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Forest community structure and composition following containment treatments for the fungal pathogen oak wilt

Scott R. Abella · **LaRae A. Sprow** · **Timothy W. Walters** · **Timothy A. Schetter**

Abstract In temperate oak forests in Ohio, USA, we examined variability in forest communities within containment treatment sites for oak wilt (*Bretziella fagacearum*), a fungal pathogen lethal to susceptible oak species. Containment treatments included quarantine lines in soil for limiting belowground fungal spread and sanitation cutting of 1–3 mature black oak (*Quercus velutina*) trees within oak wilt infection patches. At 28 sites, we compared tree structure and understory plant communities across a gradient of 1- to 6-year-old treatments and reference forest (untreated and without evidence of oak wilt). While oak seedlings were abundant, oak saplings (1–10 cm in diameter) were absent. In contrast, many native understory plant community measures were highest in oak wilt treatments. Plant species richness 100 m^{-2} doubled in treatments, regardless of age, compared with reference forest. Plant cover increased with treatment age, with 6-year-old treatments exhibiting 5 × more cover than reference forest. Non-native plants averaged only a small proportion (< 0.12) of cover across treatments and reference forest. Variability in understory communities was mostly predictable using treatment age, tree canopy cover, and geographic location, as 20 of 25 understory measures had at least 72% of their variance modeled. While oak wilt treatments did not facilitate oak regeneration nor many conservation-priority species of open savanna-woodland habitats, the treatments did diversify and increase cover of native understory communities with minimal invasion of non-native plants.

Keywords Herbaceous layer · Pest · *Quercus velutina* · Resilience · Secondary invasion · Understory

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Introduction

Fungal pathogens are among the most invasive, damaging, and difficult to manage forest pests (Liebhold et al. 2017). Some of numerous examples include

chestnut blight (*Cryphonectria parasitica*), Dutch elm disease (*Ophiostoma ulmi* and *O. novo-ulmi*), beech bark disease (*Neonectria faginata*), and butternut canker (*Sirococcus clavigignenti-juglandacearum*), all of which have altered populations of host tree species and changed forest structure and function (Loo 2009). Fungal pathogens can pose unique management challenges given their spread belowground, cryptic nature of initial infection resulting in new invasions often undetected until trees are symptomatic and the pathogen already widespread, and complexities with implementing containment treatments such as those requiring limiting root connectivity (Roberts et al. 2020). Moreover, as the containment treatments often involve soil disturbance to attempt to slow fungal spread belowground and sanitation cutting of infected trees, resulting in rapid formation of canopy gaps, combinatorial influences of both the pathogen and containment measures could trigger forest change (Dunstan et al. 2010). Several authors have noted that effects of fungal pathogens and containment efforts on forest ecosystems beyond the pathogen and host tree are generally not well understood, particularly concerning long-term forest changes (Loo 2009; Wardle and Peltzer 2017; Wingfield et al. 2017). Further knowledge of changes in key features of forest communities such as in tree regeneration, species diversity, understory plant communities including floral resources provided to pollinators, non-native plants, and spatial heterogeneity could improve understanding effects of invading fungal pathogens and associated containment treatments. In turn, this could assist understanding forest resiliency, abilities of aftermath forests to provide critical ecological services, and potential needs for managing secondary invasions of non-native plants or for restoration activities of native forests (Cale et al. 2017; Valachovic et al. 2017; Wardle and Peltzer 2017).

Here, we focused on this knowledge gap of forest community conditions in aftermath forests following a combination of a fungal pathogen outbreak (oak wilt, *Bretziella fagacearum*) and implementation of a containment treatment protocol in a temperate oak (*Quercus*) forest susceptible to pathogen invasion (Menges and Kuntz 1985; Juzwik et al. 2011; Meunier et al. 2019). In reviewing the potential origin and invasion history of oak wilt, Juzwik et al. (2008) reported that genetic analyses suggest that the pathogen originated in South or Central America or

Mexico. Given the cryptic nature of many plant pathogens, uncertainties in the origin and potential invasion history are not uncommon for fungal pathogens (Liebhold et al. 2017). Oak wilt was first documented in the United States in 1944, in Wisconsin, and by 2009, it was reported in 24 eastern states (Koch et al. 2010). By 2020, oak wilt occurred within 1 km of the Canadian border, prompting an ecological distribution and economic impact scenario analysis if oak wilt were to invade Canada (Pedlar et al. 2020). Highlighting the pathogen's potential spread and impacts within landscapes, Pellegrini et al. (2021) reported that within the 2,200-ha Cedar Creek Ecosystem Science Reserve in Minnesota, USA, oak wilt began spreading exponentially in 2010–2018 and increased oak mortality by 765%. Species of the red oak group are most susceptible to oak wilt and can die within weeks of infection (Juzwik et al. 2011). Oak wilt spreads in the red oak group two main ways: underground when root grafts connect trees, and overland when fungal-feeding beetles or other vectors transport the fungus from infected trees to wounds on other trees (Gibbs and French 1980).

Oak wilt management activities have focused on limiting long-distance spread (e.g., quarantining contaminated wood), avoiding wounding trees, and local containment (Wilson 2005). A main containment protocol includes severing root connectivity around infected trees followed by sanitation cutting of infected trees (Bruhn and Heyd 1992; Koch et al. 2010). This containment treatment can at least locally slow oak wilt spread (Juzwik et al. 2010). However, forest community changes–including tree regeneration, native understory communities, and potential for non-native plant invasions–following the combination of oak wilt-triggered canopy gap formation from oak loss and the implementation of containment measures are uncertain.

Our study sought to expand upon the small literature on oak wilt effects on community composition in eastern North American temperate forests (Tryon et al. 1983; Collada and Haney 1998; Pellegrini et al. 2021) by examining aftermath forest communities following oak wilt infestation and containment protocols. Knowledge of pest-aftermath and quarantined forest conditions is fundamental to identifying influences of increasingly expanding forest pest invasions and potential mitigation strategies for forest resilience (Liebhold et al. 2017; Abella 2018;

Roberts et al. 2020). The specific objective of our study was to assess tree structure; understory composition including metrics such as native diversity, modeled floral resources for pollinators as a forest function, and non-native plant abundance; and spatial diversity in forest communities along an age gradient of containment treatments for oak wilt. We assessed the following hypotheses of variation in forest communities across 1- to 6-year-old oak wilt containment treatments and as compared with reference forest without evidence of oak wilt: (i) tree regeneration would be more prevalent in treatments and increase with treatment age; (ii) understory plant species richness, cover, and modeled pollinator resources would be highest in treatments and increase via colonization by light-demanding, short-lived, or ruderal species accompanied by persistent increases in non-native plants; (iii) understory species composition would display persistent multivariate differences between treated areas and reference forest; and (iv) spatial diversity in post-treatment forest conditions would be associated with treatment age, residual tree canopy cover, and site location.

Methods

2.1 Study area

The study area was the 200-ha Wildwood Preserve (41°40′53″N, − 83°40′26″W), administered by Metroparks Toledo and within the 45,000-ha Oak Openings region in northwestern Ohio, USA (Schetter et al. 2013). The temperate climate, recorded at the Toledo Airport 15 km southwest of the study area, averages 86 cm/year of precipitation and daily temperatures of − 8/0 °C (low/high) in January and 17/29 °C in July (1955 through 2019; Midwestern Regional Climate Center, Champaign, IL, USA). Topography is flat to undulating. Sandy upland soils are classified as Udipsamments of the Ottokee and Oakville series (Stone et al. 1980). Early 1800s land survey records indicated that uplands in the preserve, like much of the region, contained open oak woodland, savanna, and prairie largely maintained for millennia by anthropogenic-ignited fires (Brewer and Vankat 2004). Contemporary vegetation in the preserve consists of predominately century-old oak forest, mostly black oak (*Quercus velutina*) with some white

oak (*Quercus alba*; Table 1). The forests were not known to have experienced fire since at least 1975 when the preserve was established. Land use surrounding the preserve is urban-suburban.

Oak wilt treatments

It is uncertain how long oak wilt was present in the preserve, but by the mid-2010s, helicopter- and ground-based surveys identified oak tree individuals and groups (usually 1–3 trees) incipiently declining or dead after displaying symptoms consistent with oak wilt infection (Koch et al. 2010). Based on confirmation that tissue samples from symptomatic trees tested positive for oak wilt (C. Wayne Ellett Plant and Pest Diagnostic Clinic, Ohio State University, Reynoldsburg, Ohio, USA), we identified infected trees using diagnostic symptoms (e.g., necrosis on wilting leaves at the margins and tip progressing inward to the main vein and leaf base in black oak) and signs (e.g., fungal evidence on the inner bark or outer wood) of oak wilt infection (Juzwik and Appel 2016). In 2015, Metroparks Toledo began mapping infected and declining oak trees, which were scattered throughout the preserve then and in subsequent years, and implementing a treatment protocol for oak wilt containment (Juzwik et al. 2010). The protocol's first step was using a vibratory plow blade, mounted on a Ditch Witch RT125Q Quad Ride-On Tractor (Charles Machine Works, Inc., Perry, OK, USA) to establish a containment line 1.5 m deep and 4 cm wide in the soil. The line was designed to sever root connectivity 30 m from, and encircling, the scattered pockets of 1–3 declining oak trees. The second step was sanitation cutting of infected and declining oak trees within the containment lines. Trees were cut to within 10 cm above ground level using chain saws. Tree boles were chipped or the cambium layer was removed and chipped, allowing for other uses of the wood, and slash was chipped (Wilson 2005). Wood and chips of all cut trees were removed from the treatment areas. Except for one white oak, all the trees removed were black oak, likely reflecting its proportional dominance in the forest and greater susceptibility to oak wilt (Koch et al. 2010).

Table 1 Vegetation characteristics of 28 study sites in 1- to 6-year-old containment treatment areas for oak wilt and untreated reference forest without oak wilt in oak forests in northwestern Ohio, USA. There were 7 sites in each oak wilt treatment and in reference forest. The focal *Quercus velutina* was the centrally located tree removed during sanitation cutting in treatment areas. The first three understory species are natives with the most cover followed by non-natives with the most cover. Higher values of the floristic quality index indicate sites with conservative species typifying high-quality natural habitats (Andreas et al. 2004)

	1 year	3 year	6 year	Forest
Trees		Mean ± SEM (range)		
Diameter focal *Quercus velutina* (cm)	59±7 (29-90)	73±7 (45-100)	67±12 (35-130)	66±9 (45-107)
Canopy cover (%)	48±6 (22-67)	51±7 (23-77)	51±9 (23-78)	93±1 (90-97)
Basal area (m^2 ha^{-1})	18±5 (1-36)	18±5 (5-43)	28±11 (1-61)	51±10 (32-99)
Understory				
Carex pensylvanica cover (%)	8±2 (0.1-15)	23±10 (0.5-80)	30±11 (0-80)	8±4 (0.1-30)
Rubus allegheniensis cover (%)	2±1 (0-8)	6±4 (0-30)	24±12 (0.5-90)	0.1±0.1 (0-0.1)
Prunus serotina cover (%)	1±1 (0.1-3)	11±5 (1-35)	10±4 (0.3-25)	2±1 (0.3-5)
Euonymus alatus cover (%)	1±1 (0-4)	0.2±0.1 (0-0.5)	0.2±0.1 (0-1)	0.8±0.4 (0.1-3)
Celastrus orbiculatus cover (%)	0.3±0.1 (0.0-1.0)	0.2±0.1 (0.1-0.5)	0.9±0.4 (0.1-3)	0.4±0.3 (0-2)
Frangula alnus cover (%)	0.1±0.1 (0-0.3)	0.2±0.1 (0-1)	0.6±0.6 (0-4)	0.1±0.1 (0-0.1)
Shannon diversity index	2.3±0.2 (1.9-3.6)	2.0±0.2 (1.4-2.6)	1.6±0.2 (0.8-2.6)	1.9±0.2 (1.1-2.8)
Floristic quality index	16±1 (13-20)	18±1 (16-22)	18±1 (15-23)	16±1 (12-20)
Native species (proportion)	0.79±0.03 (0.67-0.90)	0.83±0.02 (0.74-0.90)	0.88±0.01 (0.84-0.92)	0.83±0.02 (0.78-0.92)
Native cover (proportion)	0.89±0.03 (0.73-0.98)	0.97±0.01 (0.95-0.99)	0.97±0.02 (0.89-0.99)	0.88±0.03 (0.77-0.98)
Full-partial sun species (proportion)	0.36±0.04 (0.23-0.50)	0.32±0.03 (0.22-0.42)	0.36±0.03 (0.28-0.48)	0.14±0.03 (0-0.25)

Data collection

For sampling, we defined oak wilt treatment sites as containing 1–3 sanitation-cut oaks (all of which were mature trees $\geq$ 29 cm in diameter) encircled by a containment line (Table 1). The age of treatments was designated as the growing season year following the dormant season completion of treatments and included 2015, 2018, and 2020. These years represented 6-, 3-, and 1-year-old treatments in summer 2020 during sampling. We sampled 28 sites, with seven sites in each treatment age and in reference oak forest, untreated and without evidence of oak wilt. All available treatment sites for each age were sampled, except that seven of the nine available 6-year-old treatments were randomly selected so that samples were equal among ages. Sample sites in reference forest were located using randomly generated coordinates. At each site, a circular 100-m^2 plot (11.28 m in diameter) was established. Treatment plots were centered on the stump of a sanitation-cut oak tree. If a treatment plot contained multiple sanitation-cut trees, it was centered on the largest stump. The 100-m^2 plot size was selected to fit underneath the 100–200-m^2 canopy gaps from the removal of 1–3 mature trees encircled by a containment line. In reference sites, plots were centered on the southeastern edge of the trunk of a mature, living oak tree nearest the random coordinates. Plots among treatment ages and reference forest were interspersed and the average between-plot distance was 0.3 km (maximum = 1.0 km).

Between 24 June and 25 July, during peak plant cover in summer 2020, we inventoried trees (stems $\geq$ 1 cm in diameter at a height of 1.4 m for tree species) and understory communities (vascular herbs, shrubs, and stems < 1 cm in diameter at 1.4 m for tree species) in each plot. We recorded the species and diameter at 1.4 m of each tree in plots. In the center and in the four cardinal directions along the perimeter of each plot, we recorded tree canopy cover to the nearest 5% using a densitometer above a height of 3 m. We averaged the canopy cover measurements

into one plot value. To inventory understories, we recorded the aerial cover of each vascular plant species rooted in plots as 0.1, 0.25, 0.5, and 1% up to 1% cover, 1% intervals to 10% cover, and 5% intervals above 10% cover. Cover could not exceed 100% for a species on a plot, but could exceed 100% in sum for all species if foliage of different species overlapped. Nomenclature and classification of growth form (e.g., shrub) followed Natural Resources Conservation Service (2020). Plants were identified to species, except for some *Crataegus* and *Viola* lacking required reproductive material and thus retained as genera for analyses. Early to mid-summer (May through July) precipitation preceding and during our data collection period in 2020 was 24 cm, 92% of the 26-cm, 1955 through 2020 average for the period (Toledo Airport station; Midwestern Regional Climate Center, Champaign, IL, USA).

Data analysis

We compared tree and understory response variables across treatments (oak wilt treatment ages and reference forest) using sets of univariate and multivariate analyses. To compare tree diameter distributions (10-cm classes) across treatments, we used Epps-Singleton tests in PAST 4.02 (Hammer 2020). We analyzed mean density of trees 1–10 cm in diameter (sapling-sized regeneration layer) using analysis of covariance (ANCOVA), consisting of treatment with tree canopy cover as the covariate, in SAS 9.4 (PROC GLM). If the covariate was not significant at $P < 0.05$, it was dropped and the model re-run as a one-way analysis of variance (ANOVA) including treatment. If treatment was significant at $P < 0.05$ in ANCOVA or ANOVA, means were separated using Tukey tests. Using the same approach, we analyzed understory mean species richness (species 100 m^{-2}) and cover, and richness and cover means by species groups. Species were grouped by nativity (native, non-native to the Oak Openings region), potential life span (short: annuals and biennials; intermediate: short-lived perennials; and long: long-lived perennials), fidelity to high-quality natural habitats (ruderal with 0–2 coefficients of conservatism, facultative with 3–5, and conservative with 6–10; Andreas et al. 2004), and light requirement (herbaceous or shrub species that at maturity require full or partial sunlight or adventitious). Nativity and potential life span followed

Natural Resources Conservation Service (2020). Light requirement was derived from Mack (2009), with the adventitious category mainly including non-native species. We also analyzed, based on a previous study in the Oak Openings region, cover of plants (divided as *Rubus* spp. and other bee host plants) favored by bees as an indicator of pollinator resources (Arduser 2010). We analyzed multivariate species composition using relative cover (cover of species$_i$/$\sum$ cover of all species) and species presence-absence by calculating mean Sørensen similarities among treatments, performing permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), and computing a non-metric multidimensional scaling (NMS) ordination for presence-absence data that displayed significant variation in PERMANOVA. To identify species associated with treatments and distinguishing their species composition, we used indicator species analysis with relative cover (Dufrêne and Legendre 1997). We performed multivariate and indicator analyses in PC-ORD 7.07, and for NMS, used Sørensen distance and "thorough" settings (McCune and Mefford 1999).

To model spatial variation in understory response variables and sapling density (1–10 cm diameter), we used variance partitioning (Jaeger et al. 2017). We partitioned variance hierarchically by sequentially fitting linear mixed models including either treatment, tree canopy cover, or plot as a random variable in SAS 9.4 (PROC GLIMMIX). Plot was a random variable for this analysis assuming that study sites with oak wilt were a subset of those that could have been infected or that could be infected in the future.

Results

Trees

Tree structure did not consistently vary among treatments. For example, mean density of saplings (1–10 cm in diameter) did not differ across oak wilt treatment ages and reference forest ($F = 0.76$, $P = 0.526$). The only difference in diameter distributions among treatments for all sizes of trees was in the 3-year-old treatment (Fig. 1). This treatment's unique diameter distribution primarily resulted from numerous witch hazel (*Hamamelis virginiana*) sapling-size stems on a few plots. The largest trees (≥ 40 cm in

Fig. 1 Diameter distributions for tree species in oak wilt containment treatments (1, 3, and 6 years old) and untreated reference forest without oak wilt in oak forests of northwestern Ohio, USA. The inset graph in (d) shows Epps-Singleton tests for equality of diameter distributions among treatment ages and reference forest
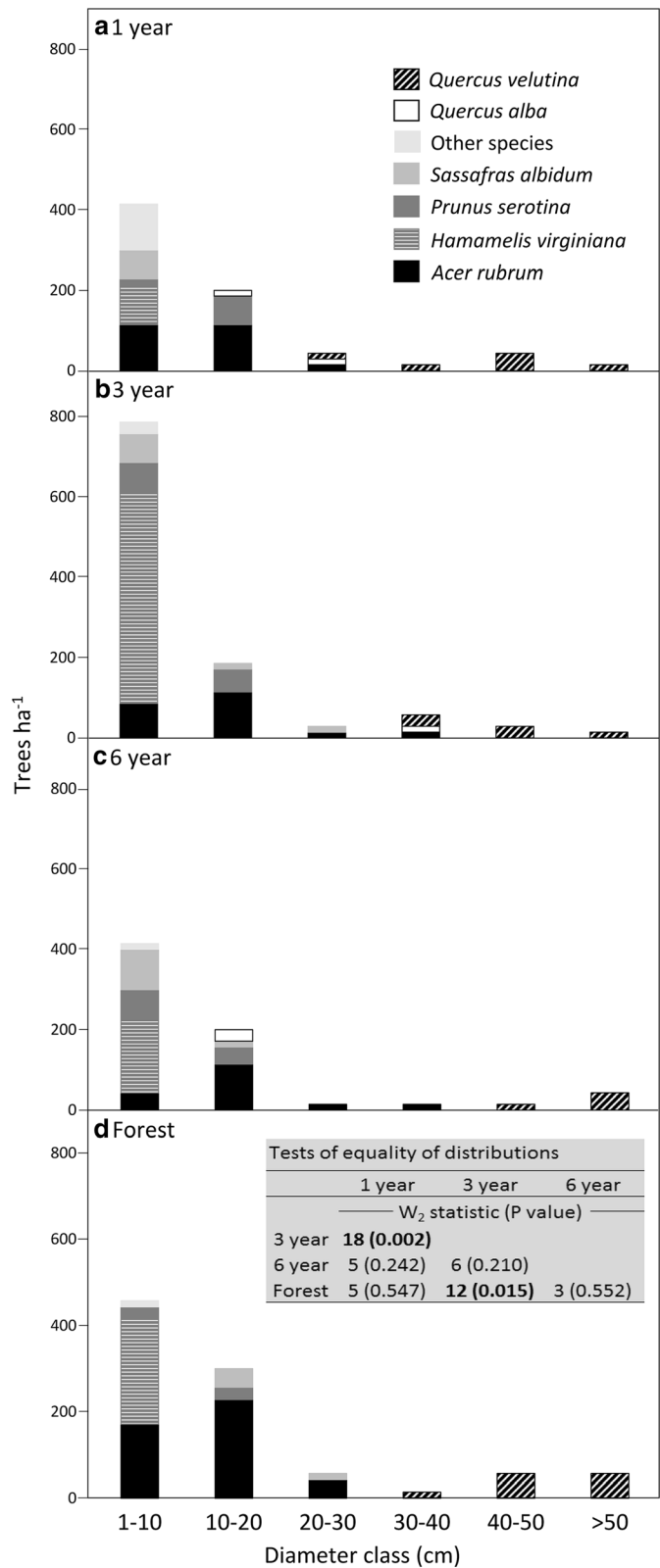
diameter) in all treatments were all black oaks, the predominant oak species among plots. The few white oaks present ranged from 16 to 33 cm in diameter. No sapling-sized (1–10 cm in diameter) oaks of either species occurred on any of the 28 plots. In addition to witch hazel, red maple (*Acer rubrum*), sassafras (*Sassafras albidum*), and black cherry (*Prunus serotina*) were abundant in the 1–10 cm diameter class.

Understory communities

Species richness was higher in oak wilt treatments of any age than in reference forest, and this increase was primarily driven by native and perennial species (Table 2, Fig. 2). Except for the 1-year-old treatment containing more non-native species than reference forest, non-native species richness did not differ significantly among treatments. Across 1- to 6-year-old treatments, native species on average proportionately comprised 79–88% of species, equaling or exceeding that in reference forest. There was a small increase in the number of short-lived species (annuals-biennials) in the 1-year-old treatment relative to reference forest, while richness of ruderal and full sun-requiring species was elevated in all treatments compared with reference forest.

The 3- and 6-year-old treatments had greater understory plant cover than 1-year-old treatments or reference forest (Fig. 3). Long-lived perennials and native species provided almost all the cover across all treatments. Cover of annuals-biennials plus short-lived perennials never averaged above 0.8%. Non-native species cover did not differ among treatments, averaging < 2.4% and proportionately comprising only 0.03–0.12 of total cover. Cover of ruderal and facultative species with respect to association with disturbance progressively increased from 1- to 6-year old treatments. Cover of species requiring full or partial sun followed a similar pattern. *Rubus* comprised most of the cover for species requiring full sun. As indicators of resources for pollinators, cover of *Rubus* in the 6-year-old and cover of other plants favored by bees in the 3-year-old treatment were significantly higher than in reference forest.

Of 142 total species detected on plots, Pennsylvania sedge (*Carex pensylvanica*), Allegheny blackberry (*Rubus allegheniensis*), and black cherry were the three natives with the most cover (Table 1, Online Resource 1). Burningbush (*Euonymus alatus*), oriental bittersweet (*Celastrus orbiculatus*), and glossy buckthorn (*Frangula alnus*) comprised the most non-native cover. Species compositional differences among treatments were generally subtle, with nearly as much variation among plots within a treatment as among treatments (Fig. 4). On average, plots within and among treatments shared about half their species. In PERMANOVA, species composition did not differ among treatments using relative cover but did using presence-absence. In follow-up pairwise comparisons, species composition of all ages of treatments differed from that of reference forest, and composition of 1- and 6-year-old treatments differed. An ordination displaying these differences indicated some separation of plots by treatments and that some species, such as wrinkleleaf goldenrod (*Solidago rugosa*) and black raspberry (*Rubus occidentalis*), correlated with community compositional variation. As only five species had indicator values ≥ 50 with P < 0.05, most species did not display fidelity to one treatment. However, there were species, such as wrinkleleaf goldenrod, that were absent from reference forest and only inhabited oak wilt treatments regardless of treatment age (Online Resource 1).

Spatial variability

Variance partitioning indicated that most variance for most variables was accounted for by treatment, tree canopy cover, and plot as a random variable representing site location (Table 2). About half (12 of 25) of understory variables had at least a third of their variance accounted for using only treatment and canopy cover. By adding the plot random variable, 20 of 25 understory variables had at least 72% of the variance modeled. Moreover, the two variables with the least variance accounted for (cover of short- and intermediate-lived species) had low cover across plots and therefore little variance to model. For tree sapling density, 17% of variance was attributable to tree canopy cover (none to treatment) and 83% to site location.

Discussion

One of the a priori hypotheses was rejected, one was only partly supported, and two were at least mostly

Table 2 Analysis of variance or covariance for effects of oak wilt containment treatments and partitioning of variance attributable to treatment, tree canopy cover, and location (site) influences on understory plant community response variables in oak forests in northwestern Ohio, USA.

| Response variable	Treatment and covariate[a]			Variance partitioning (%)			
	F	P value	r^2	Treatment	Canopy	Site	Residual
Nativity	————————— Species richness —————————						
Native	**7.80**	**< 0.001**	**0.49**	43	0	55	2
Non-native	**4.02**	**0.019**	**0.29 (0.45)**	20	15	58	7
Life span							
Short	**3.94**	**0.021**	**0.28 (0.46)**	18	17	50	15
Intermediate	0.53	0.668	0.06	0	0	55	45
Long	**7.99**	**< 0.001**	**0.50**	44	0	54	2
Distribution fidelity							
Ruderal	**8.46**	**< 0.001**	**0.51**	45	1	49	5
Facultative	2.95	0.053	0.27	18	0	76	6
Conservative	0.55	0.654	0.06	0	23	38	39
Light requirement							
Full sun	**9.50**	**< 0.001**	**0.50 (0.60)**	43	9	40	8
Partial sun	2.80	0.062	0.26	15	3	54	28
Adventitious	2.53	0.0822	0.21 (0.36)	11	14	66	9
Total	**6.34**	**0.003**	**0.44**	37	0	62	1
Nativity	———————————— Cover ————————————						
Native	**13.38**	**< 0.001**	**0.63**	58	4	38	0
Non-native	0.62	0.611	0.07	0	0	72	28
Life span							
Short	2.00	0.141	0.20	2	1	11	86
Intermediate	0.90	0.457	0.10	0	3	28	69
Long	**13.37**	**< 0.001**	**0.63**	58	4	38	0
Distribution fidelity							
Ruderal	**3.52**	**0.030**	**0.31**	22	7	71	0
Facultative	**6.64**	**0.002**	**0.45**	39	0	61	0
Conservative	2.37	0.096	0.23	13	0	78	9
Light requirement							
Full sun	**3.22**	**0.040**	**0.29**	20	9	71	0
Partial sun	**4.57**	**0.012**	**0.36**	26	1	46	27
Adventitious	0.56	0.647	0.07	0	0	77	23
Floral resources							
Rubus group	**3.08**	**0.047**	**0.28**	19	4	77	0
Bee host plants	**4.07**	**0.018**	**0.34**	25	9	60	6
Total	**13.68**	**< 0.001**	**0.63**	58	4	37	1

Bold values signify analysis of variance/covariance significant at $P < 0.05$

[a] Analysis of covariance with tree canopy cover as a covariate when it was significant at $P < 0.05$. When the covariate was not significant, the model was a one-way analysis of variance comparing oak wilt containment treatments (ages 1, 3, and 6 years old) and untreated reference forest without oak wilt. The r^2 column includes the partial r^2 of the covariate in parentheses for analysis of covariance

supported. The first hypothesis, that tree regeneration would be greatest in oak wilt containment treatments and increase with treatment age, was rejected. Density of saplings did not vary significantly among treatment ages, instead mostly varying with plot location as a random variable. The second hypothesis, that species richness and cover of understory plants would be higher in treatments than in reference forest, was only partly supported. Species richness in total and for ruderal, light-demanding species was higher, as hypothesized, in treatments compared with reference forest. However, it was perennials, rather than shorter-lived species as hypothesized, that produced most of the increase. Contrasting with the hypothesis that non-native plants would be highest in oak wilt patches, cover of non-natives was minimal. Species
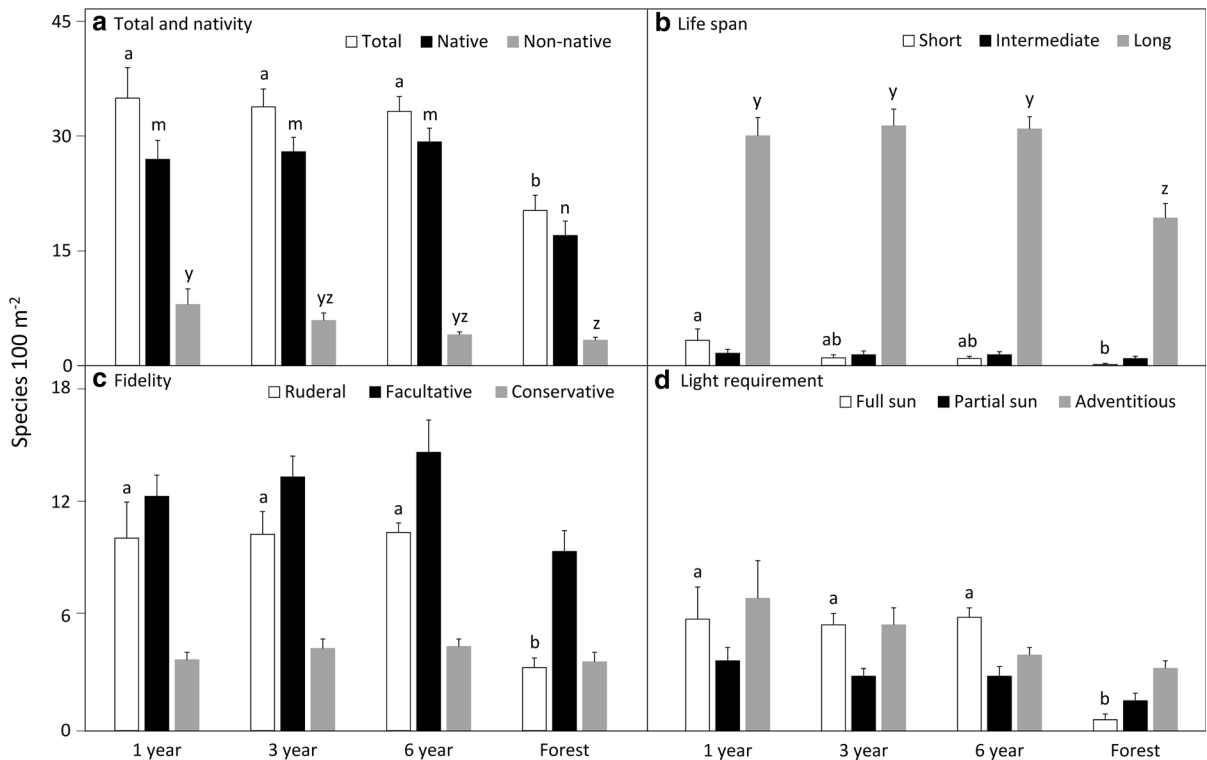
Fig. 2 Understory plant species richness in oak wilt containment treatments (1, 3, and 6 years old) and untreated reference forest without oak wilt in oak forests of northwestern Ohio, 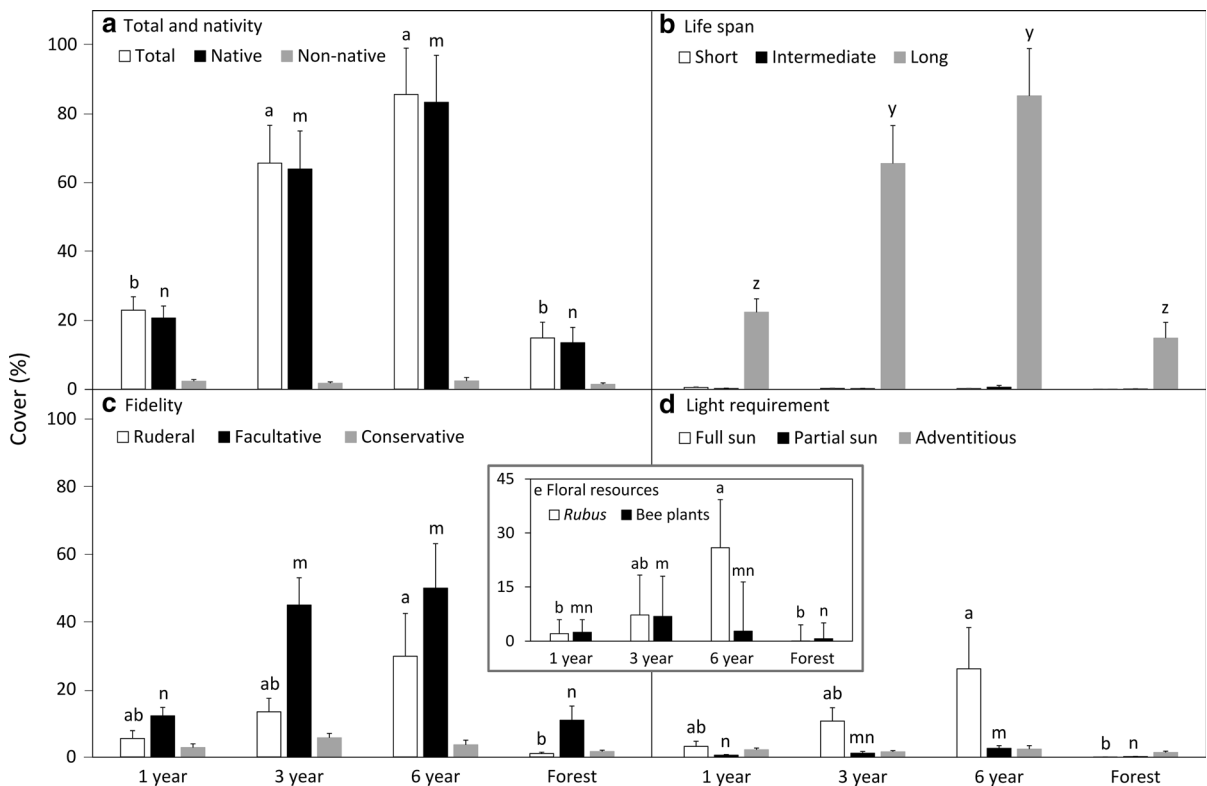USA. Within each panel and separately by response variable (each with different sets of associated letters), means without shared letters differ at $P < 0.05$. Error bars are one standard error of means

composition, for presence-absence data, differed between treatments of all ages and reference forest, thus supporting the third hypothesis. Lastly, 80% of response variables had at least 72% of their variability modeled using tree canopy cover, treatment age, and site location, largely supporting the fourth hypothesis regarding predictability of spatial variability.

Oak regeneration

None of the 28 sites across treatment ages and reference forest contained oak saplings (1–10 cm in diameter). Given that oak seedlings were ubiquitous, results were consistent with the idea that the bottleneck to oak regeneration in eastern forests is failure of seedlings to advance into saplings (Lorimer et al. 1994). In our study area, it seems oak recruitment could be limited by four main possibilities including oak wilt, herbivory, lack of fire, or insufficient size of canopy gaps. Young oak seedlings and sprouts, less than three years old, can become infected with oak

wilt, suggesting that oak wilt could limit transitions to saplings especially for more oak wilt-susceptible black oak (Jacobi and MacDonald 1980). It is noteworthy, however, that a recent study in Wisconsin reported that after clearcutting mixed-oak forests infected with oak wilt, sprouts from cut stumps survived through a 10-year study period and recruitment of new saplings also occurred (Meunier et al. 2019). Their results suggested that abundant oak regeneration can arise from oak-wilt infected sites, including in the absence of fire given open canopy conditions (Meunier et al. 2019). Severe browsing by dense populations of white-tailed deer (*Odocoileus virginianus*) can limit oak recruitment (e.g., Granger et al. 2018). However, deer populations in our study area have been relatively low (Horsley et al. 2003) since oak wilt treatments began, ranging from 6 deer/km^2 in 2016 to 3/km^2 in 2020 (Metroparks Toledo, Toledo, Ohio). Winter deer browse assessments (Abella et al. 2020b) revealed that in 2020, less than 20% of oak seedlings were severely browsed and 30%

Fig. 3 Understory plant cover in oak wilt containment treatments (1, 3, and 6 years old) and untreated reference forest without oak wilt in oak forests of northwestern Ohio, USA. Within each panel and separately by response variable (each with different sets of associated letters), means without shared letters differ at $P < 0.05$. Error bars are one standard error of means

exhibited no damage (Metroparks Toledo, Toledo, Ohio). While fire can act synergistically with canopy openings to promote oak regeneration, oak regeneration can occur without fire, particularly on dry sites, if canopy gaps are sufficiently large (e.g., Iverson et al. 2017; Lhotka et al. 2018; Abella et al. 2020c).

Canopy gap size can be a primary factor related to advancement of oak seedlings to saplings, raising a question as to the minimum gap size required for oak sapling recruitment (e.g., Heitzman and Stephens 2006; Lhotka et al. 2018). Published literature in eastern North American oak forests has generally articulated that at the extremes of gap sizes, oak regeneration is poor in the smallest gaps (e.g., single-tree gaps) while it can be profuse in large gaps of several hundred square meters to hectares (Shure et al. 2006). Thresholds conducive to oak regeneration in the middle part of this range are poorly understood, however. Gap-size thresholds vary with numerous factors in space and time such as with climate, edaphic

factors, severity of herbivory, density of competing trees, and fire frequency (Spetich 2020). While a consistent minimum gap size needed to support oak recruitment has yet to be identified, a previous study of oak wilt and published literature of oak response to other pests and silvicultural manipulations provide ranges of gap sizes at which oak recruitment could occur. For example, in West Virginia oak wilt-affected forests, Tryon et al. (1983) reported that oak saplings inhabited 8-year-old openings 0.04–1 ha in size. In New Jersey, 7-year-old gaps of at least five mature oaks killed by gypsy moths (*Lymantria dispar*) contained oak saplings, whereas closed-canopy, mature oak forests did not (Ehrenfeld 1980). Exemplifying silvicultural literature, group selection or patch cuts ≥ 200–400 m^2 triggered oak sapling growth at some sites (e.g., Rudolph and Lemmien 1976; Jenkins and Parker 1998; Shure et al. 2006; Lhotka et al. 2018). These observations suggest that in our study, it is possible that the gaps of 100–200 m^2
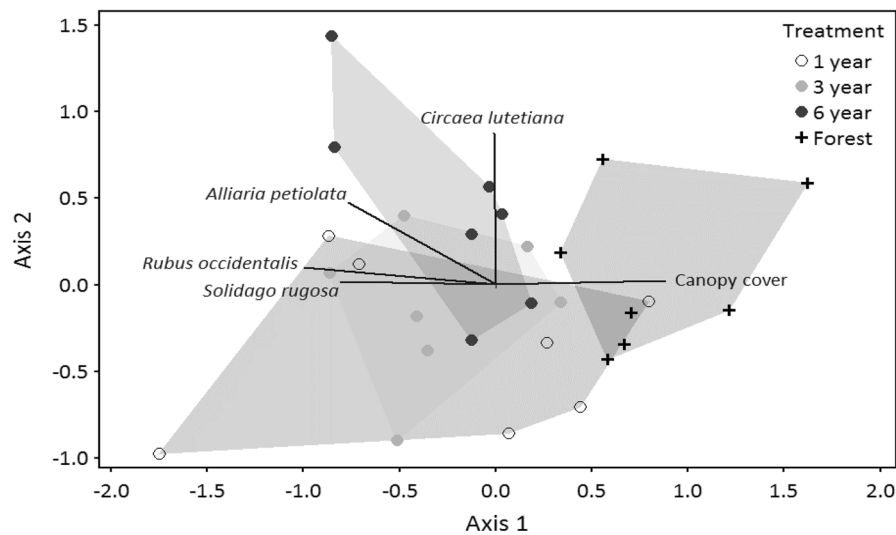
a Percent similarity

	1 year	3 year	6 year	Forest
1 year	**40** 49	52	48	48
3 year	**39**	**41** 59	58	52
6 year	**36**	**40**	**38** 57	51
Forest	**39**	**40**	**36**	**41** 59

b Permutational multivariate analysis of variance

Source	Relative cover		Presence/absence	
	Sum of squares	F (P value)	Sum of squares	F (P value)
Treatment	0.9	1.48 (0.088)	0.8	2.53 (< 0.001)
Residual	4.7		2.4	
Total	5.6		3.2	

c Presence/absence ordination

Fig. 4 Understory plant species composition in oak wilt containment treatments (1, 3, and 6 years old) and untreated reference forest without oak wilt in oak forests of northwestern Ohio, USA. **a** Sørensen similarity based on relative cover (lower triangle, bolded) and presence-absence data (upper triangle). **b** Test of no difference in species composition comparing treatments and reference forest. **c** Non-metric multidimensional scaling ordination where each point represents a site and vectors display species correlated ($r^2 \geq 0.40$) with compositional gradients

created by removing 1–3 mature oak trees were too small for oak recruitment. The gaps apparently were sufficiently large, however, to stimulate increases in understory herbaceous plants and shrubs (c.f. Pavlovic et al. 2006).

Understory composition

Understory compositional variation among treatments suggested that communities in treatment areas were consistent with an approximately equally split reorganization-colonization model and that colonization occurred by ruderal species that were primarily perennials rather than short-lived species. A reorganization-colonization model provides a framework whereby post-disturbance community changes arise from reshuffling quantities of resident and colonizing species in varying proportions (e.g., Goldblum 1997; Abella et al. 2019). In our study, species richness 100 m^{-2} in treatments was about double that of reference forest, suggesting nearly equal contributions of residents and colonizers to community richness. Almost all (39 of 43, 91%) of the total species present in reference forest plots inhabited treatment plots, indicating persistence of residents and that oak wilt treatments did not eliminate species (Online Resource 1). The four species detected only in reference forest plots included one non-native and

three native species common regionally (Online Resource 1). Relatively long-lived perennials were the primary colonizers of canopy gaps, differing from ecological succession models for disturbances such as abandoned agricultural fields frequently initially colonized by annuals (Inouye et al. 1987).

Based on published literature, most of the dominant species in treatment sites utilize multiple strategies for recruitment that, while in some cases capable of long-distance seed dispersal, emphasize reproduction from on-site propagules. For example, the shrubs *Rubus* spp., abundant in treatment sites, form persistent soil seed banks and also employ vegetative reproduction (Widen et al. 2018). Two of the major forbs, wrinkle-leaf goldenrod and enchanter's nightshade (*Circaea lutetiana*), form persistent seed banks (Abella et al. 2020a) and reproduce via rhizomes (Verburg and During 1998). Dominants common in treatment sites as well as in reference forest also rely on multiple strategies, exemplified by Pennsylvania sedge, Virginia creeper (*Parthenocissus quinquefolia*), and Blue Ridge blueberry (*Vaccinium pallidum*) forming seed banks and utilizing vegetative reproduction, and the trees black cherry and sassafras capable of forming moderately long-lived soil seed ($\sim$ 4–5 years) and "seedling banks" (Haywood 1994). Species more reliant on seed dispersal from off site, exemplified by wind-dispersed American burnweed (*Erechtites hieraciifolius*), were present in treatment sites but were not dominants. It is possible that the scattered distributions of canopy gaps and the resulting need for seeds to disperse through intact forest constrained primarily dispersal-reliant species in favor of those more effectively regenerating locally.

While treatment sites contained some species associated with full sun or moderately sunny habitats, they generally did not contain rare species or those most characteristic of the region's open habitats (Online Resource 1, 2). In total, the 21 treatment plots contained only one state-listed rare species (racemed milkwort [*Polygala polygama*]), the only species with a coefficient of conservatism exceeding 7 representing high fidelity to the region's natural open habitats. Based on comparing species in treatment sites with a floristic inventory of four prairie-savanna sites in the study area, overlap in flora was mainly for generalist species also inhabiting reference forest (Online Resource 2). Conservative and state-rare species found in the study area's prairies-savannas, such as

sweet fern (*Comptonia peregrina*), wild lupine (*Lupinus perennis*), and Canada frostweed (*Helianthemum canadense*), were absent in treatment sites. Our finding of few conservative prairie-savanna species in treatment sites is not unlike that of a previous study in Wisconsin, where prairie-savanna species were relatively infrequent in oak wilt patches consisting of > 3–4 dead trees (Collada and Haney 1998). Given that prairie-savanna species are sparse in soil seed banks of mature oak forests in our study region (Abella et al. 2020a), factorial experiments varying potential limiting factors such as propagule availability and canopy gap size may help identify colonization constraints to these species in oak wilt-affected forests. It is also possible that species of more densely treed portions of savannas and woodlands, such as the moderately shade-tolerant woodland sunflower (*Helianthus divaricatus*), could be most capable of colonizing small oak wilt canopy gaps (Brewer and Vankat 2004).

Spatial variation

Including plot as a random variable representing site location increased the variance models accounted for in many response variables. While pinpointing causal factors in location effects was beyond our study's scope, there were at least qualitatively likely associates with location. For example, in assessing spatial variation in non-native plants, which were more correlated with location than with treatment or canopy cover, the five plots containing the most non-native species (9–16 species 100 m^{-2}) were closest to edges of the preserve nearest suburban developed areas. Similarly, five of the six plots containing the most non-native cover (3–7%) were closest to the preserve's edges. This distributional pattern is consistent with many studies relating non-native plant abundance to distance from dispersal vectors (e.g., Honu and Gibson 2008; Schetter et al. 2013). Tree canopy cover accounted for variance in some response variables but was not as generally important as might be expected based on its potential effect on microclimate and light (Pavlovic et al. 2006; Levy-Varon et al. 2014). One reason for this could be that simply the presence or absence of a canopy gap was paramount, rather than variation in gap size or canopy cover because these were not especially variable among treated plots or may not have crossed thresholds such

that quantities then mattered (Heitzman and Stephens 2006).

Forest conservation and adaptation

This study of forest conditions in oak wilt containment treatments offers considerations for aiding forest adaptation to a fungal pathogen. First, secondary invasion by non-native plants did not appear to be a major part of forest response to oak wilt and containment treatments. As a result, a strategy encouraging forest adaptation could focus on early detection and treatment of non-native plants, to keep the oak wilt patches at least dominated by native plants including those offering floral resources for pollinators. Second, compared with black oaks, white oaks were sparse in treatments and in reference forest. This is consistent with broader patterns in the region and across many eastern North American oak forests where white oaks have disproportionately declined since Euro-American settlement (Abrams 2003; Brewer and Vankat 2004). Strategies that encourage white oak could both re-balance abundance of the oak species while fostering forest adaptation because white oak is less susceptible to oak wilt (Koch et al. 2010). Third, given we found no oak sapling recruitment, a likely priority for future research is evaluating whether general management strategies (creating open canopies and reintroducing fire) that promote oak could also be used to foster resistance and resilience to oak wilt. Compared with dense, unburned forest, Pellegrini et al. (2021) found that oak wilt killed proportionally fewer trees in open, frequently burned oak savanna. Whether this resulted from the more widely spaced trees in savanna, the fires interacting with oak wilt, or other factors remained unclear, as did potential interactions of oak wilt with fire-free windows needed for future oak recruitment and with growth of saplings in the presence of the pathogen (Pellegrini et al. 2021). More open oak forests, as well as woodlands and savannas, could all meet habitat conservation goals in our study given the historical prevalence of these open ecosystems and the unique habitats they provide (Brewer and Vankat 2004). Our study suggests that if oak decline and small, dispersed oak wilt containment treatments continue *status quo*, an existing trend for conversion from oak- to red maple-dominated forest would likely continue via this "piecemeal" appearance of gaps individually too small for oak

recruitment and already filled with red maple and other tree species. This slow, incremental change may differ from what could occur with oak regeneration if fewer but larger gaps appeared faster. However, small oak wilt containment treatments can be expected to produce native plant diversity hotspots persistent for 6 + years and abundant floral resources for pollinators compared with reference forest.

Declarations

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