RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration

RESEARCH ARTICLE

Invasion by the annual grass, *Ventenata dubia* does not impact mycorrhizal fungal abundance in an endangered prairie

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One of the challenges in ecological restorations is that some native plant species are difficult to reestablish in disturbed or invaded landscapes. Some invasive plant species negatively impact soil biota, such as arbuscular mycorrhizal (AM) fungi, which can lead to feedbacks that facilitate their dominance in a landscape. The winter annual grass, *Ventenata dubia*, is rapidly invading North American grasslands, however, the effect of its invasion on AM fungi, plant communities, and soil properties is not well known. In a greenhouse and field experiment, we tested (1) whether the abundance of viable AM fungal propagules is altered in soil invaded by *V. dubia*; (2) how plant community composition, species richness, and/or diversity may be impacted by *V. dubia*; and (3) whether soil properties, such as pH and organic matter (OM) vary across invasion levels. We found that the effect of *V. dubia* on the abundance of mycorrhizal propagules varied among sites and did not reduce the species richness or diversity of resident plant communities. Higher canopy cover of *V. dubia* was associated with higher soil pH and OM, which suggests either *V. dubia* invasions change these soil properties, or that *V. dubia* preferentially invades these areas. Our findings suggest *V. dubia* does not consistently alter above- or belowground communities; however, the potential link between *V. dubia* invasions and soil pH and OM deserves additional investigation. A better understanding of how invasive annual grasses impact plant and soil communities will be useful in informing restoration efforts in landscapes impacted by invasive species.

Key words: arbuscular mycorrhizal fungi, invasive annual grass, mycorrhizal inoculation potential, Palouse prairie, *Ventenata dubia*

Implications for Practice

- Practitioners should ask whether there is evidence that the dominant invasive plant species at their site alters soil biota before including a microbial inoculant in their restoration plan.
- Testing the impact of invasive species on soil biota in greenhouse assays can be a useful method for determining whether microbial inoculations are necessary for restoration.
- Because invasive species may affect soil properties such as pH and organic matter, pairing greenhouse assays with data collected on plant communities and soil properties in the field will help to determine the broader ecological consequences of species invasion.

Introduction

Invasive plant species can alter grassland function (Vitousek et al. 1997; Zhao et al. 2020) by decreasing native plant species richness (Pyšek et al. 2011) and diversity (Prevéy et al. 2010). Invasions can also impact belowground systems by altering soil biotic communities (Mummey & Rillig 2006; Lekberg et al. 2013; Zubek et al. 2016) and altering soil properties, such as pH (Kourtev et al. 2003; Weidenhamer & Callaway 2010) and organic matter (OM) (Saggar et al. 1999; Koutika et al. 2007). Impacts of invasive plant species on belowground systems can be direct, through changes in root exudation or litter quality (reviewed in Zhang et al. 2019; Weidenhamer & Callaway 2010), or indirect through changes in plant community structure (Řezáčová et al. 2021). Furthermore, impacts vary by plant species (Jordan et al. 2008) and plant functional group (Bunn et al. 2015). With such variable effects, it is difficult to predict how new invaders will alter functioning in a novel ecosystem.

Author contributions: TEC conceived the research; TEC, AAS, RB designed the experiment; AAS performed the experiment; AAS analyzed the data with input from RB, TEC; AAS, RB, TEC wrote and edited the manuscript.

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doi: 10.1111/rec.14008
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.14008/suppinfo

One group of soil biota that can be impacted by invasive plants is arbuscular mycorrhizal (AM) fungi. AM fungi form a symbiotic relationship with 80% of land plants (Smith & Read 2008). In this symbiosis, plants benefit from increased uptake of nutrients such as phosphorus (P) and other putative services such as increased drought tolerance and pathogen protection; in exchange, AM fungi receive carbon from the plant (Smith & Read 2008). Invasive plant species vary in their impacts on AM fungi, with effects ranging from negative (e.g. Vogelsang & Bever 2009) to positive (e.g. Reinhart & Callaway 2006), but their impacts are not always different from those of native plant species (e.g. Bunn et al. 2015). In some cases, changes to the abundance or community composition of AM fungi from invasive plant species can promote further invasions (Mummey & Rillig 2006; Reinhart & Callaway 2006; Shah et al. 2009), while in other contexts, inoculations with AM fungi can improve the survival and growth of native plant species in disturbed landscapes (e.g. Koziol et al. 2018). Given the variability in how different invasive plant species impact and respond to AM fungi, more research on specific invaders is needed to inform practitioners as to whether or not microbial inoculations will benefit a specific restoration project.

Some invasive plant species can alter soil abiotic characteristics, such as soil pH and OM. Soil pH and OM are important to both aboveground and belowground biota because they contribute to the availability of soil nutrients, such as P (Brady & Weil 2008; Penn & Camberato 2019). Levels of pH and OM in the soil may also play a role in the invasibility of a habitat. For example, Gilbert and Lechowicz (2005) found that invasive plant species were positively correlated with high soil pH. Some invasive plant species have been shown to alter OM by changing the quality or abundance of inputs, such as leaf litter, into the soil (Norton et al. 2004; Maurel et al. 2010). Due to the important role that both soil pH and OM play in plant and soil biotic communities, evaluating the levels of both in invaded and native habitats is important in understanding potential drivers or consequences of invasion.

The winter annual grass, *Ventenata dubia* (African wiregrass), is rapidly invading North American grasslands. Since its introduction to the Pacific Northwest (PNW) in 1952, *V. dubia* has spread from Spokane County, Washington, United States throughout the PNW and into some areas of the Intermountain West (Barkworth et al. 2007; Consortium of Pacific Northwest Herbaria 2019). The invasion of *V. dubia* has negatively impacted agricultural systems (Wallace et al. 2015; Fryer 2017), Conservation Reserve Program lands (Wallace & Prather 2013; Wallace et al. 2015; Fryer 2017), and sensitive grasslands, including the endangered Palouse prairie of eastern Washington and northern Idaho (Fryer 2017).

To better understand the impacts of *V. dubia* invasions, we identified replicate "Native," "Invaded," and "Transition" plots at three field sites in the Palouse prairie. In each plot, we characterized the plant communities and measured viable AM fungal propagules, OM, and pH of rooting zone soil. We asked (1) is the abundance of viable AM fungal propagules altered in soil invaded by *V. dubia*? (2) Is plant community composition, species richness, and/or diversity impacted in communities invaded by *V. dubia*?, and (3) How do soil pH and OM vary across invasion levels?

Methods

Field Sites

Site Description. The Palouse prairie of southeastern Washington and north central Idaho, United States is particularly vulnerable to being lost or degraded through plant invasions due to its rarity and patchiness (Black et al. 1998). It is considered a critically endangered grassland with less than 1% of its original acreage remaining (Noss et al. 1995; Black et al. 1998). The remaining prairie primarily exists as disconnected fragments on shallow, rocky soils (Looney & Eigenbrode 2012). The expanding invasion of *Ventenata dubia* in the PNW threatens the Palouse prairie, thus, successful restoration and conservation of the Palouse prairie will require further knowledge of the impact of *V. dubia* on above and belowground communities.

Three field sites were identified for this study: Smoot Hill (46°81′729.3″N, 117°0.23′693.4″W), Kamiak Butte (46°85′394.3″N, 117°0.20′214.0″W), and Steptoe Butte (47°01′54.0″N, 117°18′08.0″W) (Fig. S1). These sites were chosen because they are representative of the Palouse prairie bunchgrass communities and are threatened by *V. dubia* invasion. The dominant native grasses in the Palouse prairie are *Pseudoroegneria spicata* (bluebunch wheatgrass) and *Festuca idahoensis* (Idaho fescue). Ground cover consists of bare soil and moss-dominated biological soil crusts. Smoot Hill, Kamiak Butte, and Steptoe Butte are all xeric surface and mesic subsurface soils consisting of Tekoa series soil from the parent material loess, metasedimentary residuum, and colluvium (NRCS 2020). Kamiak Butte and Smoot Hill are both gravelly silt loam, and Steptoe Butte is stony silt loam (NRCS 2020). Each site receives annual precipitation of 500–630 mm (PRISM 2021).

Plot Design and Plant Percent Cover. We established nine plots (4 m × 4 m) varying in percent cover of *V. dubia* and dispersed throughout a 50 m × 50 m area at each of the three sites. Field plots were established based on the presence of two focal plant species: *V. dubia* and *P. spicata*. We chose the invasive grass *V. dubia* because of its concern to local land managers, its expanding range in the Palouse prairie, and the lack of information on its interactions with soil microbes such as AM fungi. We chose *P. spicata* because it is one of the iconic native bunchgrasses in the Palouse prairie and is known to associate with AM fungi (Cheeke et al. 2021). Within each field site, we set up three replicate "Invaded" plots (>30% relative cover of *V. dubia*), three replicate "Transition" plots (10–20% relative cover of *V. dubia*), and three replicate "Native" plots (<5% relative cover of *V. dubia*) for a total of 27 field plots (3 sites × 3 invasion levels × 3 replicates). The Transition plots were included in the study design to examine the impacts of *V. dubia* on soil and plant communities at different stages of invasion, ranging from less than 5% invasive *V. dubia* cover to greater than 30% *V. dubia* cover at each site. Plots were on the same slope, on

the same aspect, and within similar plant communities, but spaced at least 5 m apart. We established the plots during peak flowering time (May–June 2021) and measured the percent cover of each plant species in each plot (Table S1). We followed the percent canopy cover protocol from the Bureau of Land Management Assessment, Inventory, Monitoring Protocols (Herrick et al. 2017), except we recorded the percent cover of individual plant species in the entire 4 m × 4 m plot, rather than a 1 m × 1 m quadrat placed in sections in the 4 m × 4 m plot. Because total percent plant cover can lead to values exceeding 100%, we standardized the percent cover in each plot before analysis. Thus, the percent cover reported represents the relative plant cover for each species.

Soil Abiotic Factors. In June and July 2021, 16 soil cores (2 cm diameter, 10 cm depth) were collected in an x-pattern across each plot and homogenized. One composite soil sample per plot was analyzed for nutrient concentrations and soil properties, including pH and OM (measured as percent by mass of dry soil, Kuo Testing Laboratories, Othello, WA, U.S.A.; Table S2).

Mycorrhizal Inoculation Potential Assay

A mycorrhizal inoculation potential (MIP) assay measures the abundance of viable AM fungal propagules in a sample (Moorman & Reeves 1979). This differs from AM fungal spore density (e.g. as measured by spore extractions) because not all spores are viable and the relationship between spore numbers and colonization levels in roots is inconsistent (Moorman & Reeves 1979). Roots and/or hyphal fragments can also serve as viable infection propagules, so an MIP assay is a more holistic measure of infectivity. Lower percentage mycorrhizal colonization of roots in assay plants grown with invaded versus native field soil inocula would suggest that invasion reduces the abundance of viable AM fungal propagules.

Background Soil. The background soil was collected from Smoot Hill (46°49′38.7″N, −117°14′0.21″W, Pullman, WA, U.S.A.) and consisted of Thatuna series silt loam derived from the parent material of loess and ash (NRCS 2020). Soil was mixed 1:1 (by volume) with medium-coarse sand (Beaver Bark, Richland, WA, U.S.A.) to improve drainage, and autoclaved twice at 120°C for 2 hours with a 24-hour resting period to eliminate soil biota. Nutrient data from the autoclaved background sand/soil mix are included in Table S3 (Kuo Testing Labs, Othello, WA, U.S.A.).

Inocula. The soil inocula used for the MIP experiment were collected from the root zone (2 cm diameter, 10 cm depth) of the focal plant species in each plot. In the Invaded plots, we collected soil cores from the root zone of 10 individual *V. dubia* plants and composited them into one soil inocula sample per Invaded plot. In the Transition plots, soil cores were collected from the root zone of five individual *P. spicata* plants and from the root zone of five individual *V. dubia* plants and composited into one soil inocula sample per Transition plot. In the Native plots, we collected soil cores from the root zone of 10 individual *P. spicata* plants and composited them into one soil inocula sample per Native plot.

Greenhouse. Rayleech containers (Stuewe and Sons, Tangent, OR, U.S.A.) were filled with 75 cm^3 (cc) of autoclaved 1:1 background/sand soil mix and 15 cc of live soil inocula (soil from Invaded, Transition, or Native plots), then topped with another 75 cc of autoclaved 1:1 background/sand soil mix to minimize contamination and/or splashing across pots. The uninoculated controls contained 165 cc of the autoclaved background/sand soil mix. Two *Zea mays* seeds (Peaches & Cream Sweet Corn, Seeds Needs, New Baltimore, MI, U.S.A.) were planted in each pot and thinned to one plant per pot after plants reached 5 cm tall. We used *Z. mays* because it a mycotrophic generalist host, has easy-to-stain roots for visualization of fungal structures (INVAM 2023), and was used in the development of the MIP assay (Moorman & Reeves 1979). We had 10 replicates from each field plot and 10 replicates of the control (3 plots × 3 sites × 3 replicate plots per site × 10 replicate pots + 10 control pots = 280 experimental units). Containers were placed in a randomized complete block design and rotated twice per week to reduce effects of localized environmental variables. Six weeks after planting, seedlings were harvested. Approximately 0.25 g of washed roots (fresh weight) was collected from each plant for assessment of AM fungal structures. Root samples were cleared using 10% potassium hydroxide and stained in a trypan blue solution to visualize fungal structures (Phillips & Hayman 1970). Percentage colonization of AM fungal hyphae, vesicles, and arbuscules were scored out of 100 root intersections at 200× total magnification using a compound microscope (McGonigle et al. 1990). Total percentage mycorrhizal colonization of roots was calculated by dividing the number of intersections with AM fungi present by the total number of root intersections assessed and multiplying by 100.

The MIP assay was conducted from August to October 2021 at Washington State University (Richland, WA, U.S.A.). Plants received natural lighting and environmental conditions were as follows: average daily high humidity 62%, average daily low humidity 20%, average daily high 37°C, and average daily low 21°C. Plants were watered to capacity daily.

Statistical Analysis

All statistical analyses were done in R (version 4.1.2, R Core Team 2022).

Is Abundance of Viable AM Fungal Propagules Altered in Soil Invaded by *V. dubia*?. We used a two-way analysis of variance (ANOVA) to compare the mean AM fungal colonization (%) in assay plants across sites and invasion levels, as well as their interaction using the "aov" function. Pairwise differences among groups were determined using a post hoc Tukey's honest significant difference (HSD) test with $\alpha = 0.05$ using

"TukeyHSD." We confirmed that data met assumptions of homoscedasticity and normality by running Levene's test on all groups using "leveneTest" from the cars package (Fox & Weisberg 2019) and running Shapiro–Wilks test on the ANOVA residuals using "shapiro.test."

Is Plant Community Composition, Species Richness, and/or Diversity Impacted by Invasion of *V. dubia*?. Because we set up our field plots based on the presence of two focal species, *V. dubia* and *P. spicata*, we assessed the impact of site and invasion level on plant community composition, species richness, and diversity in two ways—one dataset with the focal plant species removed (main manuscript) and one dataset with the focal species remaining (Supplement S1). In our community analysis, we used several functions from the vegan package (Oksanen et al. 2022). First, we standardized our percent cover data to relative abundance using the "decostand" with method "total." To visualize the communities, we ran two-dimensional nonmetric multidimensional scaling with Bray–Curtis dissimilarities using "metaMDS." The ordinations fit well, with stress of 0.19 and 0.17 and nonlinear r^2 of 0.96 and 0.96 for the datasets that excluded and included focal plant species, respectively. Next, we tested if mean composition differed among sites and/or invasion levels using Bray–Curtis dissimilarities and permutational multivariate ANOVA with sequential sums of squares and 1,000 permutations (community ~ site × invasion) via the "adonis2" function. We tested equality of dispersion among community × site combinations using "betadisper." We calculated plant species richness using the "specnumber" function and the Shannon–Weiner index of plant diversity using the "diversity" function. Differences among mean richness and diversity were tested with two-way ANOVA and pairwise comparisons as described above.

How do Soil pH and OM Vary Across Invasion Levels?. To determine if soil pH and OM varied across sites and invasion levels, we used a two-way ANOVA and pairwise comparisons as described above.

Results

Is the Abundance of Viable AM Fungal Propagules Altered in Soil Invaded by *Ventenata dubia*?

We found no evidence that *Ventenata dubia* invasion consistently altered the abundance of viable AM fungal propagules ($F_{[2,18]} = 1.72, p = 0.21$; Table S4; Fig. 1). However, the abundance of viable AM fungal propagules differed by site ($F_{[2,18]} = 17.8, p < 0.001$; Table S4; Fig. 1) and was highest in soils collected from Steptoe Butte (Table S5; Fig. 1). We found weak evidence of interactions between invasion levels and sites on the abundance of AM fungal propagules ($F_{[4,18]} = 2.71$, $p = 0.06$; Table S4). Within Steptoe Butte, the abundance of AM fungal propagules varied in the way that we had predicted, with lower abundance of viable AM fungal propagules in soil inocula from Invaded plots compared to soil inocula from Native plots (Fig. 1).

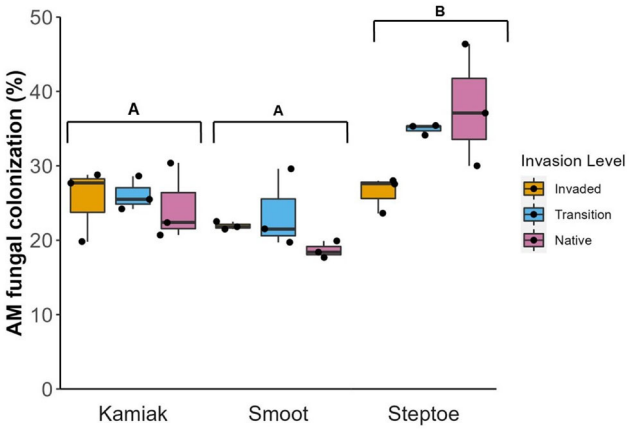


Figure 1. Results from mycorrhizal inoculation potential (MIP) assay: percentage colonization by arbuscular mycorrhizal (AM) fungi across sites and invasion levels. Box plots show distribution of AM fungal colonization, with the horizontal line representing the median, and whiskers representing minimum and maximum values. Each dot represents the average mycorrhizal colonization level from replicate greenhouse pots ($n = 10$ greenhouse pot replicates per plot) that were inoculated with live soil from a particular site and invasion level in the greenhouse experiment. Letters indicate Tukey's honest significant difference test comparing means across sites ($p \leq 0.05$).

Is Plant Community Composition, Species Richness, and/or Diversity Impacted in Communities Invaded by *V. dubia*?

When the focal plant species *V. dubia* and *Pseudoroegneria spicata* were excluded from the dataset, plant community composition varied by invasion level (pseudo-$F_{[2,18]} = 8.18, p = 0.001$; Table S6; Fig. 2) and by site (pseudo-$F_{[2,18]} = 20.35$, $p = 0.001$; Table S6; Fig. 2), with invasion level and site interactions (pseudo-$F_{[4,18]} = 2.86$, $p = 0.001$; Table S6). In addition to differences among mean communities, we also found the variance of communities differed ($F_{[8,18]} = 0.02$, $p = 0.026$). For instance, within Smoot Hill, Invaded communities were distinct from, but more variable than, Transition and Native communities (Fig. 2).

Plant species richness did not differ among invasion levels ($F_{[2,18]} = 2.10$, $p = 0.15$; Table S7), however it did differ among sites ($F_{[2,18]} = 3.92, p = 0.04$; Table S7). Plant species richness was higher in Kamiak Butte (mean = 11.7 ± 1.4 standard error) than in Steptoe Butte (mean 9.3 ± 0.8 standard error; Tukey's HSD $p = 0.03$; Table 1) but did not differ from Smoot Hill (mean 9.9 ± 0.6 standard error; Tukey's HSD $p = 0.13$; Table 1), and there were no differences in plant species richness between Steptoe Butte and Smoot Hill (Tukey's HSD $p = 0.80$). There were no site by invasion level interactions on plant species richness ($F_{[4,18]} = 2.44, p = 0.08$; Table S7).

Plant diversity differed among invasion levels ($F_{[2,18]} = 8.50, p = 0.002$; Table S7) and sites ($F_{[2,18]} = 4.37$, $p = 0.03$; Table S7), but there were no site by invasion level interactions ($F_{4,18} = 1.16, p = 0.36$; Table S7). Plant diversity

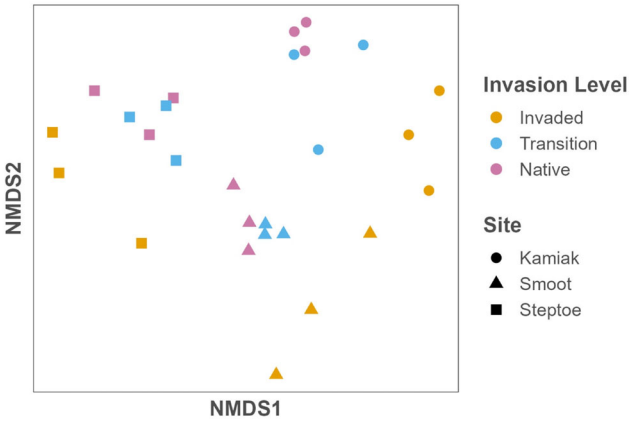


Figure 2. Plant communities by site and invasion level. Nonmetric multidimensional scaling (NMDS) showing plant communities (with focal plant species *Ventenata dubia* and *Pseudoroegneria spicata* removed) at each site (Kamiak Butte, circles; Smoot Hill, triangles; Steptoe Butte, squares) and invasion level. Stress = 0.19; nonlinear $r^2 = 0.96$.

was higher in Transition plots compared to Invaded or Native plots (Tukey's HSD $p = 0.01$, $p = 0.003$, respectively; Table 1). Plant diversity was higher in Kamiak Butte than in Steptoe Butte (Tukey's HSD $p = 0.02$; Table 1) but not compared to Smoot Hill (Tukey's HSD $p = 0.70$; Table 1). Results for the dataset with the focal plant species included are shown in Supplement S1, Tables S8–S10; and Figure S2.

How Do Soil pH and OM Vary Across Invasion Levels?

Soil pH differed among invasion levels depending on site ($F_{[4,18]} = 4.81$, $p = 0.008$; Table S11). Within Steptoe Butte, soil pH was higher in Invaded and Transition plots compared to the Native plots (Tukey's HSD $p < 0.001$, $p = 0.03$, respectively; Fig. 3). OM also differed among invasion levels, depending on site ($F_{[4,18]} = 3.85$, $p = 0.02$; Table S11; Fig. 4). At Smoot Hill, OM was higher in Invaded plots compared to Native and Transition plots (Tukey's HSD $p = 0.01$, $p = 0.05$, respectively; Fig. 4).

Discussion

Ventenata dubia Invasion Does Not Consistently Reduce the Abundance of Viable AM Fungal Propagules in Soil

The abundance of viable AM fungal propagules at Steptoe Butte was lower in Invaded plots than Transition or Native plots, as we had predicted. However, this pattern did not hold for Kamiak Butte or Smoot Hill. The lack of a consistent trend was unexpected given that *Ventenata dubia* is only weakly colonized by AM fungi (Cheeke et al. n.d. unpublished data; Luedloff et al. 2022) and has a neutral growth response to AM fungal inoculation (Cheeke et al. n.d. unpublished). *V. dubia*'s limited association with AM fungi does not necessarily mean that it suppresses AM fungi. Indeed, not all invasive grasses alter AM fungal abundance (e.g. Bunn et al. 2015; Cheeke et al. 2021). Perhaps the differences in AM fungal abundance at Steptoe Butte compared to the other sites had less to do with *V. dubia* specifically, and more to do with the other plant species in the plots. Steptoe Butte was the only site containing the native shrub, *Eriogonum heracleoides* (Wyeth buckwheat), and its cover was highest in the Native plots. This shrub has a strong positive growth response to local field soil containing AM fungi (Cheeke et al. n.d. unpublished data) and may be a good host plant. The invasive annual grass, *Bromus tectorum*, which is known to reduce AM fungal abundance (Busby et al. 2012; Lekberg et al. 2013), was also in greater abundance at Steptoe Butte compared to the other two sites, particularly in the Invaded sites with raw cover counts reaching 95%. At Steptoe Butte, AM fungi may have been promoted by *E. heracleoides* at the Native and Transition plots and depressed by *B. tectorum* at the Invaded plots. The Steptoe Butte plant community may have been different because of different management practices. The Steptoe Butte plots were on private property, but within a state park and accessible to public foot traffic; foot traffic is a vector for introducing invasive plants. In contrast, the Smoot Hill plots

Table 1. Average percent cover of focal native and invasive plant species, plant species richness, and plant diversity for each invasion level at each site. The focal species were the invasive annual grass, *Ventenata dubia*, and the native bunchgrass, *Pseudoroegneria spicata*. Plots containing greater than 30% relative cover of *V. dubia* were labeled as Invaded, plots containing 10–20% relative cover of *V. dubia* were labeled as Transition, and plots containing less than 5% relative cover of *V. dubia* were labeled as Native. Relative percent cover of the focal plant species, as well as species richness and diversity of the non-focal plants (dataset with the focal plant species removed), were averaged across the three replicates plots for each invasion level (Native, Invaded, and Transition) within each site (Smoot Hill, Steptoe Butte, and Kamiak Butte, Pullman, WA, U.S.A.; 27 plots in total, $n = 3$ replicate plots of each invasion level per site). Mean and ± standard error are reported.

Site	*Focal species*	*Invasion level*	*% Cover of* V. dubia	*% Cover of* P. spicata	*Species richness*	*Shannon diversity*
Kamiak	*V. dubia*	Invaded	33.2 (1.0)	0.2 (0.1)	13.0 (1.2)	1.59 (0.09)
	Both	Transition	17.0 (1.6)	18.5 (2.7)	11.3 (1.8)	1.74 (0.05)
	P. spicata	Native	0.3 (0.3)	35.4 (3.0)	10.7 (1.2)	1.37 (0.07)
Smoot	*V. dubia*	Invaded	35.7 (6.3)	2.0 (1.0)	9.7 (0.7)	1.47 (0.06)
	Both	Transition	13.3 (2.5)	12.5 (1.8)	9.0 (0.0)	1.67 (0.05)
	P. spicata	Native	0.0	12.9 (6.4)	11.0 (1.0)	1.41 (0.17)
Steptoe	*V. dubia*	Invaded	36.9 (3.0)	0.1 (0.1)	11.0 (1.5)	1.14 (0.12)
	Both	Transition	15.9 (4.4)	14.3 (3.3)	10.3 (0.3)	1.58 (0.15)
	P. spicata	Native	0.0	25.0 (6.0)	6.7 (0.7)	1.31 (0.05)

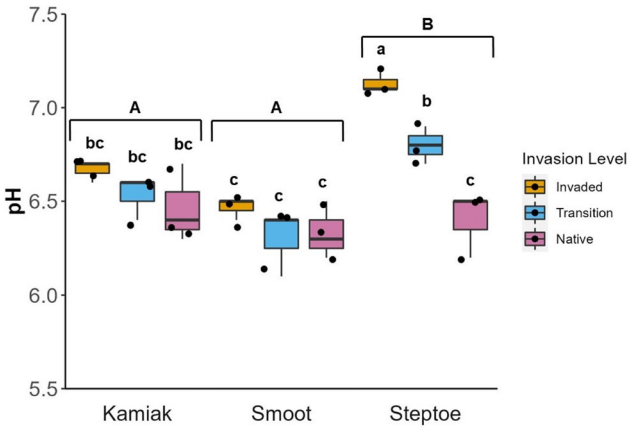


Figure 3. Soil pH across invasion levels and sites. Box plots show distribution of soil pH in each invasion level at each site (Kamiak Butte, Smoot Hill, and Steptoe Butte), with the horizontal line representing the median, and whiskers representing minimum and maximum values. Each dot represents the soil pH level of each plot in each field site. Capital letters indicate Tukey's honest significant difference test comparing means across sites, lowercase letters indicate Tukey's honest significant difference test comparing means across site × invasion levels ($p \leq 0.05$).

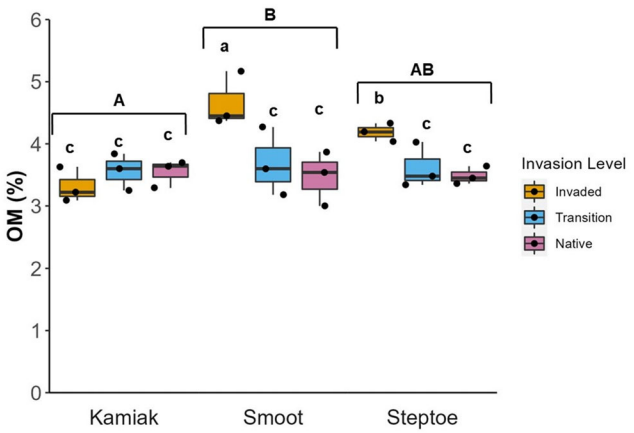


Figure 4. Organic matter (OM) across invasion levels and sites. Box plots show distribution of OM (%) in each invasion level at each site (Kamiak Butte, Smoot Hill, and Steptoe Butte), with the horizontal line representing the median, and whiskers representing minimum and maximum values. Each dot represents the OM level (%) of each plot in each field site. Capital letters indicate Tukey's honest significant difference test comparing means across sites, lowercase letters indicate Tukey's honest significant difference test comparing means across site × invasion levels ($p \leq 0.05$).

were on a university-owned preserve and Kamiak Butte plots were on private conservation property. We found no record of when *V. dubia* first entered each site, and they may have had different invasion timelines. Given that longer periods of invasion are more likely to negatively impact AM fungi than shorter periods of invasion (Edwards et al. 2022), if Kamiak and Smoot Hill plots are in earlier stages of invasion than Steptoe Butte, then *V. dubia* impacts may not be measurable yet. In any case, the lack of a consistent response to *V. dubia* levels suggests that AM fungal propagules are not directly repressed by *V. dubia*. More work is needed to understand whether this invader would have an impact given additional time, or if the impacts observed at Steptoe Butte are due to factors other than *V. dubia*.

Invasion by *V. dubia* Altered Plant Community Composition, but Did Not Reduce Species Richness or Diversity

Invasions by *V. dubia* consistently shifted plant community composition, but in different ways for each site. The dominant native bunchgrasses (*Pseudoroegneria spicata* and/or *Festuca idahoensis*) were similar in the Native plots across sites, but the co-invading annual grasses differed at each site, with *B. tectorum* at Steptoe Butte, *B. japonicus* (Japanese brome) at Smoot Hill, and *Taeniatherum caput-medusae* (medusahead) at Kamiak Butte. Our data also provide some evidence that the native buckwheat, *E. heracleoides* may be particularly sensitive to invasion by *V. dubia* and/or other invasive annual grasses as its relative abundance dropped from 14% relative cover in the Native plots to 1% relative cover in the Invaded plots at Steptoe Butte.

Even though plant communities shifted, *V. dubia* invasion did not reduce total plant species richness or diversity. Our results contrast with Jones et al. (2018) who found that total plant species richness, total plant diversity, as well as native plant species richness, was lower in plots with greater than 12.5% *V. dubia* cover compared to plots with no *V. dubia* cover. The average relative percent cover of *V. dubia* in our study ranged from 33 to 37% in the Invaded plots, 13 to 17% in the Transition plots, and 0 to 0.3% in the Native plots, so we had expected to see similar patterns. However, while measuring percent canopy cover, we observed that *V. dubia* established in the interstitial spaces of other plant species and thus may not have been actively competing for space with the native plants at the time of sampling. We documented the highest level of plant diversity in the Transition plots, suggesting that native plant species persist through the early stages of *V. dubia* invasion.

Soil pH and OM Were Higher in Invaded Plots

Soil pH and OM differed among invasion levels, but results varied within each site. At Steptoe Butte, soil pH was higher in the Invaded plots compared to the Transition and Native plots but there was no difference in soil pH among invasion levels within Kamiak Butte or Smoot Hill. At Steptoe Butte, there was an inverse relationship between soil pH and MIP mycorrhizal colonization levels, in which the abundance of viable AM fungal propagules was lowest in soil inocula collected from plots with the highest soil pH (Invaded plots mean pH = 7.1), followed by Transition plots (mean pH = 6.8), and highest in Native plots (mean pH = 6.4). This result is consistent with other studies that showed a decrease in mycorrhizal colonization at higher soil pH levels (e.g. Van Aarle et al. 2002). Soil pH can drive mycorrhizal fungal community composition (Hazard et al. 2013; Davison et al. 2021; Bodenhausen et al. 2023) and can affect AM fungal communities and plant growth directly by modifying enzymatic

activities or indirectly by affecting soil nutrient availability (Haynes 1990; Marschner 1995). Our results at Steptoe Butte are consistent with studies showing that some invasive plant species (e.g. *Centaurea stoebe*, *Euphorbia esula*) are associated with increased soil pH (Gibbons et al. 2017; Soti et al. 2020). Plants can alter soil pH through root exudates and the production of leaf litter that varies in quality, quantity, and/or composition (Weidenhamer & Callaway 2010). OM also differed among invasion levels and was higher in Invaded plots compared to Native plots at Steptoe Butte and Smoot Hill. The invasive annual grass, *B. tectorum*, has been associated with increased OM (Gibbons et al. 2017). This could partially explain the results we observed at Steptoe Butte which had a higher relative abundance of *B. tectorum* compared to the other sites. Higher OM in the Invaded plots could also be due to the low-quality leaf litter generated by *V. dubia*. The leaf litter of *V. dubia* is high in silica (Pavek et al, 2011), and because it is relatively slow to decompose, can accumulate on the soil surface and in the upper layers of soil (DiTomaso et al. 2013). However, whether *V. dubia* preferentially invades areas of higher soil pH and higher OM, or whether its invasion leads to higher soil pH and OM, remains an open question and could be a fruitful area for future research.

Impacts of Invasion by *V. dubia* on AM Fungi and Native Grasslands Are Still Being Revealed

Our research provides evidence that AM fungal propagules may not be reduced by *V. dubia* directly, but instead shift plant communities which then alter the abundance of AM fungal propagules. We also found that the canopy cover of *V. dubia* was positively associated with higher soil pH and soil OM. This finding adds to the growing list of ecological characteristics that have been identified with *V. dubia* invasion, including shallow, clay soils, and soils with low levels of potassium and phosphorus (Jones et al. 2018; Jones et al. 2020). Studies investigating the effect of time since *V. dubia* invasion on soil biota (whether through direct or indirect means) and the impact of *V. dubia* invasion on AM fungal community composition via molecular sequencing will be helpful in understanding the broader impacts of *V. dubia* invasion on both above and belowground communities.

Understanding how invasive plant species, such as *V. dubia*, impact AM fungi is important for informing restoration efforts. For example, if AM fungi in a restoration site have been negatively impacted by invasive species, microbial inoculations may be beneficial to reestablish the AM fungal communities that are important for the survival and growth of some native plant species. On the other hand, if there is no detectable effect of an invasive plant species on AM fungi, investing the time, resources, and effort in microbial inoculations at that site may not be necessary. Incorporating the use of small-scale MIP assays into a restoration management plan could help to determine if soil microbial communities have been disrupted (e.g. by invasion, tillage, and fertilizer) and whether inoculations may be beneficial. Taken together, our findings contribute to the growing knowledge about the direct and indirect impacts of *V. dubia* invasion on above and belowground communities and provides additional evidence that the abundance of AM fungal propagules are not always negatively affected by invasion.

ACKNOWLEDGMENTS

The authors thank J. Folwell, I. Clark, and Washington State University for allowing us to conduct our field survey and make collections on their property, J. Jensen for knowledgeable insight into the Palouse prairie and *Ventenata dubia*, as well as Dr. R. Learn for guidance with statistical analysis. We thank L. McKinley Nevins, R. Berner, A. Winward, E. Arredondo, G. Wickenhagen, M. Lueck, R. Huachambé, M. Nunnelee, and other Cheeke lab members who helped with the project. This work was supported by an Elling Endowed Graduate Fellowship to A.A.S. and Washington State University Tri-Cities new lab start-up funds to T.E.C. We thank R. D. Evans, E. Crespi, and members of the Cheeke lab for valuable feedback on an earlier version of this manuscript.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Plant community data.
Table S2. Field soil data.
Table S3. Soil nutrient data from the autoclaved sand:soil mix (1:1 by volume) used as the background substrate in the greenhouse experiment.
Table S4. ANOVA results for average percentage arbuscular mycorrhizal (AM) fungal colonization, with site and invasion levels.
Table S5. Mean percent colonization of arbuscular mycorrhizal (AM) fungal structures in each site and invasion level.
Table S6. PERMANOVA results for dataset with focal plant species removed.
Table S7. ANOVA results for plant species richness and total Shannon diversity (*H*) by site and invasion level, with focal plant species removed.
Table S8. PERMANOVA results for plant dataset with focal plant species included.
Table S9. ANOVA results for total plant species richness and total Shannon diversity (*H*) as affected by site and invasion level, with focal plant species included.
Table S10. Average relative percent cover of focal native and invasive plant species, and average plant species richness and plant diversity shown for each invasion level at each site.
Table S11. ANOVA results for soil pH and OM % with site and invasion level.
Figure S1. Palouse Prairie field sites.
Figure S2. NMDS of plant communities by site and invasion level.

Coordinating Editor: Valter Amaral

Received: 10 March, 2023; First decision: 9 May, 2023; Revised 23 August 2023 and 24 July 2023; Accepted: 23 August, 2023