Received: 4 October 2021 | Accepted: 9 May 2022

DOI: 10.1002/2688-8319.12157

Ecological Solutions and Evidence | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Herbicide control of the invasive Amur honeysuckle (*Lonicera maackii*) does not alter soil microbial communities or activity

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Funding information
Friends of Nachusa Grasslands Scientific Research Grant; Center for Biodiversity Research SEED Grant; Department of Biological Sciences at University of Memphis; NSF, Grant/Award Number: 1937255

Handling Editor: Errol Douwes

Abstract

1. Invasive plants are a major problem for land managers and have widespread and lasting environmental impacts. The invasive shrub Amur honeysuckle (*Lonicera maackii*) is a pervasive and noxious plant in the Midwest region of the United States.
2. Despite this, many land managers may be uncomfortable with herbicide control of this and other invasive plants due to unknown impacts on ecosystem components including soils.
3. To examine if herbicide control of Amur honeysuckle impacts soil enzyme activity and soil communities, we treated Amur honeysuckle with Garlon® 4 (triclopyr) suspended in Basal Bark Oil, Basal Bark Oil alone and untreated controls, then assessed soil community, soil enzyme activity and arbuscular mycorrhizal density changes among treatments and across the subsequent growing season.
4. We found that basal bark herbicide treatments of Amur honeysuckle do not negatively impact soil enzyme activity, nor do they impact fungal, prokaryotic or oomycotan diversity or community structure. There was a slight but likely ecologically unimportant effect on community structure associated with basal bark oil applications, but not with herbicide applications. Arbuscular mycorrhizal colonization was negatively affected by herbicide use but this is likely due to reduction in host health and/or mortality.
5. Taken together, this suggests that herbicide control of Amur honeysuckle does not impact soils and land managers can treat these invasive plants without concern for negative soil outcomes.

KEYWORDS
bacteria, fungi, herbicide, invasive plants, land management, oomycota, soil communities

1 | INTRODUCTION

Invasive plants have broad and long-lasting impacts on invaded ecosystems. It is generally assumed that invasive plants can outcompete and replace native plants (Daehler, 2003), but the strength of this community turnover is not well understood (Oduor, 2013; Thomas & Palmer, 2015). Invasive plants are also known to negatively impact animal behaviour (Stewart et al., 2021), particularly so for native insect pollinators (Sunny et al., 2015). The clearest evidence for negative ecosystem impacts of invasive plants comes from studies demonstrating that invasive plants dramatically and rapidly alter soil properties (Gibbons et al., 2017; Stefanowicz et al., 2017), soil microbial communities (Zhang et al., 2018, 2019) and soil functionality (Carey et al., 2017; Jo et al., 2017), potentially having long-lasting effects, even after plant removal (Corbin & D'Antonio, 2012).

A major invasive plant of concern in the Midwestern region of the United States is Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder.; Caprifoliaceae), which is listed as a state controlled noxious weed in the states of Illinois, Indiana, Minnesota, Ohio and Wisconsin (www.mipn.org). Amur honeysuckle (endemic to Manchuria, China) was initially established as an ornamental plant that became naturalized in the United States (Luken & Thieret, 1996) due to extensive seed production capability (Belcher & Hamer, 1982) and resistance to freezing (Gaffney & Belcher, 1978). Amur honeysuckle has rapid growth and environmental plasticity and may reduce native plant leaf area and impact ecosystem hydrodynamics (McNeish & McEwan, 2016). Mechanical removal alone is largely ineffective because of resprouts from remaining roots and stems (McDonnell et al., 2005), but numerous herbicide control options exist for Amur honeysuckle. These include stump cutting followed by herbicide painting (often glyphosate) (Cipollini et al., 2009; Frank, Nakatsu, et al., 2018; Frank, Saunders, et al., 2018), foliar herbicide spraying of glyphosate (Leahy et al., 2018) and basal bark application using triclopyr formulations (Baker, 2019; Kleiman et al., 2018), among others. Given that glyphosate works best as a foliar agent, and the potential for off target effects from foliar spraying is high (Leahy et al., 2018), it has been recommended that basal bark spraying using triclopyr formulations provides the highest mortality whilst being highly targeted (Kleiman et al., 2018).

Despite the targeted nature of basal bark spraying/painting that, when done correctly, does not directly contact understory plants/soil, a 'ring of death' has been documented whereby ground vegetation displays a circular mortality pattern approximately in the same area as the belowground root mass (Baker, 2019). It is uncertain by what action this ring of death manifests, but since no direct triclopyr application occurs to the understory, we presume that this action is modulated via root transport into the soil. One plausible mode of action for this mortality is via herbicide translocation from the roots either directly into the soil (exudates) or via microbial intermediates (likely from mycorrhizal transportation) whereby these herbicidal compounds are translocated from honeysuckle to neighbouring plants that are part of the same mycorrhizal network. Triclopyr is biodegraded within soils relatively quickly (Douglass et al., 2016; Wang et al., 2019) with a half-life as short as 5 days. While research into the toxicity of triclopyr on microbes is scant, it has been demonstrated to suppress growth of ectomycorrhizal fungi and bacteria in vitro (Baarschers et al., 1988; Chakravarty & Sidhu, 1987), but it is unknown if this suppression occurs in soils. Furthermore, it is poorly studied how the control of Amur honeysuckle impacts belowground communities and soils. One study (Frank, Nakatsu, et al., 2018) demonstrated changes in bulk soil chemistry following control treatments via rhizodeposition, but no study to our knowledge has investigated changes in soil microbial communities with control of Amur honeysuckle.

To investigate the belowground soil microbial impacts of controlling Amur honeysuckle with basal bark applications of triclopyr, we conducted the first examination of herbicide impacts on soil microbial communities (prokaryotes, fungi and oomycota), soil enzyme activity and arbuscular mycorrhizal density in Amur honeysuckle. We applied basal bark applications of triclopyr along with two controls and queried soil communities and enzyme activity. We anticipated that herbicide control of Amur honeysuckle would impact soil communities by facilitating a shift in communities, and decreasing diversity estimates, which are generally associated with increases in plant mortality. We further predict that herbicide application will be positively related to increases in soil enzyme activity as increased host mortality may lead to increased organic matter inputs and be negatively associated with arbuscular mycorrhizal density concomitant with host mortality.

2 | MATERIALS AND METHODS

2.1 | Study location and treatment applications

Mature Amur honeysuckle plants were located at Nachusa Grasslands (The Nature Conservancy, Franklin Grove, IL, USA) within the same wooded tract. A total of 30 plants were tagged and randomly assigned to one of three treatments (Herbicide with carrying oil, carrying oil and untouched controls), but due to unforeseen plant mortality during the course of this study unrelated to our treatments, only eight plants per treatment were retained ($n = 24$; see Table S1 for locations and application dates). Some plants were specifically selected for inclusion because we have background understory vegetation data (Baker, 2019), and we added additional plants because they were in close proximity to these targeted plants, were of the same general age and size (height and spread) and are located in similar woodland environments. The proximity of the plants means they experience the same climatic, weather and environmental conditions, therefore differences may be attributed primarily to treatment effects. Each plant was randomly assigned into treatments. Prior study at these sites documented 'ring of death' areas in which herbaceous plants died in a small area immediately around treated Amur honeysuckle plants (Baker, 2019). Treatments were implemented within 1 week of each other in late February 2020. Following Baker (2019) and Czarapata (2005),

treatments were applied using backpack sprayers exclusively dedicated to basal bark treatment. Herbicide treatment consisted of a 12% herbicide solution (Garlon® 4 Ultra [60% triclopyr]; Dow AgroSciences LLC, Indianapolis, IN, USA) diluted into basal bark oil (Bark Oil Red LT, Loveland Products, Inc, Greeley, CO, USA). Basal Bark Oil treatments consisted of only the basal bark oil, and the control treatment was not sprayed. Basal Bark Oil treatments were applied with a new sprayer with no legacy of herbicide residue. All spray applications consisted of spraying each stem with a vertical band between 15 and 30 cm from ground level upwards until the stems were fully coated but the mixture did not run off, per label instructions.

2.2 | Soil and root sampling

Samples were collected twice across the growing season in May and September ($n = 48$). We selected these two sampling points because we wanted to capture soil dynamics across a growing season at full photosynthetic capacity, so we sampled well after full leaf emergence and prior to senescence. Soils were collected using a hand-held slide-hammer coring device (Giddings Machine Company, Windsor, CO, USA). Cores were 5 cm in diameter and 10 cm deep. The core was cleaned, and a 'dummy' core was taken between each sampling plot to prevent sample contamination between plots. The observed 'ring of death' patches had an average radius of 25 cm. We sampled soil cores 15 cm from the root crown to remain firmly within the 'ring of death' zone and avoid interference with root sampling. Soils were immediately placed on ice in a cooler and frozen at −20°C within 8 h of collection. Frozen samples were shipped on dry ice overnight to the University of Memphis for processing. Previous work recommends storing soils at −20°C for soil enzyme analysis (DeForest, 2009; Turner & Romero, 2010; Wallenius et al., 2010). Soil for DNA analysis was transitioned to −80°C immediately after arriving at the lab. Roots for arbuscular mycorrhizal fungi (AMF) colonization measurements were hand collected from the target plant. Soil was gently brushed aside to follow a large root from the target plant into the soil until fine roots began branching off. Fine roots were carefully detached from the main root, shaken to remove excess soil and placed in a sterile plastic bag. Disturbed soil was pushed back into place, ensuring all exposed roots were covered. Gloves were worn at all times to protect root samples from microbial contamination. For some plants, it was impossible to sample AMF colonization because fine roots were absent due to the cessation of root production and root decomposition. Thusly, we were only able to measure AMF colonization on 29 samples (we measured five samples for AMF colonization for each treatment and each time, except we could only measure four samples for the May Bark Oil treatment). Fine roots were gently washed in distilled water, shaken off to remove excess water, placed in sterile plastic tubes, frozen (−20°C) and shipped to San Diego State University. Fine root samples were cleared in hot KOH, bleached, acidified and stained with Trypan blue. AMF colonization was quantified using the magnified gridline intersect method at 50 points per sample (McGonigle et al., 1990).

2.3 | Soil enzyme activity

Soils were sieved using a brass #10 (2 mm) sieve to homogenize samples, break-up large aggregates and remove rocks and roots. Homogenized samples were used for DNA extraction (below) and to query total soil enzyme activity. To query broad soil enzymatic activity, we used a fluorescein diacetate (FDA) hydrolysis assay, which measures the amount of fluorescein that is formed as a result of FDA hydrolysis (Adam & Duncan, 2001; Schnürer & Rosswall, 1982). FDA (3′, 6′-diacetyl-fluorescein) can be hydrolyzed by various enzymes and is considered a robust measure of broad enzymatic activities of microorganisms; FDA can be hydrolyzed by enzymes including such as proteases, lipases and esterases, among others (Green et al., 2006), and resultant fluorescence was measured in triplicate on a Synergy™HTX Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA) at wavelengths 475–510 nm. Soils (4.0 g) were placed into a 50-ml centrifuge tube and 15 ml of buffer (6.9 g NaH_2PO_4 and 41.8 ml of 1 M NaOH to a final volume of 1 L) was added. Then, 250 μl of FDA stock (2 mg fluorescein diacetate per millilitre in acetone) was added along with 15 ml of acetone and samples were agitated for 5 min and centrifuged at 8000 rpm for 10 min. Samples were then diluted with FDA Buffer/acetone (1:1).

2.4 | DNA extraction and sequence generation

Total soil genomic DNA (gDNA) was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, Germantown, MD, USA) following standard protocols. DNA was quantified using a NanoPhotometer N60 (Implen, Munich, Germany) and samples were normalized to a concentration of 50 ng/μl. Community metabarcoding libraries were generated using a Fluidigm Access Array system that uses microfluidics to isolate each sample and PCRs and indexing are done using 30 nl reactions and is a powerful way to sequence microbial communities (Brown et al., 2016) whilst minimizing cross-sample contamination. Libraries were generated targeting fungal, bacterial and oomycotan soil communities using the primer pairs ITS1F-ITS2 (targeting the ITS1 region of the rRNA operon of fungi; Gardes & Bruns, 1993; White et al., 1990), 515F(Parada)–806R(Apprill) (targeting the V4 region of 16S rRNA gene of bacteria and archaea; Apprill et al., 2015; Parada et al., 2016) and ITS30o-ITS4 (targeting the ITS2 region of the rRNA operon for oomycota; Riit et al., 2016; White et al., 1990), respectively. Final sequencing constructs were generated using native PCR parameters by using a two-step procedure with a total of 35 PCR cycles at 60°C annealing temperatures and 72°C extension temperatures. Final constructs (Brown et al., 2016) consisted of i5-CS1-Forward Primer-Target Sequence-Reverse Primer-Index-i7 (see Table S2 for sequences of primers, linkers and barcodes used), where i5 and i7 were the Illumina sequencing linkers, CS1 and CS2 were Fluidigm-specific amplification primer pads and index was sample-specific barcodes. Sequences were generated on one reaction of Illumina MiSeq (250PE). Fluidigm library construction

and sequencing were conducted at the WM Keck Center (Urbana, IL, USA).

2.5 | Bioinformatics and statistics

Sequences were processed using the program mothur (v.1.44.3; Schloss et al., 2009) following the removal of primers using Cutadapt (v.2.8; Martin, 2011). Briefly, paired reads were contiged (Bacteria and Oomycota), whereas only the forward read for Fungi was used, as the ITS1 region can be too long to contig well with 250PE reads with the inclusion of Fluidigm-specific sequence fragments. Sequences with ambiguous base pairs and with homopolymers longer than 10 were culled and retained sequences underwent pseudo-single linkage clustering (Huse et al., 2010). Sequences were classified into taxonomic lineages using a Naïve Bayesian classification (Wang et al., 2007) against the RDP training set for prokaryotes (v.10) and the UNITE non-redundant species hypothesis data set for fungi (v.6; Nilsson et al., 2019) locally modified to include plant, stramenopiles and PhiX sequences to aid in off-target sequences identification. All sequences that were not fungi or prokaryotes were culled. Oomycota reads were not screened until after Operational Taxonomic Unit (OTU) demarcations and representative sequences of each Oomycota OTU were identified using BLASTn against GenBank (nr/nt) with environmental sequences excluded. Chimeric reads were identified and removed, sequences were demarcated into OTUs using the abundance-based VSEARCH (Rognes et al., 2016) implementation within mothur at a 97% threshold and all OTUs with less than 10 sequences globally were culled to prevent the inclusion of spurious OTUs (Brown et al., 2015; Oliver et al., 2015). Final sample × OTU matrices for bacteria and archaea, fungi and oomycota were generated and used for downstream analyses.

2.6 | Analyses

Community diversity estimates (observed richness [S_{obs}]; complement of Simpson's diversity [1 – D]; and Simpson's evenness [E_d]) were calculated using an iterative subsampling approach (1000 iterations and the mean values used in all downstream analyses). Subsampling depths (Prokaryotes, 9000 sequences per sample; Fungi, 7000 samples per sample; Oomycota, 50 sequences per sample) were chosen to retain most samples whilst maximizing the number of reads sampled. Given the low abundance of Oomycota reads, we tested if doubling the subsampling depth (to 100 sequences) would result in additional OTUs identified using Boneh estimates (Boneh et al., 1998), but average additional predicted OTUs were minimal (0.23; range 0–0.66), therefore we used a subsampling depth of 50. Not all samples contained Oomycota reads and consequently, only 30 samples (out of 48) were retained for all Oomycotan analyses (all treatment and time combinations had at least three samples retained which still allowed for statistical analyses: Control Time 1 = six, Control Time 2 = four; Herbicide Time 1 = seven; Herbicide Time 2 = six; Bark Oil Time 1 = four, Bark Oil Time 2 = three). To test if honeysuckle control impacts soil microbial diversity, we used a two-way ANOVA framework (Treatment, Time and Treatment × Time). Diversity data were tested for normality using Anderson–Darling tests and richness and evenness for each microbial group were normal, but diversity was not and thus transformed using Box–Cox transformations prior to analyses ($\lambda = 2$ for Fungi and Bacteria, and $\lambda = 0.503$ for Oomycota). To test if soil enzyme activity changed across our experimental framework, we used a similar two-way ANOVA. To test impacts on AMF colonization, AMF abundance (the number of points out of 50 at which mycorrhizal structures were detected) was analysed using a generalized linear model with a quasipoisson error distribution and the same independent variables (Treatment, Time and Treatment × Time).

To examine if soil microbial communities are impacted by herbicide control of Amur honeysuckle, we used PerMANOVA (Anderson, 2001) tests using Bray–Curtis dissimilarity matrices (iteratively subsampled at 1000 interactions at the same subsampling depths as above and the average dissimilarity values used here) using the adonis function in the R package *vegan* (Oksanen et al., 2017) with 999 iterations using the model Treatment, Time and Treatment × Time. Because of the sparse Oomycotan sequence representation, we refrain from inclusion of these data for multivariate analyses. Where appropriate, post hoc tests were conducted to identify which treatments differed with the pairwise.perm.manova function in the R package *RVAIDeMemoire* (Hervé, 2021) with FDR corrected p-values and 999 iterations. Further, to visualize community-level similarities, we used nonmetric multidimensional scaling (NMDS) as implemented by mothur (solved across four axes for Prokaryotes and five axes for Fungi [Stress = 0.179 and 0.187, respectively]). Further, stacked histograms were used to visualize class-level taxonomic identities (family level for Oomycota) across our samples.

Additionally, we examined if there was an effect of Treatment, Time or Treatment × Time interactions on the relative abundance of fungal functional guilds, for which there are several good curated functional databases for species and/or genera. Using the database FungalTraits (Põlme et al., 2020), we queried obtained taxonomic identities for each OTU and assigned them into functional guilds. Functional guilds were retained where fungal OTUs could be unambiguously assigned to functional guilds, and where a functional guild consisted of at least 2.5% of all OTUs, resulting in analyses of the following guilds: Ectomycorrhizal (EcM; 47 OTUs), Mycoparasites (27 OTUs), Plant Pathogens (42 OTUs) and Saprotrophs (300 OTUs). For each sample, relative abundances of these guilds were determined and tested if they are normally distributed (Anderson–Darling tests); Saprotrophs were normally distributed ($A = 0.206$, $p = 0.862$) where EcM, Mycoparasites and Plant Pathogens were not normal and transformed to increase normality using Box–Cox transformation ($\lambda = 0.137$ for EcM, $\lambda = 0.397$ for Plant Pathogens and $\lambda = 0.381$ for Mycoparasites) prior to analyses. Using ANOVA tests, we tested if theses relative abundances change with Treatment, Time and Treatment × Time interactions.

To examine how individual OTUs responded to treatments, we identified biomarker taxa that were overrepresented in treatments using Linear Discriminant Analysis Effect Size (LEfSe; Segata et al., 2011)

tests as implemented in mothur. We aimed to find biomarker OTUs that were overrepresented with treatment (class) whilst accounting for abundance variability associated with time (subclass), and after test implementation, signed Linear Discriminant Analysis (LDA) log scores and associated *p*-values were calculated.

All statistics were conducted using a combination of JMP Pro (v.15; SAS Institute, Cary, NC, USA), R (v.3.3.3; R core team) and mothur (v.1.44.3).

3 | RESULTS

3.1 | Treatment efficacy

All plants subject to the herbicide treatment died in the course of the study. One oil plant also died from non-herbicide causes (Table S1).

3.2 | Sequence information

Initial Fluidigm sequencing generated >2.2 million prokaryotic reads, >1.7 million fungal reads and >70,000 oomycotan reads, and after quality control, 7011 prokaryotic OTUs, 1037 fungal OTUs and 23 oomycotan OTUs were retained. All sequences are deposited in the Sequence Read Archive (SRA) at NCBI under the accessions: BioProject (PRJNA767064) and BioSamples (SAMN21882608–SAMN21882751).

3.3 | Taxonomic summaries

Soil communities associated with Amur honeysuckle are diverse (Figure 1; Tables S3 and S4). Fungal communities are dominated by Ascomycotan taxa with numerous members of Basidiomycota and Zygomycota. We use 'Zygomycota' here *sensu latissimo* as this phylum has been recently restructured (Spatafora et al., 2016), but the UNITE database used does not fully encompass these changes. Prokaryotic communities are dominated by Proteobacteria, Verrucomicrobia and Planctomycetes (Figure 1; Table S3), with 44 OTUs identified as Archaea (13,465 sequences—0.8%, mainly belonging to Thaumarchaeota) (Table S4). Almost all the oomycotan taxa belong to the orders Pythiales and Saprolegniales (Table S3).

3.4 | Treatment responses

Soil enzyme activity did not differ with applied treatments, sampling dates, or their interactions ($F_{5,54}$ = 1.406, p = 0.2368), neither did microbial diversity estimates for prokaryotes (S_{obs}: $F_{5,42}$ = 1.612, p = 0.1778; 1 – D: $F_{5,42}$ = 0.805, p = 0.5522; E_D: $F_{5,42}$ = 1.188, p = 0.3314), fungi (S_{obs}: $F_{5,42}$ = 0.208, p = 0.9573; 1 – D: $F_{5,42}$ = 1.228, P = 0.3128; E_D: $F_{5,42}$ = 1.506, p = 0.2083) or oomycota (S_{obs}: $F_{5,23}$ = 0.215, p = 0.9524; 1 – D: $F_{5,23}$ = 0.073, p = 0.9957; E_D:

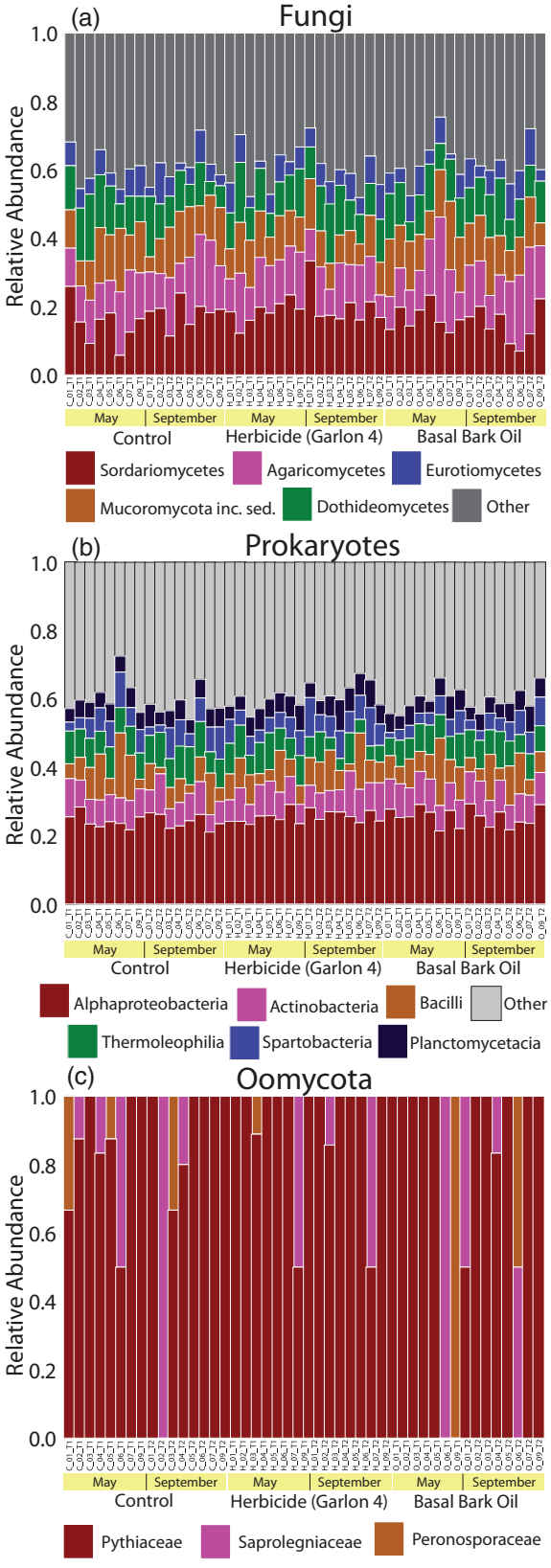

FIGURE 1 Relative abundance of class level identities (family level for Oomycota) of each sample for Fungi (a), Prokaryotes (b) and Oomycota (c) across treatments (Control, Herbicide, Control) and Time (May, September) demonstrating stability of communities within soils

TABLE 1 Results of PerMANOVA tests across Herbicide, Basal Bark Oil and control treatments (Treatment), sampling dates (Time) and their interactions for each targeted organismal group. Presented are Pseudo-F test statistics, p-values and associated R2 values. Significant results are in bold

Test	Pseudo-F_{df}	*p*-values	R^2
Prokaryotes			
Treatment	$\mathbf{F_{2,42} = 1.643}$	**0.018**	**0.069**
Time	$F_{1,42} = 0.868$	0.566	0.018
Treatment × Time	$F_{2,42} = 0.819$	0.784	0.034
Residuals			0.879
Fungi			
Treatment	$\mathbf{F_{2,42} = 1.300}$	**0.013**	**0.055**
Time	$F_{1,42} = 0.968$	0.540	0.021
Treatment × Time	$F_{2,42} = 0.763$	0.997	0.032
Residuals			0.892

TABLE 2 Results of ANOVA tests of functional guilds relative abundances across Herbicide, Basal Bark Oil and control treatments (Treatment), sampling dates (Time) and their interactions for each fungal functional guild, significant responses are in bold and italics

Test	*F*-statistic	*p*-value
Saprotrophs		
Treatment	0.881	0.421
Time	***4.798***	***0.034***
Treatment × Time	1.139	0.329
Mycoparasites		
Treatment	1.030	0.365
Time	0.107	0.744
Treatment × Time	0.434	0.650
Plant Pathogens		
Treatment	2.553	0.089
Time	***4.336***	***0.043***
Treatment × Time	0.349	0.707
Ectomycorrhizal		
Treatment	2.871	0.067
Time	0.560	0.458
Treatment × Time	0.423	0.657

$F_{5,23}$ = 0.246, p = 0.9373). PerMANOVA tests demonstrated that few significant but weak shifts in community structure occur with treatment (Table 1; Figure 2; Bacteria: $F_{2,42}$ = 1.644, p = 0.018, R^2 = 0.068; Fungi: $F_{2,42}$ = 1.300, p = 0.013, R^2 = 0.055). Post hoc pairwise PerMANOVA tests indicate that fungal and prokaryotic community structure is statistically different between control plants and oil plants (p = 0.015 and 0.042). AMF colonization of fine roots was low (6.2% ± 0.8% overall root colonization), and root AMF colonization was affected by treatment (χ^2 = 12.942, p = 0.002), but not Time (χ^2 = 0.000, p = 0.992) or their interaction (χ^2 = 0.563, p = 0.755). Post hoc pairwise tests indicate colonization was significantly reduced in the herbicide treatment compared to oil plants (p = 0.010) and marginally reduced compared to control plants (p = 0.099).

We tested if the relative abundances of OTUs that belong to fungal functional guilds changed with Treatments, Time, or Treatment × Time interactions; these results suggest that functional guilds are not affected by herbicide treatments of Amur honeysuckles (Table 2). Saprotrophs and plant pathogens did have reduced relative abundance in September compared to May, but this was independent of treatment.

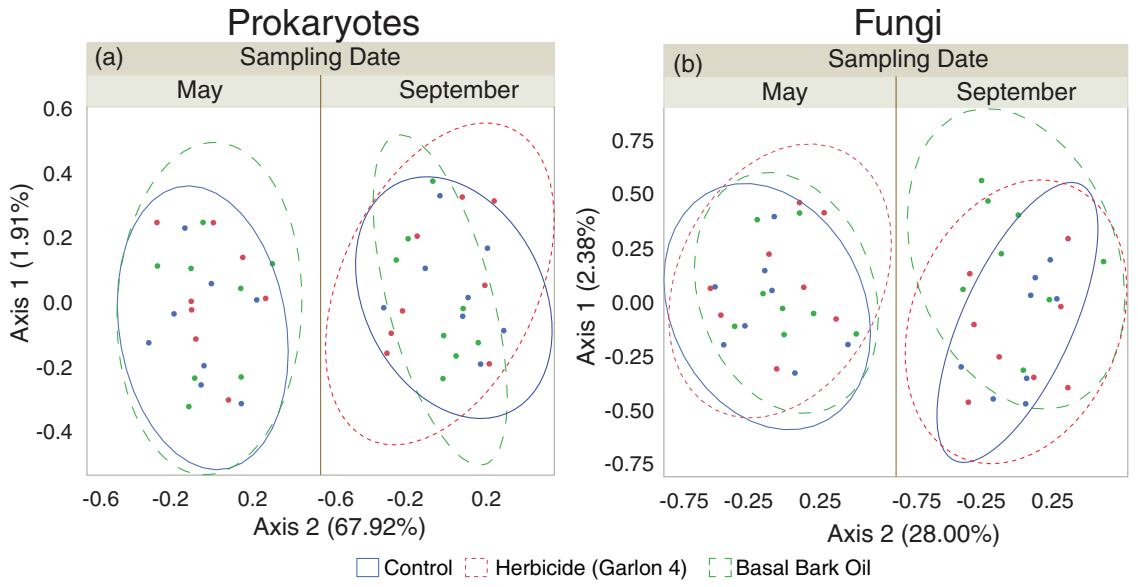

FIGURE 2 Nonmetric multi-dimensional scaling (NMDS) ordinations with 95% confidence ellipses for each treatment (Control, blue; Herbicide, red; Basal Bark Oil, green) for Prokaryotes (a) and Fungi (b), visualized separately for May and September sampling dates

TABLE 3 Biomarker OTUs identified using LEfSe analyses for treatments (class) whilst controlling for variation for sampling date (subclass). All fungal biomarkers and bacterial biomarkers that have a total sequence count >1000 are presented along with LDA tests statistics and associated *p*-values

Biomarker OTU	Treatment	LDA	*p*-value	Taxonomic ID	Ecological role
Fungi					
Otu0003	Herbicide	4.374	0.0329	*Mortierella humilis*	Saprotroph
Otu0004	Oil	3.914	0.0146	*Mortierella humilis*	Saprotroph
Otu0012	Oil	3.905	0.0277	*Cryptococcus terricola*	Saprotroph
Otu0171	Herbicide	3.765	0.0437	*Tomentella stuposa*	Ectomycorrhizal
Otu0383	Oil	3.411	0.0498	Pleomassariaceae sp.	Saprotroph
Bacteria					
Otu00002	Herbicide	3.868	0.0276	Spartobacteria *incertae sedis*	Unknown
Otu00008	Oil	3.543	0.0413	*Actinoallomurus*	Unknown
Otu00019	Herbicide	3.055	0.0289	*Roseiarcus*	Phototroph[a]
Otu00036	Control	3.164	0.0027	*Pyxidicoccus*	Soil-borne[b]
Otu00039	Control	2.883	0.0335	*Terrimicrobium*	Fermentative Saprotroph[c]
Otu00043	Oil	3.155	0.0003	*Acidisoma*	Chemoorganotroph[d]
Otu00051	Control	2.978	0.0033	*Gaiella*	Unknown
Otu00061	Oil	2.999	0.0119	Gp1	Unknown
Otu00068	Control	2.690	0.0163	*Pseudarthrobacter*	Unknown
Otu00081	Control	2.842	0.0086	Gp16	Unknown
Otu00112	Oil	2.943	0.0059	*Actinoallomurus*	Unknown
Otu00126	Herbicide	2.662	0.0002	Spartobacteria *incertae sedis*	Unknown
Otu00141	Control	2.849	0.0343	*Pedomicrobium*	Putative Mn-binding[e]
Otu00161	Oil	2.543	0.0273	Gp3	Unknown

Ecological roles, where unambiguously known, are determined for fungi using the FungalTraits database (Põlme et al., 2020) and for bacteria using primary literature.
[a]Kulichevskaya et al. (2014).
[b]Garcia and Müller (2014).
[c]Qiu et al. (2014).
[d]Reis and Teixeira (2015).
[e]Moore (1981).

Full list of functional guild relative abundances is presented in Table S5 and OTUs that belong to guilds are presented in Table S6.

3.5 | Biomarker taxa

In all, there were 79 prokaryotic OTUs (all bacteria) that are biomarker taxa for a treatment (whilst accounting for sampling date) and five fungal biomarker taxa. Of these, 13 prokaryotic and two fungal taxa are overrepresented in the herbicide treatment, 36 prokaryotic and three fungal taxa are overrepresented in the oil treatment and 30 prokaryotic taxa are overrepresented in the control plants. See Table 3 for common biomarker taxa and Table S7 for full biomarker taxa for treatments. No oomycotan OTUs were identified as biomarkers.

4 | DISCUSSION

The strategies land managers or conservationists choose to control invasive plants are often dictated by tradition, supplies and time available for control. These may include a combination of mowing, cutting and herbicide treatments, among others. Although these removal treatments may achieve their goal of invasive plant suppression, the effects of removal treatment on soil health and belowground communities remain poorly resolved. Land managers may be concerned about unintended direct and/or indirect effects of herbicides on other organisms in the environment. It is well established that invasion by non-native plants can alter soil communities and functionality (Stefanowicz et al., 2017; Zhang et al., 2019), but most investigations into herbicide impacts on soil communities focus on either

non-targeted plants across a large area or direct application onto soils (Cerdà et al., 2021; Du et al., 2018; Köberl et al., 2020; Tang et al., 2019; Thiour-Mauprivez et al., 2019). Both of these scenarios result in direct herbicide application onto soils (but see Frank, Nakatsu, et al., 2018). Of these studies, there are varied soil responses, including reduced microbial diversity and abundance of ammonia oxidation genes when exposed to mesotrione (Du et al., 2020), reduction of dehydrogenase activity with glufosinate exposure (Pampulha et al., 2007) and broad reduction in organic carbon for several herbicide classes (Lupwayi et al., 2004). However, a general consensus is that glyphosate formulations do little to impact soil microbial communities or functionality (Guijarro et al., 2018; Kepler et al., 2020; Weaver et al., 2007). This work expands on these previous studies to investigate if targeted triclopyr herbicide control of Amur honeysuckle impacts soil microbial communities and functionality. We demonstrate that by and large, herbicide control of Amur honeysuckle with triclopyr does not impact microbial community structure, functionality or diversity. This indicates that land managers can control Amur honeysuckle using cost-effective, targeted and labour-efficient basal bark treatments with triclopyr formulations without worrying about adverse effects in soils.

In this work, we examined the impacts of basal bark application of a triclopyr formulation on below ground microbial communities and soil functionality by comparing herbicide treatment against two controls, basal bark oil only spray (carrying oil for herbicide) and unsprayed, across the growing season following treatment application (May and September). We detected no discernible differences in broad enzymatic soil activity using FDA assays, which indicates that triclopyr management of Amur honeysuckle does not affect FDA hydrolysis in these soils. Previous work has reported that the presence of Amur honeysuckle does not alter the activity of several soil enzymes including peroxidases, phenol oxidase, phosphatase and β-glucosidase (Woods et al., 2019), therefore it may not be surprising that plant mortality induced by herbicides does not impact soil enzyme activity either. Additionally, we fail to identify any differences in metabarcoding-derived fungal, prokaryotic or oomycotan richness, diversity or evenness estimates and there is relative stability in taxonomic profiles across treatments (Figure 1). PerMANOVA tests indicated that communities differed with treatment for prokaryotes and fungi; however, these community shifts were slight, with low R^2 values and post hoc tests indicating that the triclopyr treatment did not differ in community structure from the control plots. Furthermore, there was no detectable changes in the abundance of fungal functional guilds with treatments, that is relative abundances of four major guilds remained stable no matter which treatment was applied. The only treatment with differing soil community composition was the basal bark oil only treatment, based on post hoc examinations. Taken together, these results indicate that while there may be some community turnover (i.e., addition, loss or replacement of taxa) with treatments across the growing season, this turnover is not associated with the herbicide use and does not impact community diversity and soil enzyme activity, likely due to functional redundancy within these communities.

This community response, albeit weak, to basal bark oil in below ground communities, but not herbicide application, is interesting and likely resulted from dose-dependent inputs. Even though the Garlon 4 used was mixed into basal bark oil, which acts as a surfactant in the herbicide application, this basal bark oil was diluted so these plots did not receive as large of a dose as the oil-only treatment. This bark oil contains petroleum distillates, tall oil fatty acids and nonylphenol ethoxylate as listed active ingredients. These chemical classes include numerous compounds with high aromaticity which suggests that utilization and/or degradation of these carbon rings requires specialized microbial organisms, which may induce community shifts, like the minor ones seen here. Tall oil fatty acids and related compounds can be biodegraded by specific microbes in soils (Prokkola et al., 2014) and functionally modify cellulolytic compounds (Setälä et al., 2020). Similarly, nonylphenol is readily biodegraded in soils (Chang et al., 2007) and has been demonstrated to shift bacterial community structure (Lozada et al., 2004) and to impact ammonia-oxidizers (Wang et al., 2014). So, it is perhaps not surprising that additions of these compounds within basal bark oil might shift microbial communities toward aromatic degraders and other chemoorganotrophs as seen for several biomarker taxa in the oil treatment (Table 3). However, it should be noted that we have no direct evidence that addition of these oils directly shifts rates of ammonia oxidation or is enriched for taxa that can degrade these compounds. Additional work is needed to confirm this mode of action. Additionally, AMF colonization did not differ between herbicide and oil-only treatments. Colonization was reduced for herbicide treatment plants, which is unsurprising because mycorrhizae likely reduce associations with dying plants as they provide few carbohydrate resources, even if minimal resources remain (Pepe et al., 2018). But the lack of AMF colonization differences between herbicide and oil-only treatments, and the lack of functional guild differences across all treatments, suggests that the small fungal community changes did not have strong effects on fungal community functionality. Future work could measure AMF colonization in surrounding plants to determine if reduced colonization of dying honeysuckle is accompanied by increased colonization of other surrounding plants, which might provide increased carbohydrates under reduced competition for sunlight.

Together, the lack of changes in broad soil enzymatic activity and microbial diversity, and only minor shifts in community composition in the oil-only application, points to the stability of these soil communities with treatments and time. This suggests that land managers may be able to treat Amur honeysuckle with basal bark spraying of herbicides without major impacts on soil communities and soil enzyme activity, likely preserving broad soil functionality. Combined with the high efficacy of the treatment (100% mortality in our experiment), our results indicate that this strategy is an important component in the restoration and recovery of invaded plant communities.

ACKNOWLEDGEMENTS

We gratefully thank The Nature Conservancy in Illinois, Bill Kleiman and Cody Considine. This work was supported by a Friends of Nachusa Grasslands Scientific Research Grant (SPB), Center for

Biodiversity Research SEED Grant (SPB), Department of Biological Sciences at University of Memphis (SPB and MS) and NSF #1937255 (NAB). We acknowledge members, both past and present, of the Peoria, Meskwaki, Sauk, Ochethi Sakowin, Myaamia, Kiikaapoi and Potawatomi tribes who call the area in and around the Nachusa Grasslands preserve home.

CONFLICTS OF INTEREST

Elizabeth Bach is employed by The Nature Conservancy, which owns and manages the land on which work was conducted. Elizabeth Bach is an Associate Editor of Ecological Solutions and Evidence, but took no part in the peer review and decision-making processes for this paper. The authors declare no other conflicts of interest.

AUTHOR CONTRIBUTIONS

Shawn Brown and Elizabeth Bach originally conceived of this project with input from Nicholas Barber. Elizabeth Bach conducted the spraying and sampling. Jonathan James, Maryam Shahrtash, Nicholas Barber, Ryan Buck, Kaleb Baker and Shawn Brown conducted the data generation and analyses portions of this project. Jonathan James and Shawn Brown wrote the contribution and all authors edited and approved the final text.

DATA AVAILABILITY STATEMENT

The data have been deposited with links to BioProject accession number PRJNA767064 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/). All BioSamples archived with this BioProject were used in this work.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/2688-8319.12157.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: James, J. J., Bach, E. M., Baker, K., Barber, N. A., Buck, R., Shahrtash, M., & Brown, S. P. (2022). Herbicide control of the invasive Amur honeysuckle (*Lonicera maackii*) does not alter soil microbial communities or activity. *Ecological Solutions and Evidence*, *3*, e12157. https://doi.org/10.1002/2688-8319.12157