Biol Invasions (2020) 22:3145–3162
https://doi.org/10.1007/s10530-020-02315-4

ORIGINAL PAPER

Amynthas spp. impacts on seedlings and forest soils are tree species-dependent

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Received: 29 October 2019 / Accepted: 9 July 2020 / Published online: 22 July 2020

Abstract Asian jumping worms (*Amynthas* spp.) are recent invaders of Upper Midwest forests. Research has highlighted the impacts of *Amynthas* earthworms on soil biogeochemistry and structure, and field observations suggest that *Amynthas* spp. decrease litter horizon depth and alter plant communities. However, the extent to which *Amynthas* spp. effects vary among forest types and with worm density and the mechanisms driving these effects are unknown. We conducted a 3-month tree seedling study to evaluate the effects of *Amynthas* spp. on tree seedling growth and a mesocosm field experiment to evaluate *Amynthas* spp. effects on soil carbon and nutrient cycling, soil structure, and leaf litter decomposition rates across forest types. In the seedling study, *Amynthas* spp. enhanced the growth of sugar maple and European buckthorn seedlings and decreased the growth of white oak seedlings. These effects were due to *Amynthas* spp.-induced changes in soil properties. In the mesocosm study, as *Amynthas* spp. density increased, carbon mineralization and carbon, nitrogen, and phosphorus availability increased in white oak forest soils and decreased in sugar maple forest soils, while decomposition rates of European buckthorn litter increased as *Amynthas* spp. density increased. *Amynthas* spp. altered soil structure similarly across all forest soil types. Taken together, our results suggest that *Amynthas* spp. have the potential to alter forest ecosystem dynamics via feedbacks among tree species, seedlings, and soil biogeochemistry. However, *Amynthas* spp. effects on tree seedlings and forest soils are largely context-dependent, and the direction and magnitude of these effects are mediated by tree species.

Keywords *Amynthas agrestis* · *Amynthas tokioensis* · Asian jumping worm · Earthworm · Forest soil · Illinois · Seedlings

Introduction

Asian 'jumping worms'—a complex of three common species, *Amynthas agrestis*, *A. tokioensis*, and *Metaphire hilgendorfi* (Chang et al. 2016a)—are recent invaders of forests in New England (Burtelow et al. 1998; Görres and Melnichuk 2012; Görres et al. 2016), the southern Appalachian Mountains (Callaham et al. 2003; Snyder et al. 2011), and the Upper Midwest (Laushman et al. 2018). As ecosystem engineers, earthworms convert organic and mineral materials

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-020-02315-4) contains supplementary material, which is available to authorized users.

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into long-lasting microstructures. In doing so, they alter the distribution and stability of organic matter, directly release nutrients, and modify microbial communities and their functions (Lavelle et al. 1997). These earthworm-induced changes in soil properties can lead to changes in plant communities via ecological cascades (Lavelle et al. 1997; Frelich et al. 2019). However, due to variations in the quality and quantity of their litters, plants also influence earthworms and their activities, thereby mediating earthworm impacts on soils. This can result in negative or positive feedbacks between earthworms and plants (Bohlen et al. 2004; Frouz 2018). While common non-native European earthworms, anecic *Lumbricus terrestris* and epi-endogeic *Lumbricus rubellus*, decrease understory plant diversity and tree growth (Hale et al. 2006, 2008; Laushman et al. 2018) and 're-engineer' soils (Frelich et al. 2006), less is known about how epi-endogeic Asian jumping worms (i.e., *Amynthas* spp.) modify plant communities and soils. Asian jumping worms are larger and reach higher densities than *Lumbricus* spp. (Greiner et al. 2012; Richardson et al. 2015; Chang et al. 2016b; Görres et al. 2016), reproduce by parthenogenesis (Chang et al. 2016a), spread rapidly (Laushman et al. 2018), and potentially competitively exclude *Lumbricus* spp. during a secondary invasion (Zhang et al. 2010; Greiner et al. 2012; Chang et al. 2016a, 2017). As such, there is increasing concern that Asian jumping worm effects on understory plants and soils will be more detrimental than those of European earthworms. Examining the effects of *Amynthas* spp. on tree seedling growth and soil properties and elucidating the mechanisms behind any observed changes are critical for predicting where Asian jumping worm impacts will be most severe.

Lumbricus spp. have been linked to sugar maple (*Acer saccharum*) canopy and seedling decline and alterations in understory plant communities (Bohlen et al. 2004; Hale et al. 2006; Laushman et al. 2018). As *Amynthas* spp. may occupy the same niche as *Lumbricus* spp. (Zhang et al. 2010), there is concern that Asian jumping worms might also have negative impacts on understory plants. In fact, negative impacts of *Amynthas* spp. are commonly reported in electronic newsletters and plant clinic alerts and on conservation websites. However, these reports are primarily based on anecdotal evidence (e.g., finding *Amynthas* spp. in forested areas that have low density and diversity understories). In contrast, observational studies have found no relationship between *Amynthas* spp. presence and understory biomass (Greiner et al. 2012; Laushman et al. 2018). Furthermore, *Amynthas* spp. may actually have positive impacts on some plants (Barbosa et al. 2017), and one recent study found an increase in plant species richness and tree seedling abundance in an *Amynthas* spp.-invaded area (Laushman et al. 2018). Experimental approaches are necessary for accurately characterizing *Amynthas* spp. impacts on tree seedling growth.

If *Amynthas* spp. do, in fact, alter tree seedling growth, these alterations may be direct effects driven by their feeding behavior and/or indirect effects due to their modification of the soil. *Amynthas* spp. have high dietary flexibility (Zhang et al. 2010), and, as such, they may directly consume mycorrhizal hyphae or fine roots, reducing seedling growth rates. Indirectly, *Amynthas* spp. might alter tree seedling growth by impacting soil nutrient availability or soil structure. However, different tree species may vary in their susceptibility to *Amynthas* spp.-driven changes in growth. For instance, if *Amynthas* spp. alter tree seedling growth via increases in soil nitrogen (N) availability, arbuscular mycorrhizal (AM) maples may exhibit positive growth responses, while ectomycorrhizal (ECM) oaks may exhibit negative growth responses (BassiriRad et al. 2015; Averill et al. 2018). AM trees and their associated fungi create and are adapted to high N soils, while ECM trees and fungi thrive in low N environments due to ECM trees' high N use efficiency and ECM fungi's ability to acquire N (Talbot et al. 2008; Phillips et al. 2013; Liese et al. 2018); these adaptations likely drive divergent seedling responses to increased soil N. Alternatively, maples may be more susceptible to structure-driven changes in soil moisture availability than oaks due to their growth strategies. *Amynthas* spp. frequently increase soil aggregation (Zhang et al. 2010; Snyder et al. 2011; Greiner et al. 2012), which may decrease soil water-holding capacity. Thus, *Amynthas* spp.-driven changes in soil structure may inhibit the growth of maple seedlings, which generally have shallow roots, but have minimal effects on oak seedlings, which have large taproots and grow deeper in the soil. Additionally, *Amynthas* spp. may create an advantageous habitat for other invasive species, such as European buckthorn (*Rhamnus cathartica*) (Heneghan et al. 2007; Ziter and Turner 2019). Overall, if *Amynthas* spp. alter tree seedling growth, it is

paramount to identify the mechanisms driving this in order to predict which tree species will be most susceptible to the positive or negative effects of *Amynthas* spp. invasion.

Further complicating the matter, *Amynthas* spp. impacts on soils may also be context-dependent. While Asian jumping worms seem to consistently alter soil structure (Zhang et al. 2010; Snyder et al. 2011; Greiner et al. 2012), their effects on soil biogeochemistry may be more variable. The limited number of studies evaluating *Amynthas* spp. impacts on soil biogeochemistry suggests that *Amynthas* spp. increase mineral N and phosphorus (P) availability (Burtelow et al. 1998; Greiner et al. 2012; Qiu and Turner 2017). However, these studies were conducted in maple-dominated ecosystems. As different tree species drive differences in forest soil biogeochemistry (Lovett et al. 2004; Hobbie et al. 2006), the magnitude of *Amynthas* spp. effects may vary across ecosystems. For instance, while Qiu and Turner (2017) found that *Amynthas* spp. increased the percentage of carbon (C), N, and P in soils, they observed greater effects in forest soils than in prairie soils, likely due to higher litter quality and soil moisture and lower bulk density of forest soils compared to prairie soils. Furthermore, inherent ecosystem properties may also mediate the direction of *Amynthas* spp. effects. *Amynthas* spp. may enhance soil organic matter content, for example, if they readily incorporate leaf litter into the soil (Greiner et al. 2012), or they may decrease soil organic matter content by stimulating microbial organic matter consumption (Burtelow et al. 1998). Because the direction and magnitude of *Amynthas* spp. effects on soils may feed back into their effects on plant growth and community dynamics, examining *Amynthas* spp. effects on soils across forest types is needed to gain insight into their effects on ecosystems as a whole.

On one hand, *Amynthas* spp. may have the greatest impact where soils are least like those they create. Invasive plant species effects on soil functions depend on the leaf litter traits of the original plant community (Kuebbing et al. 2018); invasive plant species effects are greatest when their leaf traits are most dissimilar from natives (Lee et al. 2017). As *Amynthas* spp. are commonly reported to increase soil pH and nutrient availability, they may have the greatest impacts in forest soils with low pH and nutrient availability like pine (Hobbie et al. 2006) and the least impact on high pH, high nutrient soils, like those under European buckthorn (Heneghan et al. 2007). Alternatively, *Amynthas* spp. may be unable to persist in some soils, limiting their effects (Görres et al. 2016). Several studies have shown that worm abundance, community composition, and functional groups vary according to tree community composition and leaf litter properties (Reich et al. 2005; Szlavecz et al. 2011; Schelfhout et al. 2017). Thus, *Amynthas* spp. may have the greatest impacts on soils that enhance their growth and fecundity.

Amynthas spp. presence is also commonly associated with reduced leaf litter cover (Greiner et al. 2012; Qiu and Turner 2017; but see Laushman et al. 2018). Currently, it is not known whether these reductions are driven by direct litter consumption or by indirect changes in soil properties. If *Amynthas* spp. directly consume leaf litter, they may prefer litter with specific properties. However, *Amynthas* spp. also have high dietary flexibility (Zhang et al. 2010), so they may not be selective in the litters they consume. *Amynthas* spp. may also alter leaf litter decomposition rates by modifying soils. *Amynthas* spp.-induced changes in nutrient availability may alter decomposition rates, particularly of labile leaf litters (Midgley and Phillips 2016). *Amynthas* spp. have also been shown to alter microbial biomass (Chang et al. 2016b, 2017) and the abundance of other soil decomposers (Snyder et al. 2011, 2013; Qiu and Turner 2017), which may lead to changes in leaf litter decomposition rates. Decreased leaf litter can have cascading ecosystem effects on soil fauna and food web dynamics; reduced leaf litter has been attributed to reduction in salamander breeding habitat (Moore et al. 2018). Therefore, it is critical to characterize *Amynthas* spp. effects on leaf litter decomposition, identify mechanisms driving any declines in leaf litter, and evaluate the extent to which these declines vary among leaf litters with different qualities.

In this study, we investigated the ecological consequences of *Amynthas* spp. invasion on common tree species and forest types found in the Upper Midwest. Specifically, we evaluated *Amynthas* spp. effects on tree seedling growth rates, soil biogeochemistry and structure, and leaf litter decomposition rates using two experimental set-ups: a Quonset hut tree seedling study and mesocosm field study. In addition, we assessed the extent to which *Amynthas* spp. effects were tree species-dependent. That is, we evaluated whether they varied among tree seedlings in the

Quonset hut study or across soils and litters from different forest types in the mesocosm study. To further elucidate the mechanisms driving alterations in tree seedling growth, soil properties, and litter decomposition, we posed the following questions: (1) What are the direct (i.e., seedling consumption) and indirect (i.e., modifications of soil structure and nutrients) effects of *Amynthas* spp. on tree seedling growth? (2) Do tree species mediate the effects of *Amynthas* spp. on soil C and nutrient dynamics, soil structure, and leaf litter decomposition rates? (3) Are *Amynthas* spp. impacts on litter decomposition rates direct (i.e., litter consumption) or indirect (i.e., alterations of the microbial community)? (4) Do the above effects vary with *Amynthas* spp. density?

Methods

Experiment I: tree seedlings

We conducted a three-month Quonset hut experiment to evaluate the direct (via consumption) and indirect (via changes in soil properties) effects of *Amynthas* spp. on tree seedling growth. To assess whether worm impacts on tree seedlings varied across species, we selected four species: European buckthorn (*Rhamnus cathartica*), sugar maple (*Acer saccharum*), white oak (*Quercus alba)*, and white pine (*Pinus strobus*). These four species are commonly found in the Upper Midwest (Nowak et al. 2014). We purchased 30 to 46 cm tall bare root seedlings of sugar maple, white oak, and white pine (Cold Stream Farm LLC, Free Soil, MI), and harvested European buckthorn seedlings from The Morton Arboretum that were similar in height to the purchased seedlings. We measured seedling wet biomass, stem diameter, stem height, and root length prior to planting. Stem diameter was taken at one inch above the root flare; we marked this location with black ink to ensure initial and final measurements were collected from the same location.

To evaluate indirect effects, we planted the tree seedlings in either *Amynthas* spp.-invaded or uninvaded soils. In June 2017, we collected soils from *Amynthas* spp.-invaded and uninvaded sites in Gallistel Woods at the University of Wisconsin-Madison Arboretum (43.04° N, 89.42° W) where a population of Asian jumping worms was discovered in 2013 (Laushman et al. 2018). Gallistel Woods is a remnant forest on land that was partially cleared but never plowed, and it contains trees that were planted between 1941 and 1964. The site is reflective of sugar maple-dominated mesic forests commonly found in the Upper Midwest. Soils are very-to-moderately deep and well-drained Alfisols formed from sandy loam till or loess and the underlying till. The major soil series are Military loam and Dodge silt loam. We collected soil from the top 10 cm of the soil layer from an Asian jumping worm-invaded area and a nearby uninvaded area. Invaded and uninvaded soils exhibited differences in a variety of biological and chemical properties (Table 1) that were consistent with a broader assessment of invaded and uninvaded soils at this site (Qiu and Turner 2017). Prior to use in the tree seedling experiment, we separately mixed, covered, and baked the invaded and uninvaded soils at 40 °C for 72 h to kill any worms or worm cocoons (personal communication, M. Johnston), and we periodically misted the soil to prevent drying. Soils were not sieved in order to maintain worm-induced differences in soil structure. We potted the tree seedlings into 2.84 L plastic pots.

To evaluate direct effects, we added either zero or two worms to each pot. We collected over 200 *Amynthas* spp. from the East Woods of The Morton Arboretum in Lisle, Illinois (41.81° N, 88.05°W) and sorted and removed the largest and smallest worms. *Amynthas* spp. used in our experiments had an average weight of 0.98 ± 0.09 g. While they were too juvenile to identify to species at the time of collection, *A. agrestis* and *A. tokioensis* regularly co-occur in the East Woods.

As such, individual seedlings received one of four treatments: control—planted into uninvaded soil; direct effect—planted into worm-uninvaded soil with two worms added; indirect effect —planted into invaded soil; or both effects—planted into invaded soil with two worms added. For each species, we replicated the soil x worm treatment five times ($n = 5$ for each soil type × tree species × worm treatment). Species-specific leaf litter was added to the soil surface of each pot. We covered the top of each pot with window screening and the bottom with permeable landscape fabric. We secured the window screening to the sides of the pot and left a small gap around the stem, thus allowing for the seedling to grow while ensuring the worms remained in the pot. To further prevent and monitor worm escape from the experimental area, we set up a moat under the benches.

Table 1 Properties of soils collected from *Amynthas* spp. invaded and uninvaded sites at University of Wisconsin—Madison Arboretum

	Soil type	
	Uninvaded	Invaded
Water content (%)	24	30
Organic matter (%)	4.8	8.1
pH	5.2	7.2
Total C (%)	2.1	3.5
Total N (%)	0.2	0.3
C:N	12.1	11.6
DOC (mg C g $soil^{-1}$)	0.05	0.2
MBC (mg C g $soil^{-1}$)	0.2	0.4
Ammonium (μg N-NO_3^- g $soil^{-1}$)	46	60
Nitrate (μg N-NH_4^+ g $soil^{-1}$)	1.5	1.7
N mineralization rate (μg N g $soil^{-1}$ day^{-1})	0.2	−0.05
Nitrification rate (μg N g $soil^{-1}$ day^{-1})	0.02	0.05
H1 (μg P g $soil^{-1}$)	1.8	9.3
H2 (μg P g $soil^{-1}$)	27	41

The experiment commenced in July 2017. We placed the seedlings in a Quonset hut where they received ambient light (light shade) and were monitored regularly to ensure sufficient moisture content for standard growing conditions over a 3-month period.

In October of 2017, we harvested the tree seedlings and measured total wet biomass, stem diameter, stem height, root length, and oven-dried fine root biomass. Relative changes were calculated by subtracting the initial measurement from the final measurement, dividing the difference by the initial measurement, and multiplying the quotient by 100 to obtain a percentage. As there were few-to-no fine roots present at the beginning of the experiment, we did not calculate relative change for this measurement. After the seedlings were harvested, we baked the soil at 40 °C for 72 h prior to disposal. While no worms were present at the conclusion of the tree seedling experiment, we also found no evidence of worm escape, indicating that the worms had died in the pots. However, the presence of highly-manipulated, granular soil (likely worm castings) throughout the worm addition pots indicated that the worms lived for some portion of experimental duration.

To evaluate the direct (worm addition) and indirect (worm-invaded soil) effects of *Amynthas* spp. on tree seedling growth, we ran ANOVAs with tree species (European buckthorn, sugar maple, white oak, or white pine), soil type (invaded or uninvaded), worm addition (present or absent), and all interactions as fixed effects and changes in total biomass, stem diameter, stem height, and root length, and total fine root biomass as dependent variables. Results were considered significant if they had P-values < 0.10, chosen *a priori* to balance model specificity with our relatively low sample sizes and the limited information available on *Amynthas* spp. impacts on seedlings. When we detected a significant main effect of tree species, we conducted a Tukey HSD *post-hoc* test to identify differences among groups. Because large differences among species often obscured the drivers of interaction effects, when we detected a significant tree species × soil type or worm addition interaction, we subset our data by tree species and ran t-tests to identify drivers of the interaction. For instance, if we found a significant species × soil type effect on total fine root biomass, we isolated the white oak data in our database, and we ran a t-test with total fine root biomass as the dependent variable and soil type as the independent variable to determine if white oak fine root growth differed between the two soil types. By conducting a similar test for the other three species, we were able to identify the drivers of species x soil type interactions. All statistical analyses were conducted in the base package in R (R Core Development Team 2019).

Experiment II: soil mesocosms

In order to evaluate whether or not forest types mediated the effects of *Amynthas* spp. on soil C and nutrient cycling, soil structure, and leaf litter decomposition rates, we conducted a mesocosm experiment at The Morton Arboretum. The mesocosm design was modified from an experiment conducted by Qiu and Turner (2017). We collected intact soil cores (n = 80) from four Arboretum sites: a sugar maple stand, a managed white oak savanna, a remnant white pine plantation, and a European buckthorn-dominated site adjacent to a restored wet meadow. At each location, we laid out a 100 m transect in a random direction; at 0, 25, 50, 75 and 100 m markers along each transect, we collected a set of four intact soil cores (20 cm diameter wide by 25 cm deep) within a 1 m radius of the transect markers. Additionally, we collected one soil sample (5 cm depth) from each forest type transect location with a stainless steel soil probe in order to measure soil properties (Table 2). Soils found here are deep and moderately-to-poorly drained Alfisols formed from a thin layer of loess (0.3–1 m) underlain by glacial till. The major soil series are Beecher and Ozaukee silt loams. We maintained soil cores in groups of four according to collection location and placed each group in randomized locations in a shaded field site.

In order to test the effects of worm density on soil C and nutrient cycling, soil structure, and leaf litter decomposition rates, we varied the number of worms added to each mesocosm. For each four-core group, we had one control core with no worms, one core with one worm added, one core with three worms, and one core with six worms. We collected and sorted worms as above. The maximum number of introduced worms was consistent with a study conducted by Qiu and Turner (2017); six worms approximated the maximum density of Asian jumping worms measured in an invaded forest at the University of Wisconsin-Madison Arboretum.

In order to assess the direct and indirect impacts of *Amynthas* spp. on leaf litter decomposition, we added two types of 10 cm × 10 cm mesh leaf litter decomposition bags to each mesocosm. One litterbag was made from 1 cm mesh that worms could access while the other bag was made from 1 mm mesh that the worms could not access. We filled each bag with ~1 g of senesced, air-dried, forest type-specific leaf litter (European buckthorn, sugar maple, white oak, or

Table 2 Initial properties of mesocosm soils collected from sites dominated by European buckthorn, sugar maple, white oak, and white pine at The Morton Arboretum

	Forest type			
	European Buckthorn	Sugar Maple	White Oak	White Pine
Water content (%)	29 ± 0.7^{a}	35 ± 1.4^{b}	29 ± 0.7^{a}	26 ± 1.8^{a}
Organic matter (%)	17 ± 0.8	15 ± 1.3	15 ± 1.1	18 ± 1.7
pH	7.0 ± 0.09^{a}	6.5 ± 0.12^{b}	6.5 ± 0.09^{b}	5.4 ± 0.11^{c}
Total C (%)	8.2 ± 0.3	7.3 ± 0.6	6.7 ± 0.5	8.2 ± 0.8
Total N (%)	0.6 ± 0.03	0.6 ± 0.05	0.5 ± 0.03	0.5 ± 0.05
C:N	13.7 ± 0.5^{a}	12.8 ± 0.3^{b}	12.6 ± 0.4^{b}	15.2 ± 0.3^{c}
DOC (mg C g soil^{-1})	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
MBC (mg C g soil^{-1})	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.2
Ammonium (μg N-NO_3^- g soil^{-1})	0.4 ± 0.4^{a}	-0.2 ± 0.1^{a}	0.7 ± 0.1^{ab}	2.3 ± 0.8^{b}
Nitrate (μg N-NH_4^+ g soil^{-1})	6 ± 1.0^{a}	15 ± 1.7^{b}	9 ± 1.0^{ab}	9 ± 1.5^{ab}
N mineralization rate (μg N g soil^{-1} day^{-1})	0.3 ± 0.1^{a}	1.1 ± 0.1^{b}	0.3 ± 0.2^{a}	1.0 ± 0.1^{b}
Nitrification rate (μg N g soil^{-1} day^{-1})	0.3 ± 0.1^{a}	1.1 ± 0.1^{b}	0.3 ± 0.2^{a}	1.0 ± 0.2^{b}
H1 (μg P g soil^{-1})	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
H2 (μg P g soil^{-1})	19 ± 1.1	19 ± 4.5	19 ± 1.5	24 ± 4.1

Letters denote significant differences among forest types ($P < 0.1$; n = 5)

white pine). Immediately after adding the worms, we laid the two litter bags on the soil in each core and added additional forest type-specific leaf litter to cover the bags and soil.

In order to ensure the worms remained in the cores and lived through the experiment, we took several precautions. We covered the top of each core with window screening secured with a bungee cord to allow for the circulation of air and allow for precipitation and covered the bottom of each core with a permeable landscape fabric secured with Gorilla tape to allow for water flow out of the core. The cores were mulched-in to provide moisture control and prevent the cores from drying out during the length of the experiment. Throughout the experiment, we monitored the cores weekly, and we added water consistently to all cores as needed to prevent desiccation. We maintained the mesocosm experiment from June through October 2017; the site had a temperature range from a low of 13 °C to a high of 26 °C, with total precipitation of 48.4 cm during this time period. At the end of the experiment, we collected the mesocosms and transported them to the laboratory for deconstruction and analysis.

Mesocosm processing

In the laboratory, we removed the litterbags, air-dried the litter, and weighed the litter. We calculated decomposition rates as the percent change in weight. Additionally, we extracted the top 5 cm of soil from each core. Asian jumping worms are epi-endogeic species and do not burrow vertically in the soil; they are typically found in the top 5 cm of the soil (Richardson et al. 2009; Qiu and Turner 2017). We sieved half of the soil sample through a 2 mm sieve to remove rocks and soil debris and homogenize the soil. We left the other half un-sieved and air-dried these subsamples in plastic baggies. We used sieved soils for biogeochemical analyses and air-dried, un-sieved soils for physical analyses. We stored sieved soils at 4 °C overnight before extracting C, N, and P, conducting C mineralization assays, and initiating N cycling incubations. We conducted all other assays within three months of collection. We stored soils for microbial biomass fumigations at − 20 °C prior to extraction, and we stored all solutions at − 20 °C prior to analysis. We baked the remaining soil from each core at 40 °C for 72 h to kill off any potential cocoons prior to disposal.

Soil biogeochemical analyses

We dried subsamples of soil (5 g) at 105 °C for 1 h to determine gravimetric soil moisture. We subsequently measured organic matter (OM) content by ashing dry soils in a muffle furnace at 450 °C for 16 h. To conduct soil pH analyses, we placed 5 g soil samples in 50 mL centrifuge tubes and added 40 mL of 0.01 M $CaCl_2$ to each. We shook the suspensions for 1 h and vortexed them immediately prior to analysis. We measured soil pH using a benchtop electrode pH meter (Orion 5 Star, Thermo Scientific, Beverly, Massachusetts). To measure total C and N, we dried soil samples at 55 °C and ground them to fine powders. We used the dry combustion method to measure total C and N concentrations (Vario El III, Elementar, Lengenselbold, Germany).

To assess treatment effects on C pools and fluxes, we measured extractable C, microbial biomass C, and C mineralization. We extracted organic C from 10 g soil samples with 0.5 M K_2SO_4 and quantified total organic C concentrations in extracts with high-temperature oxidation (1010 TOC analyzer, OI Analytical, College Station, Texas). We determined soil microbial biomass C concentrations by quantifying changes in extractable pools of C after 4 days of chloroform fumigation (Vance et al. 1987). We adjusted our microbial biomass C values to reflect an extractability of 45% (Beck et al. 1997). To determine C mineralization rates, we incubated 5 g soil samples in 40 mL vials fitted with setpa in the laboratory for 3 h at 23 °C. We collected 2 mL headspace samples with a syringe with at the beginning, middle, and end of the incubation and quantified the CO_2 concentrations in the subsamples (LI-6200, Li-Cor Incorporated, Lincoln, Nebraska). C mineralization rates were calculated as the change in C concentrations in the headspace samples per gram of dry soil over time.

We measured extractable inorganic N pools and N mineralization and nitrification rates to assess treatment effects on N pools and fluxes. We extracted inorganic N (ammonium and nitrate) from 4 g soil samples with 2 M KCl. We quantified ammonium concentrations using the salicylate-nitroprusside method (Sims et al. 1995) and measured absorbance at 660 nm on a microtiter plate reader (Synergy HTX,

Biotek, Winooski, VT). We quantified nitrate concentrations using the VCl_3/Griess method (Hood-Nowotny et al. 2010) and measured absorbance at 540 nm on a microtiter plate reader. Total inorganic N is the sum of ammonium and nitrate. We determined net N mineralization rates by quantifying the change in 2 M KCl-extractable pools of ammonium and nitrate in 4 g subsamples after an aerobic 14-day laboratory incubation at 23 °C. We measured net nitrification rates by quantifying the change in nitrate over the same time period. N mineralization and nitrification rates were calculated as the change in N concentrations per gram of dry soil over time.

We assessed treatment effects on soil extractable P pools using a partial Hedley fractionation (Hedley and Stewart 1982). We sequentially extracted P from 5 g subsamples with H_2O (H1; inorganic resin P) and 0.5 M $NaHCO_3$ (H2; bicarbonate P). As the bicarbonate P pool contains both inorganic and organic P, we digested organic P with persulfate prior to quantification (Rowland and Haygarth 1997). We quantified phosphate P concentrations in resin, undigested bicarbonate, and digested bicarbonate solutions using the ammonium molybdate-ascorbic acid method (Kuo 1996; Shaw and DeForest 2013), and measured absorbance at 880 nm on a microtiter plate reader. Total inorganic P is the sum of resin and undigested bicarbonate P, organic P is digested bicarbonate P minus undigested bicarbonate P, and total P is the sum of resin and digested bicarbonate P.

Soil structural analyses

We conducted physical soil analyses on the zero- and six-worm mesocosms. We quantified aggregate distributions by separating aggregates using a rotary sieve with the following sieve sizes: 8 mm, 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, and 0.053 mm. After sieving, we removed the non-soil particles (e.g., rocks and woody debris) from the soil samples, and we used the soil weights of each size fraction to calculate the proportion of the total soil weight in each size class. Because large clumps of soil were frequently found in the 8 mm size class, the 8 mm size class was excluded from subsequent calculations.

We measured the wet aggregate stability of aggregates in the 1 mm size class by gently wetting 4 g of evenly-spread soil on a size 60 mesh screen using capillary action, wet-sieving the soil over cans for 10 min (stroke is 1.3 cm, at 34 times/minute) using a wet aggregate stability tester, and wet sieving the soil remaining on the screen into a can filled with 100 ml of dispersing solution (0.003 M $(NaPO_3)_6$) for several hours until only roots and sand particles remained on the sieve. We calculated the fraction of wet-stable aggregates as the weight of soil obtained in the dispersing solution divided by the sum of the weights obtained in the dispersing solution and the water.

Statistical analysis

We used mixed linear models with forest type (European buckthorn, sugar maple, white oak, or white pine), worm density (0, 1, 3, or 6), and their interaction as fixed effects, collection location as a random effect, and the above variables of interest as dependent variables to evaluate the effects of forest type, worm density, and their interaction on soil biogeochemistry, soil structure, and leaf litter decomposition. Because we only assessed soil structural characteristics for mesocosms with 0 or 6 worms added, worm density had only two levels for these models. Results were considered significant if they had P-values < 0.10. When we detected a significant main effect of forest type or worm density, we conducted a Tukey HSD *post-hoc* test to identify differences among groups. When we detected a significant forest type × worm density interaction, we subset our data by soil type and ran mixed linear models with worm density as a fixed effect and collection location as a random effect to determine if density had an effect on the variable for a given forest type and to identify drivers of the interaction. All statistical analyses were conducted in R using the lmerTest package for mixed linear models (Kuznetsova et al. 2017) and lsmeans package for *post-hoc* tests (Lenth 2016).

Results

Experiment I: tree seedlings

Overall, worm effects on tree seedling growth were tree species-dependent and driven by worm-induced changes in soil properties (Fig. 1a). While relative changes in biomass varied among species ($F = 6.105$; $P = 0.001$), worm presence did not have a main or

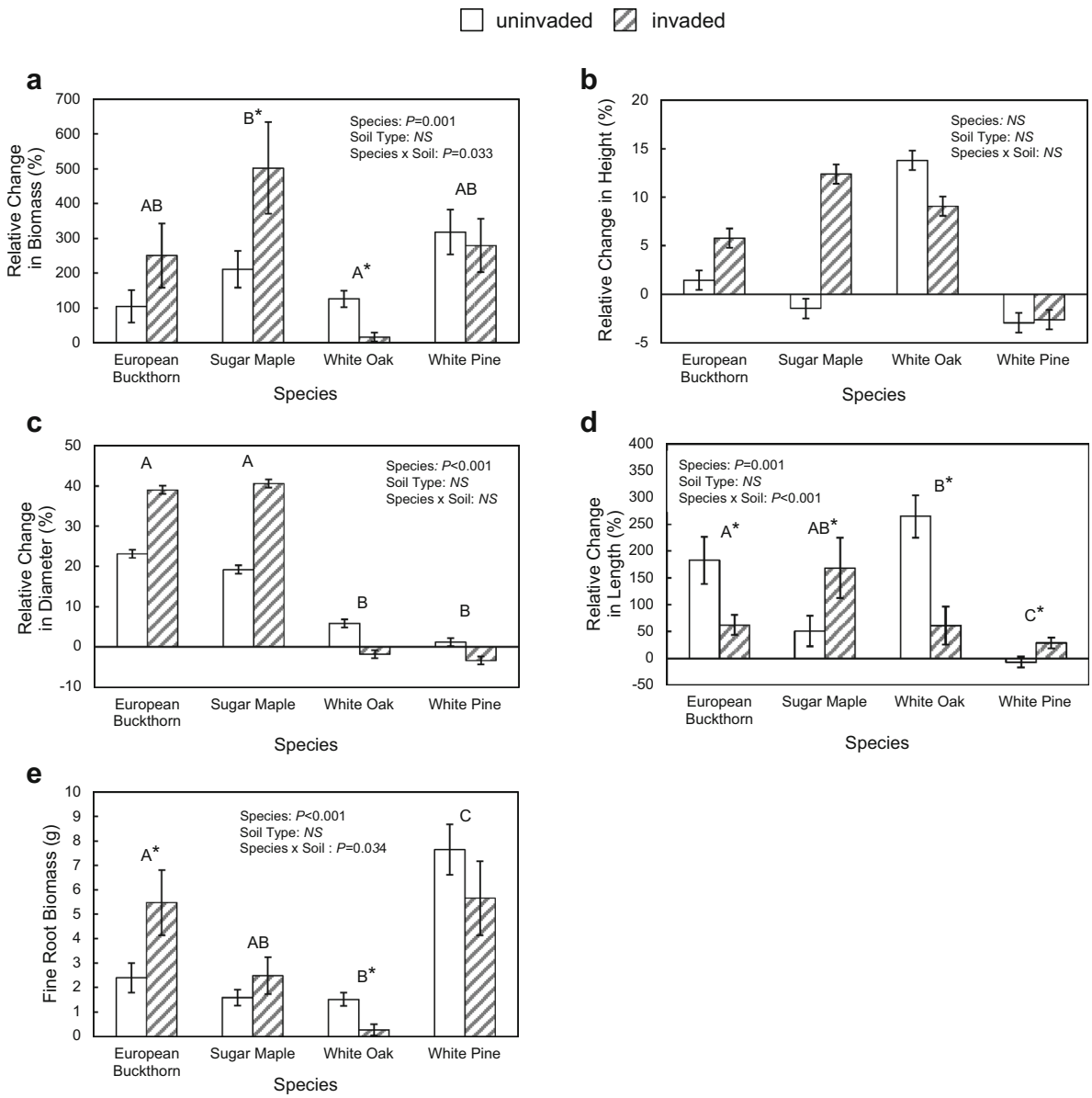


Fig. 1 *Amynthas* spp. effects on growth rates of European buckthorn, sugar maple, white oak, and white pine seedlings. **a** Relative change in total biomass, **b** relative change in stem height, **c** relative change in stem diameter, **d** relative change in root length, **e** fine root biomass. Means and one standard error of each mean are presented (n = 10). Letters denote significant differences among tree species ($P < 0.1$). An * denotes the group or groups driving soil type × worm interactions ($P < 0.1$). Uninvaded soils (open), invaded soils (lined)

interactive impact on the relative change in total seedling biomass (F ≤ 2.035, $P \geq 0.159$), and soil type did not have a consistent effect on the relative change in total seedling biomass (F = 1.956, $P = 0.167$). However, we did detect a species by soil type interaction (F = 3.086; $P = 0.033$). Specifically, worm-affected soil enhanced the relative change in biomass of sugar maple seedlings (F = 4.183; $P = 0.056$) but suppressed the relative change in biomass of white oak seedlings (F = 16.530; $P < 0.001$). Thus, *Amynthas* spp. altered the growth of tree seedlings, but the direction of this impact was tree-species dependent and driven indirectly by worm

effects on soil properties rather than directly by worm presence.

We found no significant worm effects on aboveground processes. Relative change in tree seedling height (Fig. 1b) was unaffected by soil type ($F = 0.975$, $P = 0.327$) or worm presence ($F = 1.863$; $P = 0.177$) and did not vary among species ($F = 1.137$, $P = 0.341$), and there were no significant interactions between soil type, worm presence, and species ($F \leq 1.223$, $P \geq 0.237$). Relative change in stem diameter (Fig. 1c) only varied by species ($F = 10.388$, $P < 0.001$); there were no statistically significant main effects of worm presence or soil type or any significant interactions ($F \leq 1.804$, $P \geq 0.155$).

Belowground, relative change in root length varied among species (Fig. 1d; $F = 6.250$, $P = 0.001$), and there was a species by soil type interaction ($F = 7.948$, $P < 0.001$). White oak and European buckthorn exhibited decreased relative change in root length ($F = 17.77$, $P = 0.002$ and $F = 12.065$, $P = 0.034$, respectively), while white pine and sugar maple exhibited increased relative change in root length ($F = 17.962$, $P = 0.031$ and $F = 13.346$, $P = 0.100$, respectively). Similarly, fine root biomass varied by species (Fig. 1e; $F = 14.892$, $P < 0.001$), and we again detected a species by soil type interaction ($F = 3.067$, $P = 0.034$); via their effects on soil properties (e.g., increased water content, organic matter, and increased N pools), worms had a negative effect on white oak fine root biomass ($F = 17.526$, $P = 0.002$) and a positive effect on European buckthorn biomass ($F = 12.515$, $P = 0.057$).

Experiment II: soil mesocosms

Soil biogeochemistry

Soil biogeochemistry differed among our four forest types in several ways (Online Resource 1). As anticipated, both soil pH and C:N ratio were affected by forest type ($F = 32.030$, $P < 0.001$ and $F = 9.625$, $P < 0.001$, respectively); European buckthorn had the highest pH, and white pine had the lowest pH, while sugar maple and white oak soil pH were intermediate. Similarly, white pine had a higher soil C:N than all the other forest types. In addition, microbial biomass C varied among the four forest types ($F = 5.031$, $P = 0.006$); European buckthorn soil had the greatest microbial biomass while sugar maple soils contained the lowest microbial biomass. Finally, inorganic N fluxes and P pools varied among the four forest types ($F \geq 2.476$, $P \leq 0.080$). In general, white pine soils had higher N fluxes and greater P pools than the other forest types. Forest type had no main effects on water or organic matter content, C and N concentrations, dissolved organic C, C mineralization, inorganic N pools, or inorganic P in the bicarbonate P pool ($F \leq 1.738$, $P \geq 0.176$).

While we did not detect a main effect of worm density on any of our measured soil biogeochemical properties (Online Resource 1; $F \leq 2.375$, $P \geq 0.129$), there were worm density by forest type interactions for several C, N, and P pools (Fig. 2). We found significant worm density by forest type interactions for dissolved organic C (Fig. 2b; $F = 2.270$, $P = 0.090$), nitrate (Fig. 2c; $F = 4.302$, $P = 0.008$), and organic and total P pools (Fig. 2d; $F = 5.150$, $P = 0.003$ and $F = 4.876$, $P = 0.004$, respectively). We also detected a significant worm density by forest type interaction for C mineralization (Fig. 2a; $F = 2.231$, $P = 0.095$). While we only detected significant subset effects of number of worms for nitrate and C mineralization in white oak soils ($F = 2.887$, $P = 0.068$ and $F = 2.611$, $P = 0.099$, respectively), we generally found that as worm density increased, C, N, and P pools and C mineralization decreased in sugar maple soils, but increased in white oak soils, likely driving the significant interaction terms. We did not detect any worm density by forest type interactions for any of our other soil properties, pools, or fluxes ($F \leq 1.666$, $P \geq 0.185$).

Soil structure

Worm additions consistently increased soil aggregation regardless of forest type; worm presence increased the relative abundance of aggregates in the larger size classes and decreased the relative abundance of aggregates in the smaller size classes (Fig. 3). Specifically, worm presence increased the relative abundance of aggregates in the 2 mm size class (Fig. 3b; $F = 21.009$, $P < 0.001$), while worm presence decreased the relative abundance of aggregates in the 500 µm, 250 µm and > 53 µm size classes (Fig. 3d, $F = 9.688$, $P = 0.004$; Fig. 3e, $F = 15.369$, $P < 0.001$; and Fig. 3f, $F = 12.601$, $P = 0.001$,

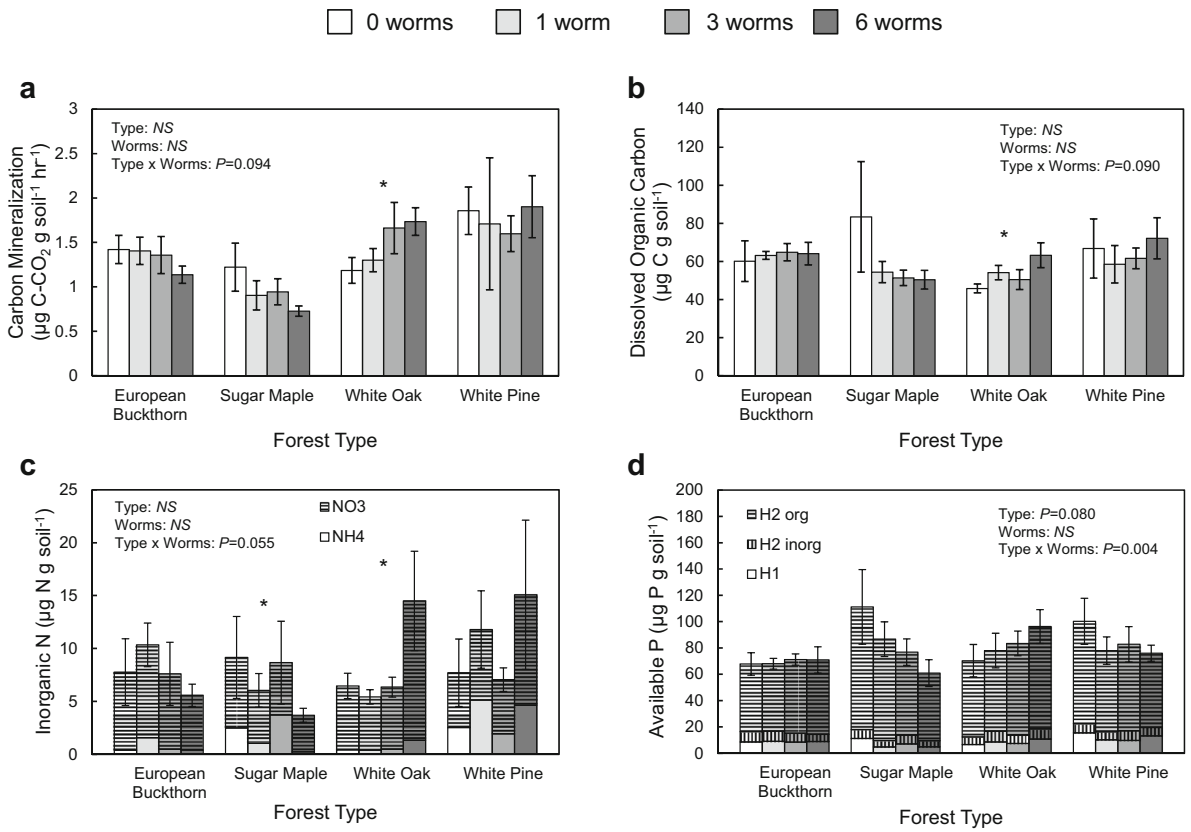


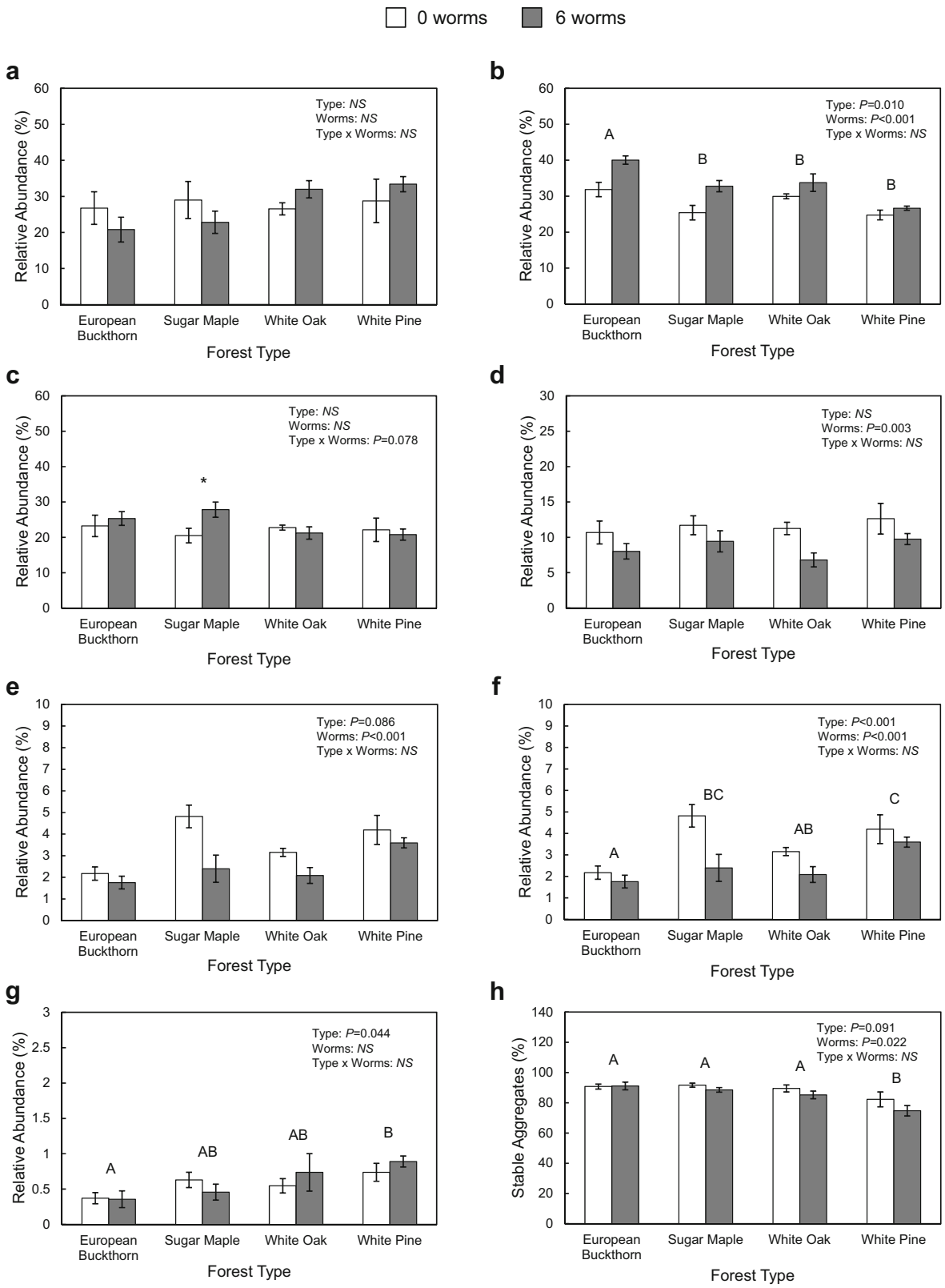
Fig. 2 *Amynthas* spp. density effects (0, 1, 3, or 6 worms) on soil biogeochemical properties in European buckthorn, sugar maple, white oak and white pine soils. **a** Carbon mineralization, **b** dissolved organic carbon, **c** inorganic N split into ammonium and nitrate components, **d** available P split into H1 (inorganic resin extracted P) and H2 (inorganic and organic bicarbonate extracted P) components. Means and one standard error of each mean are presented ($n = 5$). An * denotes the group or groups driving soil type x worm interactions ($P < 0.1$). Worm density increases with shading

respectively). We found no main effects of worms on the largest (4 mm) or smallest (< 53 µm) size classes (Figs. 3a and 3g, respectively; $F \leq 2.100$, $P \geq 0.120$). We only detected an interaction between worms and forest type in the 1 mm size class (Fig. 3c; $F = 2.700$, $P = 0.077$); worms increased the relative abundance of aggregates in the 1 mm size class in the sugar maple soils ($F = 42.905$, $P = 0.003$). In all other size classes, there were no statistically significant worm by forest type interactions ($F \leq 2.100$, $P \geq 0.120$). The relative abundance of aggregates also varied across forest types; we found differences among forest types in the 2 mm, 250 µm, and >53 µm size classes ($F \geq 2.413$, $P \leq 0.086$).

Worms also decreased the aggregate stability of aggregates in the 1 mm size class (Fig. 3h; $F = 6.426$, $P = 0.022$). Aggregate stability varied among the forest types ($F = 2.384$, $P = 0.091$); white pine soils had less stable aggregates than all other forest types. There was no forest type by worm interaction ($F = 1.286$, $P = 0.313$).

Leaf litter decomposition

Factors driving leaf litter decomposition rates varied between the coarse and fine mesh bags (Fig. 4). In the coarse mesh bags, decomposition rates were tree species-dependent (Fig. 4a; $F = 19.938$, $P > 0.001$), worm density-dependent ($F = 4.670$, $P = 0.034$), and there was a species by worm interaction ($F = 3.631$, $P = 0.017$). *Amynthas* spp. directly accelerated the rate of decomposition of European buckthorn leaf litter ($F = 4.232$, $P = 0.022$) but did not directly impact the decomposition rates of the other leaf litters. In contrast, in the fine mesh bags, decomposition rates were only tree species-dependent (Fig. 4b; $F = 9.382$,


0 worms
6 worms
a
Relative Abundance (%)
Type: NS
Worms: NS
Type x Worms: NS
European Buckthorn
Sugar Maple
White Oak
White Pine
Forest Type
b
Type: P=0.010
Worms: P<0.001
Type x Worms: NS
A
B
B
B
c
Type: NS
Worms: NS
Type x Worms: P=0.078
*
d
Type: NS
Worms: P=0.003
Type x Worms: NS
e
Type: P=0.086
Worms: P<0.001
Type x Worms: NS
f
Type: P<0.001
Worms: P<0.001
Type x Worms: NS
A
BC
AB
C
g
Type: P=0.044
Worms: NS
Type x Worms: NS
A
AB
AB
B
h
Stable Aggregates (%)
Type: P=0.091
Worms: P=0.022
Type x Worms: NS
A
A
A
B

◀**Fig. 3** *Amynthas* spp. effects on soil aggregate distribution and stability in European buckthorn, sugar maple, white oak, and white pine soils. **a** 4–8 mm size class, **b** 2-4 mm size class, **c** 1–2 mm size class, **d** 0.5–1 μm size class, **e** 250–500 μm size class, **f** 53–250 μm size class, **g** < 53 μm size class, **h** stable aggregates in the 1 mm size class. Means and one standard error of each mean are presented ($n = 5$). Letters denote significant differences among forest types ($P < 0.1$). An * denotes the group or groups driving soil type x worm interactions ($P < 0.1$). zero worms (open), six worms (filled)

$P < 0.001$). Overall, there was no worm density effect (F = 0.627, $P = 0.431$) or species by worm interaction (F = 0.631, $P = 0.597$) on leaf litter decomposition rates in the fine mesh bags.

Discussion

In this study, we evaluated the ecological consequences of *Amynthas* spp. invasions across forests varying in tree species composition. We found that *Amynthas* spp. effects on tree seedlings were tree species-dependent and indirect; specifically, *Amynthas* spp. decreased the growth of oak seedlings but increased the growth of sugar maple and European buckthorn seedlings indirectly via changes in soil properties. Similarly, while *Amynthas* spp. consistently altered soil structure, their effects on soil C and nutrient dynamics were dependent on forest soil type and worm density. Generally, as worm density increased, C and nutrient availability in sugar maple forest soils decreased, and C and nutrient availability in oak forest soils increased. *Amynthas* spp. addition had no detectable effects on white pine or European buckthorn soil biogeochemistry. Finally, *Amynthas* spp. effects on leaf litter decomposition were also species- and density-dependent. At high worm densities, *Amynthas* spp. increased decomposition rates of European buckthorn litter via direct consumption. Overall, *Amynthas* spp. alter forest ecosystem dynamics via feedbacks among tree species, seedlings, and soil biogeochemistry, but the directions and magnitudes of their effects are tree species-dependent.

Amynthas spp. impacts on tree seedling growth are indirect and tree species-dependent

Amynthas spp. might alter tree seedling growth via two mechanisms: direct consumption of fine roots and hyphae and indirect changes in soil chemistry and structure. In our study, *Amynthas* spp. did not consume the fine roots of tree seedlings, and their presence did not lead to changes in growth. If they did disrupt interactions between roots and mycorrhizal fungi, we did not detect any negative effects. Instead, *Amynthas* spp. indirectly altered seedling growth via changes in soil properties, but these effects were not consistent across tree species. Specifically, white oak growth was negatively impacted by *Amynthas* spp.-altered soil,

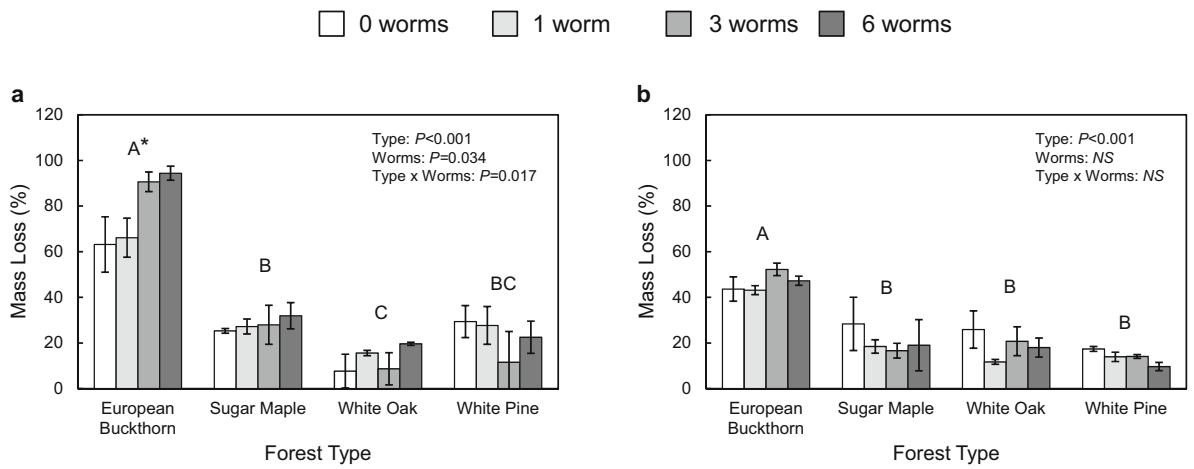


Fig. 4 *Amynthas* spp. density effects (0, 1, 3, or 6 worms) on leaf litter mass loss of European buckthorn, sugar maple, white oak and white pine litters. **a** coarse mesh bags (1 cm mesh), **b** fine mesh bags (1 mm mesh). Means and one standard error of each mean are presented ($n = 5$). Letters denote significant differences among litter types ($P < 0.1$). An * denotes the group or groups driving soil type x worm interactions ($P < 0.1$). Worm density increases with shading

while sugar maple and European buckthorn growth were positively impacted by *Amynthas* spp.-altered soil. These changes in growth were largely driven by soil effects on belowground biomass. Overall, we found that *Amynthas*-spp. effects on tree seedlings were indirect and tree species-dependent.

The indirect effects of *Amynthas* spp. on tree growth were driven by worm-induced changes in soil biogeochemistry rather than changes in soil structure. The *Amynthas* spp.-invaded soil used in this experiment had higher organic matter content, pH, and nutrient availability than the uninvaded soil, which is consistent with *Amynthas* spp.-induced changes in soil properties found in other studies (Burtelow et al. 1998; Greiner et al. 2012; Qiu and Turner 2017). These differences in soil properties also broadly reflect those created by arbuscular mycorrhizal (AM)-associated trees and ectomycorrhizal (ECM)-associated trees; AM soils generally have a higher pH and greater nutrient availability than ECM soils (Phillips et al. 2013). While we are unable to identify the soil chemical changes that drove the indirect effects of *Amynthas* spp. on tree seedling growth, soil N enrichment has been shown to positively affect sugar maple and European buckthorn and negatively affect oaks in the Upper Midwest (BassiriRad et al. 2015; Iannone et al. 2015). Similarly, N deposition generally has positive effects on AM seedling growth and negative effects on ECM seedling growth (Averill et al. 2018). Broadly, *Amynthas* spp.-induced changes in soil chemistry seem to facilitate the positive effects of invaded soils on AM seedlings (sugar maple and European buckthorn) and drive their negative effects on ECM white oaks.

In contrast, changes in soil structure did not seem to alter seedling growth patterns. While we sought to preserve initial differences in soil structure, differences in soil structure did not appear to be maintained between invaded and uninvaded soils over the course of this experiment. However, we did observe clear differences in soil structure between soils to which worms were added and those to which worms were not added; worm-added soils were more granular than soils without worm addition. As we did not detect any direct effects of worms, these visually dramatic differences in soil structure did not seem to affect growth. An important caveat is that any changes in growth would not likely have been due to changes in structure *per se*, but rather, due to structure-driven changes in water availability. During our study, we watered the tree seedlings consistently, and thus, we could not detect interactions between worm presence and enhanced drought sensitivity of our seedlings.

Amynthas spp. effects on soil biogeochemistry depend on forest type

Amynthas spp. had positive, negative, or neutral effects on nutrient availability and flux rates, and these impacts were dependent on both worm density and forest type. Specifically, C mineralization and C, N, and P availability increased as *Amynthas* spp. density increased in white oak forest soils and tended to decrease as *Amynthas* spp. density increased in sugar maple forest soils. Our finding that *Amynthas* spp. decreased mineral nutrient availability in sugar maple soils is surprising given the consistent *Amynthas*-driven increases in mineral nutrients found in other studies. In a similar mesocosm experiment, Qiu and Turner (2017) found that *Amynthas tokioensis* increased nutrient availability and mineralization rates in both prairie and forest soils. Similarly, in an observational study, Burtelow et al. (1998) found that *Amynthas hawayanus* presence was correlated with higher C and N fluxes in a maple-dominated forest. Greiner et al. (2012) also found that *Metaphire hilgendorfi* addition increased N and P availability in an oak soil in a lab mesocosm, though they did not observe this effect in a maple-dominated bottomland site. Our study shows that dominant tree species mediate *Amynthas* spp. effects on forest soil biogeochemistry within a site, but other factors may be important for extrapolating across sites.

In contrast to white oak and sugar maple soils, *Amynthas* spp. did not affect on soil biogeochemistry in white pine or European buckthorn soils. *Amynthas* spp. activity may have been suppressed in the low pH, high C:N white pine soil. Across a small-scale tree species common garden, a variety of earthworm species and functional groups were absent from pine plots due to low leaf litter quality (Reich et al. 2005). Likewise, *Amynthas* spp. may simply be unable to persist in white pine soils, driving their negligible effects on soil biogeochemistry. At the same time, *Amynthas* spp. may have little effect on soils that are already highly modified, like the high pH European buckthorn soil. We found that *Amynthas* spp. directly

consumed the European buckthorn litter but had little effect on soil biogeochemistry. *Amynthas* spp. are not obligate litter feeders, and other research suggests they may preferentially feed on soil biota and rely minimally on leaf litter (Zhang et al. 2010; Snyder et al. 2013). However, despite European buckthorn soils having the highest microbial biomass of all soil types, European buckthorn was the only litter type that *Amynthas* spp. directly consumed. This is a similar finding to that of Heneghan et al. (2007), who found that Eurasian worm abundance was greatest in European buckthorn-dominated sites, and worms preferentially consumed European buckthorn leaf litter. Thus, the *Amynthas* spp. were active in the European buckthorn mesocosms, but their activities had little effect on soil biogeochemistry. In summary, *Amynthas* spp. had no effects on forest soils with more extreme initial chemical properties, but the mechanisms preventing worm-induced biochemical modifications likely differed between the white pine and European buckthorn soils.

Unlike the forest-type specific effects of *Amynthas* spp. on soil biogeochemistry, *Amynthas* spp. effects on soil structure were uniform across soils. In our study, *Amynthas* spp. consistently increased soil aggregation; worm presence increased the relative abundance of aggregates in the larger (> 2 mm) size class and decreased the relative abundance of the smaller size classes. Our findings are consistent with a limited body of research that suggests *Amynthas* spp. alter soil structure (Zhang et al. 2010; Snyder et al. 2011) and specifically increase average aggregate size (Greiner et al. 2012). However, our study is the first to quantify the direct impacts of *Amynthas* spp. on soil aggregate stability. *Amynthas* spp. consistently decreased aggregate stability of the 1–2 mm aggregate size class. Overall, our results demonstrate that *Amynthas* spp. rapidly increase soil aggregation but that those aggregates are more susceptible to disturbance.

While we found no evidence that changes in soil structure were mediated by forest type, in a study conducted by Ziter and Turner (2019), soil 'signature' (e.g., unique blocky casts) depth was greater when *Amynthas tokioensis* were added to European buckthorn-invaded soils compared to uninvaded soils. Though we did not measure soil signature depth, our study examined *Amynthas* spp.-induced changes in soil structure across multiple soils, including European buckthorn-invaded soil, and showed a clear and distinct pattern of increased soil aggregates amongst all forest types. Our results are consistent with a study that found that *Amynthas* spp. rapidly-produce soil casts (and thus, larger aggregates) in widely varying soils types, specifically a low pH Ultisol and a neutral pH Vertisol (Bottinelli et al. 2017).

Synthesis and future directions

Overall, our results suggest that *Amynthas* spp. induce ecological cascades that vary across forest types. *Amynthas* spp. invasion may negatively affect oak seedlings via their impacts on soils in oak tree-dominated ecosystems. White oak seedling growth declined in worm-invaded soils that were richer in C and nutrients than the uninvaded soils, much like those created at high *Amynthas* spp. density in our white oak soil mesocosms. Thus, *Amynthas* spp. invasion may intensify the oak regeneration problem prevalent throughout the Eastern United States (Loftis and McGee 1992).

Surprisingly, sugar maple seedling growth was positively impacted by biogeochemical shifts in our worm-invaded soils. This is in contrast to negative impacts of *Lumbricus* spp. impacts on sugar maple forests. *Lumbricus* spp. mix organic and mineral soil layers, resulting in increased bulk density and decreased fertility, and, ultimately, decreased sugar maple tree and seedling growth (Lavelle et al. 1997; Frelich et al. 2019). In contrast, *Amynthas* spp. are largely limited to the litter and organic layers and may enhance soil fertility, leading to increased sugar maple growth. Alternatively, as mentioned above, sugar maple seedlings may be more susceptible to drought in *Amynthas* spp.-aggregated soils, which we did not evaluate in this study. Furthermore, while the soils used in the seedling study were collected from a maple-dominated forest, *Amynthas* spp. effects on sugar maple soils in our mesocosm study did not track those used in the seedling study. The inconsistent results amongst studies highlight the need for long-term *in situ* studies and experiments that examine the potential ecological cascade effects of *Amynthas* spp. across ecosystems.

Anecdotal evidence suggests that sugar maple seedlings may decline in response to Asian jumping worm invasions, and non-native worms commonly lead to increased plant mortality, especially of sugar maple seedlings (Hale et al. 2008; Laushman et al.

2018). However, little research has been conducted to determine whether *Amynthas* spp. or *Lumbricus* spp. (and *Lumbricus rubellus*, specifically) drive these observations; observations of declines in understory flora and leaf litter cover may be driven by *Lumbricus* spp. prior to Asian jumping worm invasion. Thus, while electronic newsletters, plant clinic alerts, and conservation websites commonly report that *Amynthas* spp. consume leaf litter and alter plant communities, these observations may simply be due to correlations, not causation; these reports may be misleading.

Further *in situ* studies are needed to complement and expand upon our Quonset hut and mesocosm findings. For instance, while we found that worm-invaded soil enhanced the fine root growth of European buckthorn seedlings, European buckthorn may exhibit even higher growth rates in the field given the vigorous growth habit and phenology of the plant; European buckthorn typically holds its leaves late in the growing season and maybe best able to utilize a late-season nutrient pulse from *Amynthas* spp. casts (Qiu and Turner 2017). We were unable to examine such interactions in our experiments. Similarly, while our results demonstrate that *Amynthas* spp. rapidly increase soil aggregation, those aggregates are more susceptible to disturbance; soil disturbance enhances C and nutrient fluxes, which we only observed in our oak soil mesocosms. This may be a more wide-spread phenomena in soils that have been invaded for several years. Finally, we did not evaluate other critical components of the ecological cascade, including *Amynthas* spp. effects on seed predation or seedling germination or the effects of tree species on *Amynthas* spp. abundance and persistence. *In situ* observational studies at *Amynthas* spp. invasion fronts, assessments of relationships between worm density and soil properties across forest types, and field experiments assessing worm effects on leaf litter decomposition and seeds and seedlings are needed to characterize the long-term effects of *Amynthas* spp. invasions on forest ecosystems.

Acknowledgements We thank Michelle Catania, Brendan Brown, Jeremiah Donovan, and the Soil Ecology Laboratory volunteers for their assistance in preparing materials and processing soil and seedling samples; Tom Olsen and the Natural Areas volunteer crew for their assistance in extracting eighty forest soil cores during a very hot summer day; and the members of Community Access Naperville for their assistance with mulching and watering the soil cores. Susan Lewis and Linda Williams graciously provided us with European buckthorn and white pine leaf litter. We thank Bradley Herrick and Marie Johnston at the University of Wisconsin-Madison Arboretum for their assistance in procuring soil for the tree seedling experiment and in *Amynthas* species identification. Additionally, we thank Kurt Dreisilker for allowing the field experiment to be established on The Morton Arboretum grounds. We also thank Wes Beaulieu for his statistical consultations. Jennifer Fraterrigo, Piper Hodson and Renee Gracon provided critical feedback on our experimental design, analyses, and interpretations. This project was funded and supported by the Center for Tree Science at The Morton Arboretum.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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