



Invasive plants exert disproportionately negative allelopathic effects on the growth and physiology of the earthworm *Eisenia fetida*

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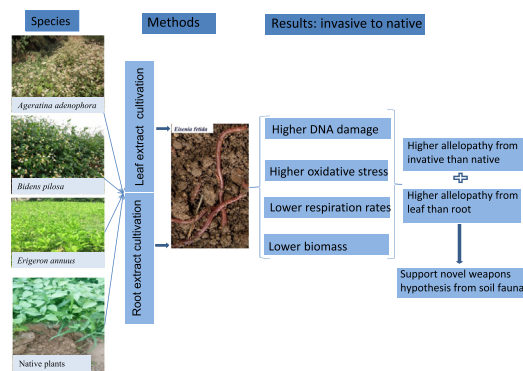
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HIGHLIGHTS

- Molecular and eco-physiological responses of *E. fetida* were sensitive to invasive plant extracts.
- Allelopathic effects of the invasive plants were stronger from leaves than those from roots.
- The inhibitory effects of plant extracts on *E. fetida* increased with extract concentration.
- We provide proof-of-concept for the utility of the earthworm *E. fetida* as a bioindicator for invasive plant allelopathy.

GRAPHICAL ABSTRACT



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ABSTRACT

Exotic invasive plants possess the capacity to disrupt and extirpate populations of native species. Native plants' increased sensitivity to invaders' allelochemicals is a mechanism by which this can occur. However, it is not clear whether and how the allelopathic effects of invasive plants affect members of the soil faunal community – particularly the important functional guild of earthworms. We used the model earthworm *Eisenia fetida* to investigate the responses to extracts from the widely invasive Asterids (*Ageratina adenophora*, *Bidens pilosa*, *Erigeron annuus*) and closely-related native species in a greenhouse experiment. We observed declines in body mass and respiration, and increases in oxidative and DNA damage biomarkers in the native earthworm *E. fetida* when grown under root and leaf extracts from these invasive plants. These effects were concentration-dependent, and worm growth and physiology was most negatively affected under the highest concentrations of leaf extracts. Most importantly, extracts from invasive plants caused significantly more negative effects on *E. fetida* than did extracts from native plant species, indicating allelopathy from invasive plants may inhibit earthworm physiological functioning. These results expand the domain of the novel weapons hypothesis to the earthworm guild and demonstrate the utility of *E. fetida* as a bioindicator for plant allelochemicals.

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1. Introduction

Globalization has led to a sharp uptick in the number of invasive species across the world, which threaten native species and cause

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tremendous economic and ecological damage (Early et al., 2016; Huang et al., 2020). There is evidence that many exotic invasive species benefit from allelopathy through their production of phytotoxic allelochemicals, such as the Asteraceae members *Ageratina adenophora* (Li et al., 2017) and *Chromolaena odorata* (Qin et al., 2013; Zheng et al., 2015). The *novel weapon hypothesis* predicts that native species which are not adapted to invaders' allelochemicals will be adversely affected or even extirpated (Callaway and Ridenour, 2004; Callaway et al., 2008; Inderjit et al., 2011a, 2011b; Qin et al., 2013; Zheng et al., 2015). Despite a rich history of research on the allelopathic effects of invasive plants on native plants (Ni et al., 2010; Becerra et al., 2018), our understanding of how invasive plants' allelochemicals affect the broader belowground community is far less developed (Inderjit et al., 2011b). Recent studies have confirmed that allelochemicals can influence the structure and functioning of soil microbial communities (Thorpe et al., 2009; Coats and Rumpho, 2014; Li et al., 2017). However, compared to the allelopathic effect of invasive plants on native plants, aboveground herbivores, and microorganisms (e.g., Cappuccino and Carpenter, 2005; Schaffner et al., 2011; Enge et al., 2013; Lucero et al., 2019), fewer studies have examined the responses of soil animals to allelochemicals (Xiao et al., 2013; Peralta et al., 2019), and whether these responses are contingent on exudates from particular plant organs (Dorning and Cipollini, 2006; Simao et al., 2010).

Earthworms can constitute up to 60% ~ 80% of a soil's total faunal biomass, and therefore play a critical role in the structuring and functioning of belowground ecosystems (reviewed in Blouin et al., 2013). Consequently, earthworms are widely used as bioindicators as they commonly exhibit molecular, cellular and physiological responses to soil environmental perturbations, such as contamination by heavy metals, organic compounds and pharmaceutical residues (Chakra and Venkateswara, 2008; Wu et al., 2012; Tang et al., 2016; Maity et al., 2018). For example, Tang et al. (2016) found that the respiration of the common earthworm *Eisenia fetida* (Savigny) was inhibited in mercury-polluted soils and Wang et al. (2016) found that *E. fetida* suffers oxidative damage when exposed to naphthenic acids. These types of abiotic environmental perturbations commonly induce oxidative stress and DNA damage (Zhang et al., 2015). Compared to these abiotic factors, far less is known about earthworms' responses to invasive plants and their allelochemicals. There is currently no consensus on the direction of earthworm population responses to invasive plants, with positive (Kourtev et al., 1999), negative (Belnap and Phillips, 2001), or no effects (Hedge and Kriwoken, 2000) having all been reported. These inconsistencies may be related to differences in the concentrations and types of plant exudates added to soils which can vary across plant organs, populations, and species.

Three Asteraceae species native to Central America – *Ageratina adenophora*, *Bidens pilosa* and *Erigeron annuus* – have been widely introduced outside their native ranges and are considered noxious invasive species in Southwestern China, forming dense monocultures in agricultural fields, disturbed forests, and along roadsides (Cui and He, 2009; Ma, 2013; Li et al., 2017). At least one of these species, *Ageratina adenophora*, exerts strong allelopathic effects leading to the extirpation of native plants (Inderjit et al., 2011a; Li et al., 2017). In a pilot survey of earthworms in the field, we encountered significantly lower earthworm densities in plots dominated by the three aforementioned invasive plant species, compared to neighboring plots dominated by closely-related native plants (Fig. S1). However, variation in the biotic and abiotic characteristics of these field plots precludes a precise identification of the mechanisms resulting in these decreased earthworm abundances. To address this, we conducted a controlled greenhouse experiment using the earthworm *Eisenia fetida* – a native species sensitive to soil environmental change and used widely in ecotoxicology (e.g., Chakra and Venkateswara, 2008; Chen et al., 2011; Tang et al., 2016) – as a model to test for allelochemical effects of the aforementioned invasive Asteraceae species. We monitored the growth, physiological responses and degree of DNA damage experienced by *E. fetida* worms cultivated

in varying concentrations of root and leaf extracts from both invasive and native Asteraceae species. We further investigate whether worms' responses to depend on the plant species, plant organ, or chemical concentration. We hypothesize that (1) invasive plants have a stronger allelopathic effects than locally co-occurring native plants on the model earthworm *Eisenia fetida*; (2) physiological responses of *E. fetida* to invasive plant extracts to differ by source plant organ and concentration. The results can answer whether the *novel weapon hypothesis* extends to the earthworm guild.

2. Materials and methods

2.1. Plant materials

In mid-August, we collected leaves and roots of invasive (*A. adenophora*, *B. pilosa* and *E. annuus*) or native (*Galinsoga parviflora*, *Sonchus oleraceus* and *Taraxacum mongolicum*) members of the Asteraceae family at eight invaded sites in Yunnan and Sichuan, southwest China (Table S1). Plants were collected at each site from eight 1 × 1 plots and at least three healthy individuals from the six aforementioned invasive species and native species were collected from each plot. The plants' roots and leaves were removed and air-dried at room temperature for three weeks. Root/leaf samples of invasive individuals from a plot were pooled by species, while dry masses of native species were pooled together to represent a 'native species' treatment. The materials were then ground into fine powder. Allelochemical extracts of leaves and roots were obtained by soaking 2.5 g of dry matter per 100 ml of sterile water (2.5%) for 48 h. Serial dilutions in water were then made to decrease the concentration of these extracts to 1.25 g and 0.625 g per 100 ml. These extract concentrations are generally used to investigate the effect of invasive plant allelopathy (Wang et al., 2014). All extracts were stored at 4 °C prior to use.

2.2. Experimental design

We conducted an earthworm cultivation experiment in a greenhouse at China West Normal University (30°48' N, 106°03' E; 276 m a. s.l.) in Sichuan, southwest China. In this area, *Eisenia fetida* is a native species and commonly grow in local farmland (Huang et al., 2006; Xie et al., 2016). Greenhouse temperature was maintained at 20 ± 1 °C, and the relative air humidity was 80%. Surface soil (0–15 cm) was collected from farmland containing no invasive species near the experimental site. The soil was a Cambisol (pH 7.9) containing 12.22 g kg⁻¹ organic carbon, 0.88 g kg⁻¹ total nitrogen, 0.92 g kg⁻¹ total phosphorus, and 74.8 mg kg⁻¹ available potassium (Liu et al., 2020). Soils were sifted through a 0.35 mm-diameter mesh and all earthworms were removed.

We obtained *E. fetida* worms of approximately similar size (ca. 5 cm in length) from a cultivator in Jiangsu, China. We purged the worms' gut contents by immersion in distilled water for 48 h in a culture dish at 20 ± 1 °C. After being rinsed in distilled water and dried on filter paper, three individual earthworms were introduced to a 1 l pot filled 90% with prepared soil. Five milliliters of liquid extracts from roots or leaves of each plant species (3 invasives or pooled natives) were added to a pot every other day for four weeks, and an equal volume of sterile water was used as a control treatment. To prevent worms from escaping, each pot was sealed with a porous transparent film. Pots were replicated 5 times, resulting in a total of 125 species × organ × concentration treatments (including water controls).

2.3. Measurement

Two and four weeks after the start of the experiment, measurements of earthworm respiration were made between 1900 and 2330 h using a gas exchange system (Li-Cor Inc., Lincoln, NE, USA). Five individuals from each treatment were randomly chosen, carefully washed in deionized water, and dried on filter paper. The individual earthworm was

placed into a plexiglass cuvette (7.5 cm diameter × 3.5 cm in length) coupled to the system, which was covered by a black cloth. Respiration was measured in the following controlled conditions: CO₂ concentration of 400 μmol mol⁻¹, and cuvette temperature of 20 °C. Respiration rate was recorded in these conditions for 30 min. After completion of the measurements, the dry mass of the earthworm was measured after oven-drying at 70 °C to a constant mass. The mass-based respiration rate was calculated as the ratio of the respiration rate to dry mass.

To further characterize the earthworm physiological changes, the activities of superoxide dismutase (SOD), glutathione S-transferases (GST) and catalase (CAT) and malondialdehyde (MDA) content were measured. One individual from each treatment was randomly chosen, and washed with sterile distilled water and transferred to clean wet filter paper to purge for 12 h. Earthworms were homogenized in 0.05 M ice-cold homogenization Na₂HPO₄-KH₂PO₄ buffer (pH 7.8). The homogenate was collected in separated tubes and centrifuged for 10 min at 13,000g under 4 °C. The supernatant was divided into several aliquots and stored at -20 °C for the determination of antioxidant enzyme activities (GST, SOD, CAT, POD), the MDA content and the protein concentration.

Activities of SOD, CAT and POD were measured according to the method described by Zhang et al. (2015). The amount of enzyme that inhibited the NBT reduction by 50% was considered as one unit (U) of SOD activity when monitored at 560 nm. The amount of enzyme that decomposed half of H₂O₂ in 100 s at 25 °C was considered as one unit of CAT activity. One unit of POD was defined as the amount of enzyme that caused an increase of 0.01 absorbance units per minute. The glutathione S-transferase (GST) activities were assayed by measuring its capacity to combine glutathione (GSH) and 1-chloro-2,4-dinitro-benzene (CDNB). One unit of GST was defined as the quantity of GST leading to combination of GSH and CDNB in per min at 340 nm. Total protein contents were assayed according to the method of Bradford (1976) using bovine serum albumin as the standard. Content of MDA determination using the thibabaturic acid colorimetric method (TBA) described by Liu et al. (2018). Briefly, 2 ml supernatants and added with SDS solution (8.1%, w/v), acetate buffer (20%, w/v), TBA solution (1%, w/v) and incubated at 95 °C for 1 h. The mixture was centrifuged at 1000g for 15 min. The absorbance of the supernatants was determined at 532 nm.

The extent of DNA damage of earthworms exposed to extract from invasive or native species was assessed from the Olive tail moment (OTM), which is the product of the tail length and the fraction of total DNA in the tail of a DNA comet assay. After exposure to extracts, earthworm coelomocytes were extracted and the alkaline comet assay was

performed following the modified method described by Zhang et al. (2015). The extent of DNA damage was categorized by the tail DNA% according to the following criteria: tail DNA < 10%, zero or minimal damage to DNA; 10–25%, low damage; 25–50%, inter mediate damage; 50–75%, high damage; and > 75%, extreme damage.

2.4. Statistical analysis

Three-way analyses of variance (ANOVA) was used to evaluate the effects of different extract treatments of species, organ and extract concentrations on the dry mass, respiration rate, antioxidant enzymes activity, malondialdehyde and OTM. The normality and homoskedasticity of model residuals were verified before proceeding. The differences in the traits among treatments were determined by Turkey’s HSD tests at a significance level of α = 0.05, after correcting for multiple comparisons. All statistical analyses were carried out using the SPSS 22.0 for Windows statistical software package (SPSS Inc., Chicago, IL, USA).

3. Results

Dry masses of *E. fetida* were different among extract treatments, and were related to plant species identity and organ (Table 1; Fig. 1). In general, we found that higher extract concentrations and extracts from invasive species corresponded to lower dry masses of *E. fetida* under both root and leaf extracts, and these results were apparent after both two and four weeks of growth. Dry masses of *E. fetida* irrigated with leaf extracts were similar to those irrigated with root extracts at each extract concentration (Fig. 1). Overall, leaf extracts from the invasive *A. adenophora* had the greatest suppressive activity on worm growth across all concentrations and organs. All *post-hoc* pairwise comparisons are listed in Table S3.

The effects of the organ extracts on *E. fetida* were further investigated by quantifying the worms’ respiration rates. With a few exceptions, respiration rates of *E. fetida* at 14 days did not differ between invasive and native extracts or between organs. However, significant differences were detected after four weeks in worms’ respiration rates under various species and organ treatments. *E. fetida* respiration rates exposed to invasive plants’ extracts were generally lower 25.2% (root extracts) and 42.5% (leaf extracts) than those exposed to native plants’ extracts after four weeks. Furthermore, respiration rates of *E. fetida* grown in invasive leaf extracts were lower 33.8% than those from root

Table 1 ANOVA results for growth and biochemical effects of extracts on *Eisenia fetida*.

	Species (S)		Organ (O)		Concentration (C)		S × O		S × C		O × C		S × O × C	
	F _(3,120)	P	F _(1,120)	P	F _(2,120)	P	F _(3, 120)	P	F _(6, 120)	P	F _(2, 120)	P	F _(6, 120)	P
14 days														
Mass	27.63	<0.001	285.96	<0.001	267.92	<0.001	5.79	0.001	2.56	0.024	29.67	<0.001	0.31	0.933
RR	7.05	<0.001	15.46	<0.001	324.61	<0.001	0.18	0.912	0.79	0.581	38.40	<0.001	2.89	0.012
SOD	15.95	<0.001	25.78	<0.001	107.31	<0.001	2.83	0.042	1.06	0.391	14.42	<0.001	0.62	0.716
CAT	25.14	<0.001	10.82	0.001	109.18	<0.001	4.24	0.007	3.19	0.007	31.89	<0.001	1.70	0.129
POD	33.08	<0.001	63.36	<0.001	238.61	<0.001	3.59	0.016	22.41	<0.001	61.83	<0.001	3.44	0.004
GST	31.56	<0.001	29.93	<0.001	144.79	<0.001	1.56	0.203	5.06	<0.001	21.52	<0.001	3.05	0.009
MDA	29.59	<0.001	93.17	<0.001	251.23	<0.001	4.43	0.006	2.95	0.011	19.42	<0.001	1.57	0.163
OMT	26.91	<0.001	91.06	<0.001	140.53	<0.001	3.96	0.010	2.16	0.054	13.29	<0.001	1.32	0.254
28 days														
Mass	142.26	<0.001	259.27	<0.001	315.17	<0.001	10.22	<0.001	6.18	<0.001	5.04	0.008	2.60	0.022
RR	64.67	<0.001	128.07	<0.001	210.64	<0.001	3.20	0.027	0.22	0.969	8.08	0.001	1.15	0.339
SOD	99.67	<0.001	192.07	<0.001	147.21	<0.001	6.82	<0.001	4.83	<0.001	15.26	<0.001	0.24	0.962
CAT	16.82	<0.001	21.55	<0.001	14.14	<0.001	1.54	0.209	4.67	<0.001	21.00	<0.001	14.57	<0.001
POD	17.01	<0.001	18.11	<0.001	67.08	<0.001	1.91	0.133	9.73	<0.001	48.05	<0.001	5.52	<0.001
GST	1.86	0.142	9.47	0.003	37.88	<0.001	5.10	0.003	26.15	<0.001	3.71	0.028	11.70	<0.001
MDA	154.16	<0.001	123.55	<0.001	202.13	<0.001	4.71	0.004	12.35	<0.001	12.25	<0.001	0.72	0.636
OMT	75.86	<0.001	178.39	<0.001	173.97	<0.001	14.83	<0.001	7.31	<0.001	95.23	<0.001	3.82	0.002

RR, respiration rate; SOD, superoxide dismutase, CAT, catalase; POD, guaiacol peroxidase; GST, glutathione S-transferases; MDA, malondialdehyde; OMT, Olive tail moment.

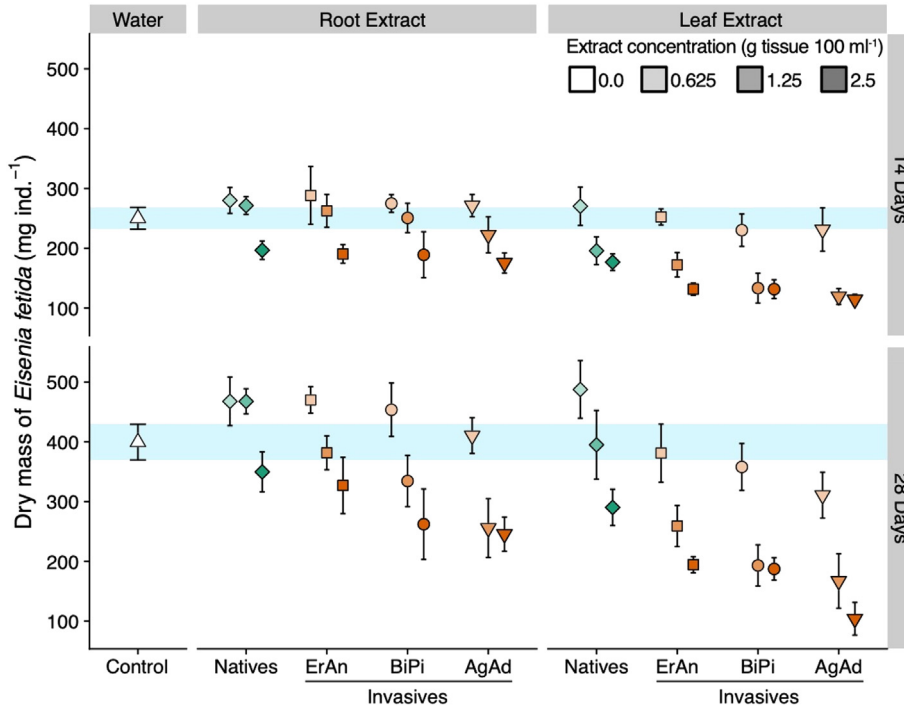


Fig. 1. Dry mass of *Eisenia fetida* incubated under each concentration from roots and leaves extract of invasive and native species treatments in two weeks (a) and four weeks (b). Mean \pm 95% CI ($n = 5$). Invasive plants include *Erigeron annuus* (ErAn), *Bidens pilosa* (BiPi), and *Ageratina adenophora* (AgAd). The native pool of plants included *Taraxacum mongolicum*, *Galinsoga parviflora*, and *Sonchus oleraceus*. Horizontal blue area is 95% CI of the control (no extract). Post-hoc comparisons are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extracts after four weeks, while this difference was not detected between extracts of native species (Fig. 2).

Activities of antioxidant enzymes SOD, CAT, POD and GST of *E. fetida* differed among different extract treatments, and were related to plant

species, plant organ, and exposure time (Table 1; Fig. 3). At both two and four week time points, SOD, CAT, POD and GST activities of *E. fetida* exposed to native plants' extracts at 0.625% and 1.25% concentrations did not differ from water controls. However, these traits increased in

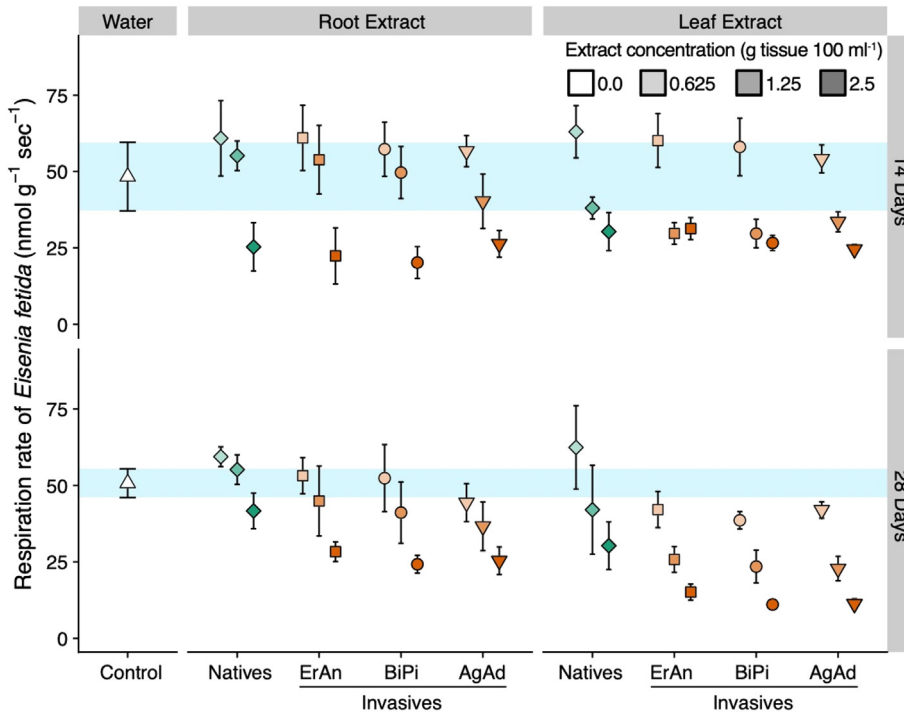


Fig. 2. Respiration rate (20 °C) of *Eisenia fetida* incubated under each concentration of roots and leaves extract of invasive and native species in two weeks (a) and four weeks (b). Mean \pm 95% CI ($n = 5$). Invasive plants include *Erigeron annuus* (ErAn), *Bidens pilosa* (BiPi), and *Ageratina adenophora* (AgAd). The native pool of plants included *Taraxacum mongolicum*, *Galinsoga parviflora*, and *Sonchus oleraceus*. Horizontal blue area is 95% CI of the control (no extract). Post-hoc comparisons are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

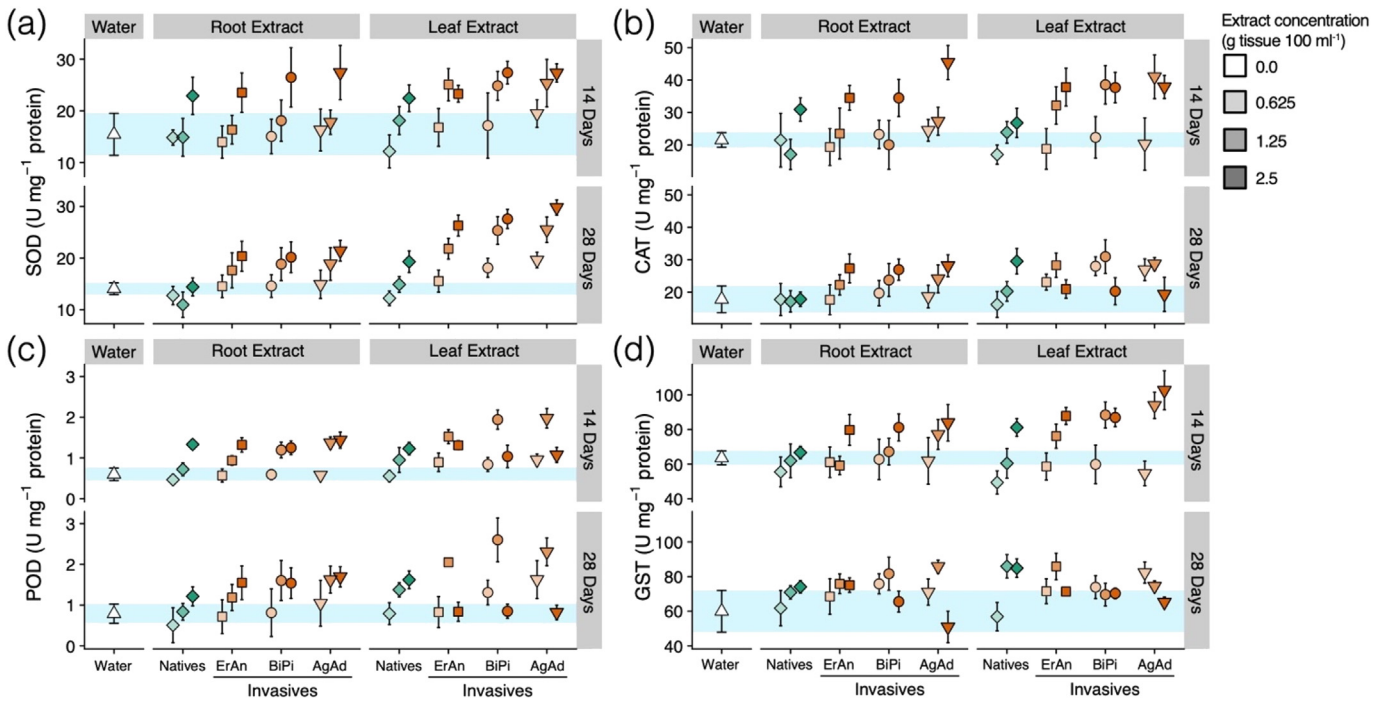


Fig. 3. Antioxidant enzyme uperoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) activities of *Eisenia fetida* incubated under each concentration of roots and leaves extract of invasive and native species in two weeks (a–d) and four weeks (e–h). Mean \pm 95% CI ($n = 5$). Invasive plants include *Erigeron annuus* (ErAn), *Bidens pilosa* (BiPi), and *Ageratina adenophora* (AgAd). The native pool of plants included *Taraxacum mongolicum*, *Galinsoga parviflora*, and *Sonchus oleraceus*. Horizontal blue area is 95% CI of the control (no extract). Post-hoc comparisons are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

worms grown under 1.25% leaf extracts and 2.5% root and leaf extracts (Fig. 3). Antioxidant enzyme activities of *E. fetida* were generally higher under extracts from invasive plants than from native plants the same

concentration, though these differences were greatest at the highest extract concentrations. Leaf and root extracts of each species had generally similar effects on enzyme activity. Positive, unimodal, and negative

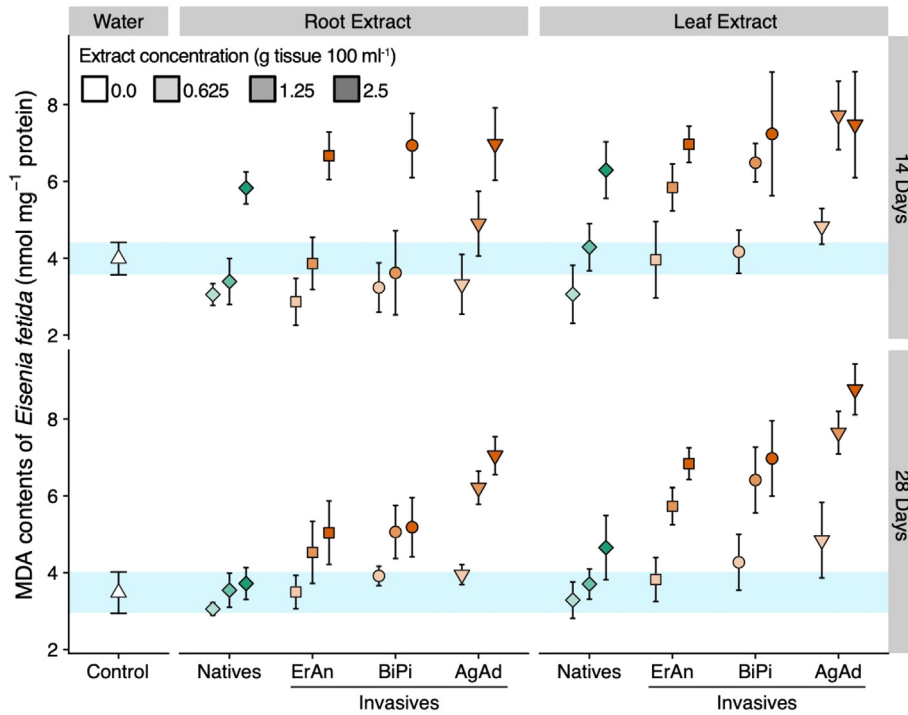


Fig. 4. Malondialdehyde (MDA) content of *Eisenia fetida* incubated under each concentration of roots and leaves extract of invasive and native species in two weeks (a) and four weeks (b). Mean \pm 95% CI ($n = 5$). Invasive plants include *Erigeron annuus* (ErAn), *Bidens pilosa* (BiPi), and *Ageratina adenophora* (AgAd). The native pool of plants included *Taraxacum mongolicum*, *Galinsoga parviflora*, and *Sonchus oleraceus*. Horizontal blue area is 95% CI of the control (no extract). Post-hoc comparisons are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration-dependence was detected for CAT, POD, and GST, particularly in invasive plant leaf extracts, as well as root extracts for GST (Fig. 3).

The oxidative damage biomarker MDA significantly varied across species, organ and concentration treatments (Table 1; Fig. 4). Water controls did not generally differ from native plant extracts, but MDA contents from invasive plant extract treatments were significantly higher at moderate to high concentrations, and particularly at 28 days. No systematic significant differences were detected in MDA expression between root and leaf extracts, though leaf extract treatments showed marginally greater MDA contents. Overall, the highest MDA contents of *E. fetida* in each extract treatment were recovered from *A. adenophora*.

Olive tail moment (OTM), an indicator of DNA damage, varied among organ, species, and concentration treatments (Table 1). Compared to controls, OTM of *E. fetida* was not affected by 0.625% extract, increased under 1.25% leaf and 2.5% invasive leaf and root extracts, with the highest OTM being found in extracts from *A. adenophora* (Fig. 5). Conversely, OTM from native species' extracts only differed from water at the highest concentrations.

4. Discussion

4.1. Earthworms sensitivity to invasive plant extracts

In this study, we found that the growth, respiration, antioxidant activity and DNA damage of *E. fetida* were sensitive to plant leaf and root extracts. Our results contribute evidence for the increased allelopathic capacity of invasive species – a prediction of the novel weapon hypothesis (Callaway and Ridenour, 2004) – on members of the soil faunal community. Compared to extracts from native species, invasive plant extracts significantly decreased growth and respiration of *E. fetida*, supporting the results of our pilot surveys showing suppressed earthworm abundances in plots dominated by invasive plants (Fig. S1). In concert, these results suggest that compared to native plants, invasive plants exert stronger inhibitory effects on *E. fetida* – an observation

consistent with previous studies (Belnap and Phillips, 2001; Thorpe et al., 2009). However, we encountered positive effects on *E. fetida* mass under low-concentration root extracts, which may be related to increased nutrient inputs from invasive plant via roots (Kourtev et al., 1999). While our study was conducted on only one earthworm taxon, other species may respond differently to our experimental treatments, and we recommend future studies expand on our findings to assess how interspecific differences in earthworm tolerances to allelochemicals might help structure the local earthworm community as a whole. The success of a plant invasion is likely linked to its allelopathic effects on native plant species (Bais et al., 2003; Simao et al., 2010), and our results contribute new evidence for the broader allelopathic effects of exotic invasive plants on soil fauna.

Previous studies on the antioxidant enzyme activities of *E. fetida* have mainly concerned xenobiotic contaminants such as heavy metals (Chen et al., 2011; Zhang et al., 2015; Liu et al., 2018). Antioxidases play important roles in preventing oxidative damage to cellular components by eliminating reactive oxygen species (ROS). For example, superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals (O_2^-) to H_2O_2 , which is subsequently detoxified by peroxidase (POD) and catalyzed by catalase (CAT) into O_2 and H_2O (Chen et al., 2011; Zhang et al., 2015; Chen et al., 2016). In earthworms, elevated levels of these antioxidant enzymes signal environmental stress are being prioritized at various time points under chronic stress treatments (Zhang et al., 2015; Maity et al., 2018).

Increases in MDA, a major oxidation product of cellular lipid membranes, was also increased in moderate and high extract concentrations. MDA is another signal of oxidative stress in the earthworm toxicology (Chen et al., 2011). After two weeks of exposure, the MDA contents of worms grown in invasive plant extracts were similar to those grown under native plant extracts, suggesting that increases in antioxidant enzymes were suppressing oxidant stress (Zhang et al., 2015). After four weeks, however, MDA contents in invasive plant extracts surpassed those from native extracts, suggesting that *E. fetida* suffered a higher oxidant stress under invasive species than that native species during

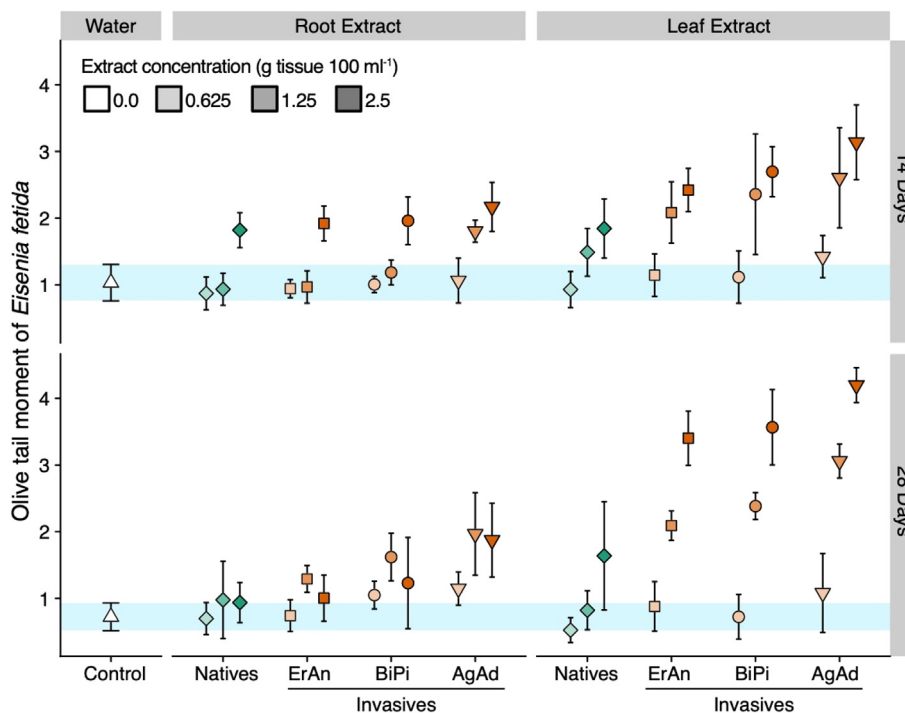


Fig. 5. The OTM responses of coelomocytes in *Eisenia fetida* incubated under each concentration of roots and leaves extract of invasive and native species in two weeks (a) and four weeks (b). Mean \pm 95% CI ($n = 5$). Invasive plants include *Erigeron annuus* (ErAn), *Bidens pilosa* (BiPi), and *Ageratina adenophora* (AgAd). The native pool of plants included *Taraxacum mongolicum*, *Galinsoga parviflora*, and *Sonchus oleraceus*. Horizontal blue area is 95% CI of the control (no extract). Post-hoc comparisons are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chronic exposure, which may be due the decreases in CAT and POD (Chen et al., 2011; Zhang et al., 2015; Wang et al., 2016). In addition, the activity of GST, an important enzyme in cellular detoxification, showed negative concentration-dependent responses in both leaves and roots, which may be associated with the interference of lipid biosynthesis (and MDA levels) in earthworms (Zhang et al., 2015).

Our results suggest that higher extract concentrations may tilt the balance between the production and removal of ROS which can result in cellular oxidative damage (Wu et al., 2012). Oxidative damage inflicted by excess ROS has important pathophysiological effects for earthworms, including cell injury, decreased metabolic processes and increased mortality risk (Dittbrenner et al., 2010). Our results support this causal chain, with exposure to invasive plant extracts resulting in DNA damage, lower respiration rates, lower body masses, and decreased population densities. However, our proposed causal chain remains correlative, and further studies are needed to investigate the roles of the individual biomolecules in plant extracts responsible for the observed allelopathic effects. Furthermore, we note that while our field surveys were designed to mitigate variation in soil quality between paired invasive and native-dominated sites, unobserved systematic variation in certain environmental factors cannot be discounted from our results, and so our density surveys remain speculative. Nonetheless, we encountered detrimental effects of invasive plants independent of the soil environment in our greenhouse studies, which helps explain our field survey results. The earthworm *E. fetida* is increasingly being used as a bioindicator for soil contaminants (Chen et al., 2011; Duan et al., 2017; Liu et al., 2018), our results show that the species may also be a useful bioindicator for allelopathy by invasive plants.

4.2. The allelopathic effects of different plant organs

Both leaf leachates and root exudates can contribute allelochemicals to the soil (Bais et al., 2003; Xu et al., 2020). Organ extracts provide a good way to demonstrate the potential allelopathic effects of different plant organs (Dorning and Cipollini, 2006), however, the differential effects of leaf and root-derived allelochemicals are not well understood, as experimental tests are scarce (*but see* Dorning and Cipollini, 2006; Cipollini et al., 2008). Our experimental results suggest that leaf extracts of invasive plants have stronger allelopathic effects than root extracts — an observation in line with a previous study (Dorning and Cipollini, 2006). This outcome may be explained by an adaptive strategy hypothesized to contribute to plant invasion success. In their nonnative ranges, such species often exhibit above an “aboveground first” strategy by increasing shoot:root biomass ratios, which can lead to invaders' enjoying competitive advantages and increased herbivory tolerance, and, as a byproduct, increased litter leachate contributing to allelopathy (Huang et al., 2012). Previous studies on the allelopathic effects of invasive plants have focused primarily on root exudates and less so on leaf inputs. Our results highlight the potentially important allelopathic contributions of invasive plant leaves on earthworms, and we encourage further research on plants' organ-dependent allelopathic effects on soil fauna.

4.3. A potential feedback between plant invasions and earthworms

Exotic invasive plants can alter the structure and function of soil ecosystems (Ehrenfeld, 2010; Liebhold et al., 2017). Likewise, earthworms perform important roles in the breakdown of soil organic matter and contribute to soil structure and fertility (Zhang et al., 2013), thereby influencing plant growth (Zhao et al., 2013; Biswas et al., 2018; Xiao et al., 2018). Because of this, our findings of exotic plant inhibition of earthworms highlight the potential for a cascading effect of exotic plants on the physicochemical properties of soil, mediated through earthworms' responses (Bardgett and Van Der Putten, 2014; Xiao et al., 2018; Xu et al., 2020). Such a cascade might then amplify invasion opportunities for nonnative plants, which may be better able to colonize

these earthworm-depauperate soils (Belnap et al., 2005). However, in line with previous studies (Kourtev et al., 1999; Madritch and Lindroth, 2009), we also encountered some positive effects of low-concentration plant extracts on worm responses. Thus, further studies — particularly field experiments — are encouraged to examine the potential for dynamic feedbacks between invasive plant and soil faunal communities.

5. Conclusions

In summary, we have demonstrated the suppressive potential of exotic invasive plant species on the growth, physiology and DNA repair of the widely-distributed earthworm *E. fetida*. These effects were related to the species, organ source, and concentration of plant extracts. Stronger negative effects were observed in worms reared in leaf compared to root extracts, though this result only manifested at higher concentrations. These results may be explained by our findings that invasive plants' exudates negatively impacted *E. fetida* growth rates, physiological traits, and DNA repair, compared to blank and native plant controls. We suggest that our focal invasive plant species imposed relatively higher allelopathic effects on *E. fetida* than did related native plants. Together, these results contribute evidence for a disproportionate allelopathic effect of invasive plants on earthworms' physiology and growth. Given the importance of earthworms in belowground systems worldwide, these findings motivate the potential for earthworm-mediated cascading effects triggered by plant invasions, and we encourage future studies to quantify these effects and potential mitigation strategies.

CRedit authorship contribution statement

Junyan Liu: Resources, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Gang Xu:** Resources, Investigation. **Lingzi Yin:** Resources, Investigation. **Xiao Xu:** Methodology. **David W. Armitage:** Formal analysis, Writing - original draft, Writing - review & editing. **Tingfa Dong:** Methodology, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.141534>.

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