effective against pathogens such as *Fusarium* species. AMF interact in the rhizosphere and compete with co-occurring pathogens for the same colonization sites (Newsham et al. 1995; Smith and Read 2008). Increases in AMF colonization with fludioxonil may be due to reduced competition with aggressive pathogens for those colonization sites (Murillo-Williams and Pedersen 2008). Here, increased biomass with fludioxonil application is most likely due to a reduction in infection by soilborne root pathogens while having no negative effect on AMF, so the ratio of AMF to

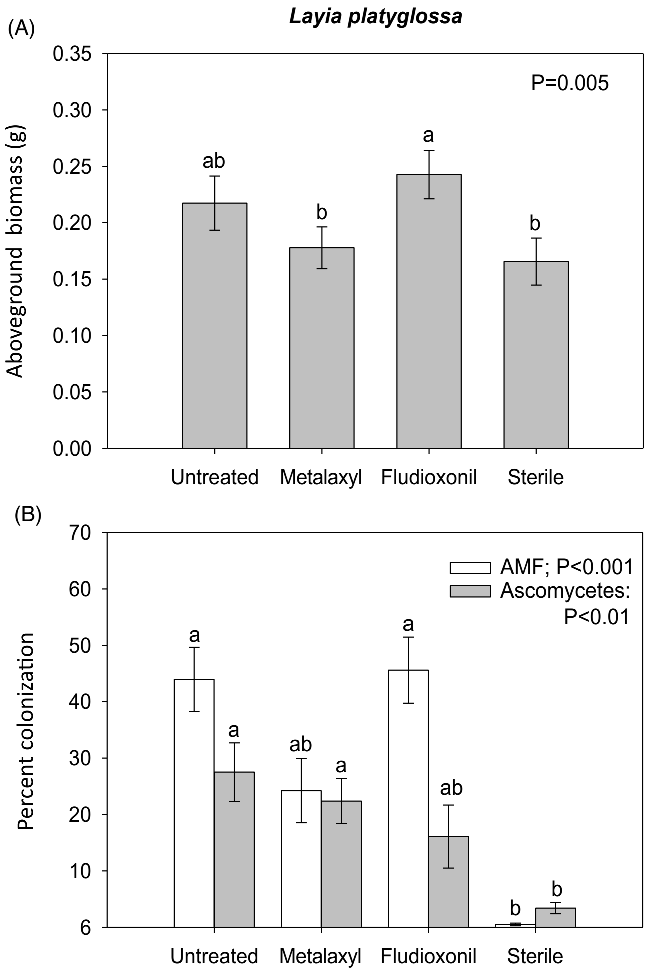

**Figure 1.** (A) Aboveground biomass and (B) percent root colonization of arbuscular mycorrhizal fungi (AMF) and ascomycete fungi in *Layia platyglossa* at week 5 in untreated, nonsterile field soil, sterile soil, and soils treated with fludioxonil or metalaxyl. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha = 0.05$. Superscript letters denote significant difference across soil treatments.

non-mycorrhizal fungi is increased, as seen in *L. californica* and *L. platyglossa*.

Conversely, application of metalaxyl reduced biomass compared with biomass of plants treated with fludioxonil. While we did not observe oomycetes in our greenhouse roots, previously cultured and sequenced oomycete (*Pythium* and *Phytophthora*) and ascomycete (*Fusarium*) pathogens from the field (Hilbig 2015; Hilbig and Allen 2015) lent credibility to the use of both a fungicide and an oomyceticide to manipulate soilborne pathogen communities in this abandoned citrus orchard. However, microscopic observation indicated metalaxyl targeted mycorrhizal fungi. AMF colonization was reduced in *L. californica* when soils were treated with metalaxyl compared with the untreated and fludioxonil-treated agricultural soils ($P << 0.001$; Figure 2B). *Layia platyglossa* showed similar trends of decreased AMF colonization with the application of metalaxyl (Figure 1B). *Lasthenia californica* plants grown in metalaxyl and untreated agricultural soil had significantly higher ascomycete colonization compared with plants grown in the sterile soil ($P << 0.001$; Figure 2B). Overall, *A. intermedia* had lower AMF colonization than other native forbs in untreated agricultural soils, with an average of 12% colonization compared with ~40% in other native forbs grown in fludioxonil-treated soils (Figure 3B). Additionally, the reduction in AMF with metalaxyl seen in the other native forbs did not occur in *A. intermedia* (Figures 1B, 2B, and 3B). Hilbig and Allen (2015) demonstrated that *A. intermedia* is less colonized by AMF than other native forbs in this system; therefore the application of metalaxyl might not negatively affect *A. intermedia* growth as it does native forb species more heavily colonized by, or dependent upon, AMF.

The observed negative effects of metalaxyl on plant growth might better be explained by non-target effects on AMF, as AMF colonization was significantly reduced with this treatment in *L. californica* and trending in *L. platyglossa* ($P < 0.1$) compared with untreated agricultural soils and fludioxonil. The reported effects of metalaxyl on AMF are highly variable. Most studies have demonstrated that low levels of metalaxyl, such as the 1.9 mg ai pot$^{-1}$ used in our study, increase AMF colonization (Afek et al. 1991; Groth and Martinson 1983; Hetrick and Wilson 1991; Johnson and Pfleger 1992; Shetty and Magu 1997). The beneficial effects of metalaxyl on mycorrhizal colonization could be due to the suppression of organisms, such as oomycetes, that are antagonistic towards AMF (Johnson and Pfleger 1992). Alternatively, metalaxyl has been demonstrated to restrict AMF when applied as soil drenches (Carrenho et al. 2000; Jabaji-Hare and Kendrick 1987). Jabaji-Hare and Kendrick (1987) found that soil drench applications of metalaxyl, or applications directly to the base of the plant, reduced mycorrhizal colonization in leeks

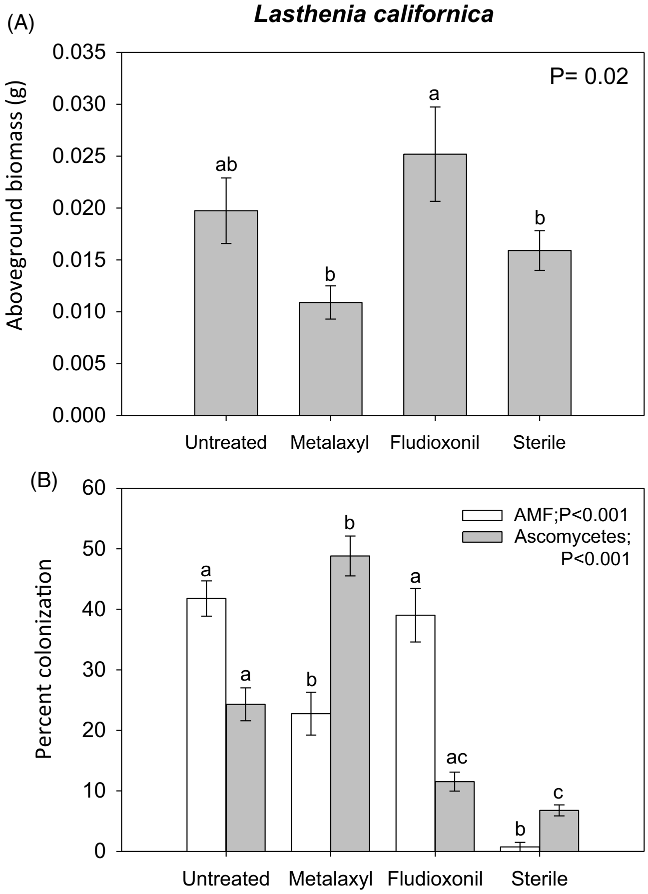

**Figure 2.** (A) Aboveground biomass and (B) percent root colonization of arbuscular mycorrhizal fungi (AMF) and ascomycete fungi in *Lasthenia californica* at week 5 in untreated, nonsterile field soil, sterile soil, and soils treated with fludioxonil or metalaxyl. Note values on *y* axis, with *L. californica* biomass an order of magnitude lower than other species. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha = 0.05$. Superscript letters denote significant difference across soil treatments.

[*Allium ampeloprasum* (L.)] at 0.5, 1.0, and 2.0 mg ai per plant. Similarly, Oliveira (1992) found low levels of mycorrhizal colonization in Rangpur lime [*Citrus limonia* (Osbeck)] when metalaxyl was applied at 1.0 and 2.0 mg ml$^{-1}$.

The mechanisms behind suppression or enhancement of mycorrhizal colonization with applications of metalaxyl or other pesticides to control root pathogens are poorly understood (Johnson and Pfleger 1992). It has been suggested that response to fungicide is AMF species specific and specific to host plant species (Jabaji-Hare and Kendrick 1987). Additionally, the chemical used, the rate of application, and method of application account for the observed different AMF responses to metalaxyl. In this study, Subdue® was applied as a soil drench at the manufacturer's recommended level at the beginning of the experiment. The benefits of oomycete pathogen protection from metalaxyl were offset by its detrimental effects on AMF in these highly mycorrhizal native forbs (Hilbig and Allen 2015), as seen in *L. californica* and *L. platyglossa*, and the subsequent reduction in the ratio of AMF:ascomycetes in both species ($P < 0.001$ and $P < 0.001$, respectively). Soil-drench applications of metalaxyl could have resulted in the accumulation of the fungicide within the root zone of soils. This systemic pesticide is taken up by plant roots at the application site and affects timing of AMF infection.

In contrast to the forbs, *B. diandrus* had varying aboveground biomass across all soil treatments ($P << 0.001$; Figure 4A). Aboveground biomass was greatest in sterile soil and lowest in soils treated with metalaxyl ($P << 0.001$; Figure 4A). Individuals grown in metalaxyl-treated soils had the highest colonization of AMF, which was significantly greater than AMF colonization in sterile soils but not untreated agricultural soils or fludioxonil soils ($P = 0.004$; Figure 4B). *Bromus diandrus* grown in untreated agricultural soils had significantly greater ascomycete colonization than in sterile soil, but not in fludioxonil- or metalaxyl-treated soils ($P = 0.03$; Figure 4B).

Pesticides have both negative and positive effects on mycorrhizal fungi due to differences in their modes of action, chemical structures, release of soil mineral nutrients, and methods of application (Jin et al. 2013; Johnson and Pfleger 1992). Release of soil nutrients following ascomycete mortality due to fludioxonil was likely minimal in this study, because only a thin layer of live soil inoculum was used. Additionally, if increased nutrient availability from fludioxonil application was driving plant growth responses, we would expect greater plant biomass in fludioxonil-treated soils compared with sterile soils in all species. However, *B. diandrus* had highest aboveground biomass in sterile soils. This suggests that the changes in plant biomass among soil treatments were due to differential effects of fludioxonil on the soil biota and not nutrient availability.

The use of fludioxonil during restoration of abandoned agriculture might result in increased establishment and growth of native forb species. However, *B. diandrus* also had increased

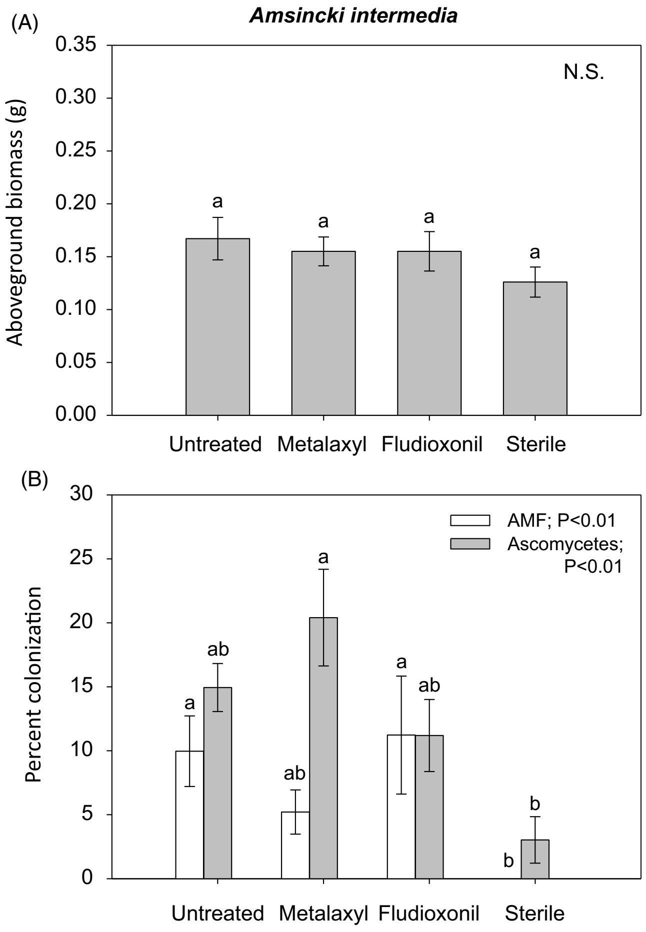

**Figure 3.** (A) Aboveground biomass and (B) percent root colonization of arbuscular mycorrhizal fungi (AMF) and ascomycete fungi in *Amsinckia intermedia* at week 5 in untreated, nonsterile field soil, sterile soil, and soils treated with fludioxonil or metalaxyl. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha = 0.05$. Superscript letters denote significant difference across soil treatments.

**Table 1.** Ratio of percent root colonization of arbuscular mycorrhizal fungi (AMF) to percent root colonization of ascomycetes in all four plant species × four soil treatments.

| AMF:ascomycete fungi[a] | | | | |
|---|---|---|---|---|
| Treatment | *Amsinckia intermedia* | *Lasthenia californica* | *Layia platyglossa* | *Bromus diandrus* |
| Untreated | $1.05^{a}$ | $2.39^{c}$ | $4.32^{c}$ | $0.89^{a}$ |
| Metalaxyl | $0.35^{ab}$ | $0.58^{ab}$ | $2.35^{ac}$ | $4.21^{c}$ |
| Fludioxonil | $1.55^{a}$ | $5.33^{c}$ | $8.31^{c}$ | $6.39^{c}$ |
| Sterile | $0.20^{b}$ | $0.15^{b}$ | $0.25^{ab}$ | $0.41^{ab}$ |

[abc] Superscript letters denote significant difference across rows and columns.

biomass in soils treated with fludioxonil and in sterile soils, suggesting the release of *B. diandrus* from soilborne pathogens with the application of fludioxonil. Yet root colonization by ascomycetous fungi was not significantly reduced in *B. diandrus* with fludioxonil. The observed *B. diandrus* biomass response to fludioxonil could be due to decreased colonization by aggressive pathogens targeted by fludioxonil, while other non-mycorrhizal fungi were not affected by fludioxonil applications.

We believe our study is the first of its kind to determine that soilborne pathogenic fungi in abandoned agricultural fields impact native species during restoration efforts in these fields. The emphasis in other studies has been to identify potential rhizosphere pathogens in plant–soil feedback studies (Callaway et al. 2011; Klironomos 2002). Kulmatiski et al. (2006) used the fungicide benomyl to control soil fungi in an abandoned agricultural site and showed abandoned agricultural soil fungi enhance exotic plant cover. Benomyl is a general fungicide that is effective on ascomycetes and AMF, but is considered to affect mutualistic mycorrhizal fungi more negatively (Callaway et al. 2004b). Through the application of different pesticides, we were able to show the potential of some soilborne fungal pathogens to inhibit both native and invasive plant growth. Therefore, applications of fungicides such as fludioxonil during restoration may benefit the already competitively superior invasive species at the same time it benefits some native species. Fludioxonil may be beneficial in restoration when invasive species have no naturalized enemies. Successful restoration of abandoned agricultural fields may rely on a greater understanding of soil biotic legacy

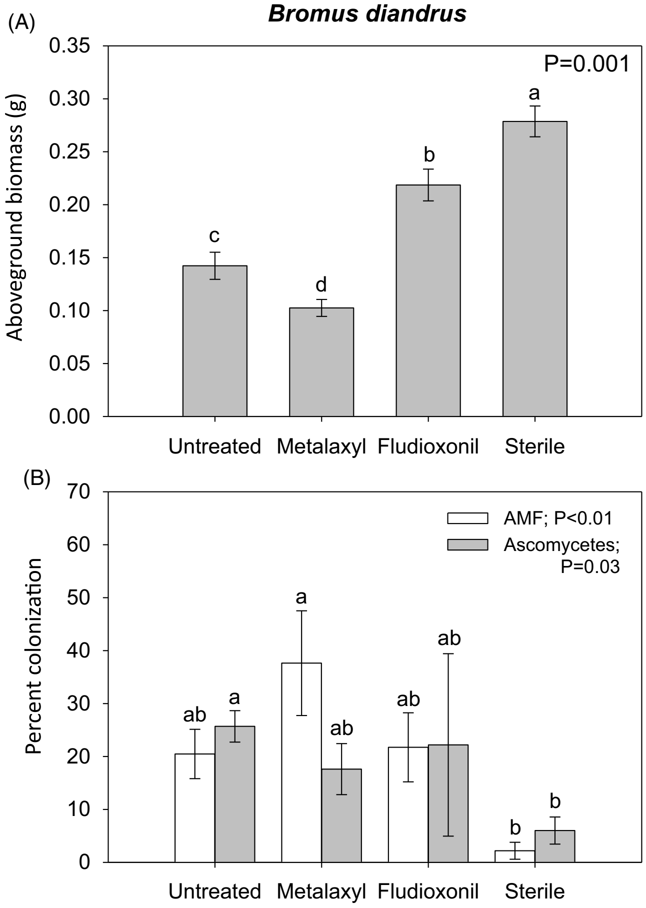

**Figure 4.** (A) Aboveground biomass and (B) percent root colonization of arbuscular mycorrhizal fungi (AMF) and ascomycete fungi in *Bromus diandrus* at week 5 in untreated, nonsterile field soil, sterile soil, and soils treated with fludioxonil or metalaxyl. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha = 0.05$. Superscript letters denote significant difference across soil treatments.

effects on native reestablishment and the successful control of soilborne pathogens that target native species more heavily than invasive species.

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## References

Afek U, Menge JA, Johnson ELV (1991) Interaction among mycorrhizae, soil solarization, metalaxyl, and plants in the field. Plant Dis 75:665–671

Allen EB, Cox RD, Tennant T, Kee SN, Deutschman DH (2005a) Landscape restoration in southern California forblands: response of abandoned farmland to invasive annual grass control. Isr J Plant Sci 53:237–245

Allen MF, Allen EB, Gomez-Pompa A (2005b) Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. Restor Ecol 13:325–333

Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agric Ecosyst Environ 74:19–31

Bever JD (1994) Feedback between plants and their soil communities in an old field community. Ecology 75:1965–1977

Bonet A (2004) Secondary succession of semi-arid Mediterranean old-fields in south-eastern Spain: insights for conservation and restoration of degraded lands. J Arid Environ 56:213–233

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068

Bullock DG (1992) Crop rotation. Crit Rev Plant Sci 11:309–326

Callaway RM, Bedmar EJ, Reinhart KO, Silvan CG, Klironomos J (2011) Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. Ecology 92:1027–1035

Callaway RM, Thelen GC, Barth S, Ramsey PW, Gannon J E (2004a) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85:1062–1071

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004b) Soil biota and exotic plant invasion. Nature 427:731–733

Carrenho R, Bononin VLR, Graciolli LA (2000) Effect of the fungicides Fosetyl-Al and Metalaxyl on arbuscular mycorrhizal colonization of seedlings of *Citrus sinensis* (L.) Osbeck grafted onto *C. limon* (L.) Burmf. Acta Sci 22:305–310

Cohen Y, Coffey MD (1986) Systemic fungicides and the control of oomycetes. Annu Rev Phytopathol 24:311–338

Cramer VA, Hobbs RJ, Standish RJ (2008) What's new about old fields? Land abandonment and ecosystem assembly. Trends Ecol Evol 23:104–112

Davidse LC, Gerritsma OCM, Ideler J, Pie K, Velthuis GCM (1988) Antifungal modes of action of metalaxyl, cyprofuram, benalaxyl and oxadixyl in phenylamide-sensitive and phenylamide-resistant strains of *Phytophthora megasperma* f. sp. *medicaginis* and *Phytophthora infestans*. Crop Prot 7:347–355

Filser J, Fromm H, Nagel RF, Winter K (1995) Effects of previous intensive agricultural management on microorganisms and the biodiversity of soil fauna. Plant Soil 170:123–129

Fisher DJ, Hayes AL (1982) Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. Pestic Sci 13:330–339

Groth DE, Martinson CA (1983) Increased endomycorrhizal infection of maize and soybeans after soil treatment and metalaxyl. Plant Dis 67:1377–1378

Hersh MH, Vilgalys R, Clark JS (2012) Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. Ecology 93:511–520

Hetrick BAD, Wilson GWT (1991) Effects of mycorrhizal fungus species and metalaxyl application on microbial suppression of mycorrhizal symbiosis. Mycologia 83:97–102

Hilbig BE (2015) Ecological Assembly Rules and Soil Legacy Effects in the Restoration of an Invaded Plant Community. Ph.D thesis. Riverside: University of California–Riverside. http://escholarship.org/uc/item/42g359z9 122 p. Accessed: June 11, 2016

Hilbig BE, Allen EB (2015) Plant–soil feedbacks and competitive interactions between invasive *Bromus diandrus* and native forb species. Plant Soil 392:191–203

Jabaji-Hare SH, Kendrick WB (1987) Response of an endomycorrhizal fungus in *Allium porrum* L. to different concentrations of the systemic fungicides, metalaxyl (Ridomil®) and fosetyl-al (Aliette®). Soil Biol Biochem 19:95–99

Jin H, Germida JJ, Walley FL (2013) Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. Appl Soil Ecol 72:22–30

Johnson NC, Pfleger FL (1992) Vesicular-arbuscular mycorrhizae and cultural stresses. Pages 71–99 *in* Bethlenfalvay GJ, ed. Mycorrhizae in Sustainable Agriculture (ASA/SSSA Special Publication No. 54). Madison, WI: American Society of Agronomy

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) Characteristics of plant winners and losers during grassland eutrophication - importance of biomass allocation and mycorrhizal function. Ecology 89:2868–2878

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. Pages 37–45 *in* Schenck NC, ed. Methods and Principles of Mycorrhizal Research. St. Paul, MN: The American Phytopathological Society

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect va-mycorrhizas. Mycol Res 92:486–505

Kulmatiski A, Beard KH (2008) Decoupling plant-growth from land-use legacies in soil microbial communities. Soil Biol Biochem 40:1059–1068

Kulmatiski A, Beard KH, Stark JM (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. J Appl Ecol 43:868–876

MacDonald D, Crabtree JR, Wiesinger G, Dax T, Stamou N, Fleury P, Lazpita JG, Gibon A (2000) Agricultural abandonment in mountain areas of Europe: environmental consequences and policy response. J Environ Manage 59:47–69

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755

Marushia RG, Allen EB (2011) Control of exotic annual grasses to restore native forbs in abandoned agricultural land. Restor Ecol 19:45–54

Middleton EL, Bever JD (2012) Inoculation with a native soil community advances succession in a grassland restoration. Restor Ecol 20:218–226

Miller RM (2002) The function of mycorrhizal fungi in soil restoration. Pages 21–22 *in* International Workshop—Microbial Function in Revegetation Process of Degraded Terrestrial Environments: From Gene to Ecosystem. Tokyo, Japan, November 29, 2002

Mills KE, Bever JD (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. Ecology 79:1595–1601

Minnich RA (2008) California's Fading Wildflowers: Lost Legacies and Biological Invasions. Berkeley, CA: University of California Press. 360 p

Murillo-Williams A, Pedersen P (2008) Arbuscular mycorrhizal colonization response to three seed-applied fungicides. Agron J 100:795–800

Nelson JW, Zinn CJ, Strahorn AT, Watson EB, Dunn JE (1919) Soil survey of the Los Angeles area, California. Washington, DC: U.S. Department of Agriculture. 78 p

Newsham KK, Fitter AH, Watkinson AR (1995) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J Ecol 83:991–1000

Oliveira AAR (1992) Effects of the fungicides metalaxyl and fosetyl-Al on endomycorrhizal development and growth of Rangpur lime. Fitopatologia Brasileira 17:75–79

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol S 40:699–715

Pywell RF, Bullock J M, Tallowin JB, Walker K J, Warman EA, Masters G (2007) Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. J Appl Ecol 44:81–94

Pywell RF, Meek WR, Webb NR, Putwain PD and Bullock JM (2011) Long-term heathland restoration on former grassland: the results of a 17-year experiment. Biol Conserv 144:1602–1609

R Development Core Team (2013) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/ Accessed: May 1, 2014

Richter BS, Stutz JC (2002) Mycorrhizal inoculation of big sacaton: implications for grassland restoration of abandoned agricultural fields. Restor Ecol 10:607–616

Rosslenbroich HJ, Stuebler D (2000) *Botrytis cinerea*—history of chemical control and novel fungicides for its management. Crop Prot 19:557–561

Shetty PK, Magu SP (1997) Influence of metalaxyl on *Glomus fasciculatum* associated with wheat (*Triticum aestivum* L). Curr Sci India 72: 275–277

Smith SE, Read D (2008) Mycorrhizal Symbiosis. 3rd ed. London: Elsevier. 800 p

Standish RJ, Cramer VA, Wild SL, Hobbs RJ (2007) Seed dispersal and recruitment limitation are barriers to native recolonization of old-fields in western Australia. J Appl Ecol 44:435–445

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091

Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. Biol Conserv 119:1–18

Yelenik SG, Levine JM (2011) The role of plant–soil feedbacks in driving native-species recovery. Ecology 92:66–74

Zhang JX (2007) The potential of a new fungicide fludioxonil for stem-end rot and green mold control on Florida citrus fruit. Postharvest Biol Technol 46:262–270

Zumkehr A, Campbell JE (2013) Historical US cropland areas and the potential for bioenergy production on abandoned croplands. Environ Sci Technol 47:3840–3847