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Can the invasive earthworm, *Amynthas agrestis*, be controlled with prescribed fire?

Hiroshi Ikeda [a, *], Mac A. Callaham Jr. [b], Joseph J. O'Brien [b], Benjamin S. Hornsby [b], Evelyn S. Wenk [b]

[a] *Faculty of Agricultural and Life Science, Hirosaki University, Hirosaki, Japan*
[b] *Center for Forest Disturbance Science, Southern Research Station, USDA Forest Service, Athens, GA, USA*

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ABSTRACT

Biological invasions are one of the most significant global-scale problems caused by human activities. Earthworms function as ecosystem engineers in soil ecosystems because their feeding and burrowing activities fundamentally change the physical and biological characteristics of the soils they inhabit. As a result of this "engineering," earthworm invasions can have significant effects on soil physical, chemical and biological properties. The species *Amynthas agrestis* (family Megascolecidae) was introduced to the United States from Asia, and has expanded its distribution range to include relatively undisturbed forests. Here, to clarify life history traits, we reared individuals under seven different conditions of food provision using litter, fragmented litter and soil, and also analyzed the stable isotope ratios of field-collected specimens to investigate their food resources in the field. Second, we examined whether prescribed fire can be used to manage invasive earthworms. We constructed eight experimental plots, each with 100 individuals of *A. agrestis* each, and burned half of the plots. The feeding experiment showed that the earthworms in units containing soil and some form of organic matter (litter and/or fragmented litter) produced many cocoons, indicating that litter and fragmented litter are important food resources for them. Stable isotope analyses also supported this result. During the experimental fires, average soil temperature at 5 cm depth increased by only 7.7 °C (average maximum of 32.2 °C). Litter mass was significantly reduced by the fires. Although numbers of *A. agrestis* and cocoons recovered from burned and unburned plots were not different, the viability of cocoons was significantly lower in burned plots. Fire may also reduce the survival rate of juveniles in the next year by depriving them of their preferred food resource. Most native earthworms in the United States live in the soil, while many invasive ones live in the litter layer and soil surface. Therefore, prescribed fire could be a viable tool for control of invasive earthworms without negatively impacting native earthworm populations.

1. Introduction

Biological invasions are one of the most significant global-scale problems caused by human activities (Vitousek et al., 1997). Whereas conspicuous or large invasive species have received much attention from researchers, news media and policy makers, many species of inconspicuous soil fauna have also been introduced around the world both intentionally and accidentally (Ehrenfeld and Scott, 2001), and their effects on native ecosystems are not well understood. These effects can be strong if the invading species has functional attributes that are not represented in the indigenous species pool (Wardle et al., 2004).

Earthworms are among the most important soil invertebrates involved in nutrient and energy cycling in various ecosystems because they strongly regulate the decomposition of leaf litter (Hendrix and Bohlen, 2002). Earthworms are often considered ecosystem engineers because their feeding and burrowing activity causes changes to the physical, chemical and biological characteristics of the soils they inhabit. Ultimately, these engineering effects can alter the community structure of other soil organism groups (Jones et al., 1994; Anderson, 1995). For example, in forested ecosystems of North America, invasive earthworms have been documented to aggressively consume leaf litter, causing changes in the native soil

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* Corresponding author. Faculty of Agricultural and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan.
E-mail address: hikeda@cc.hirosaki-u.ac.jp (H. Ikeda).

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invertebrate, microbial, and plant communities (e.g., McLean and Parkinson, 2000; Hale et al., 2006; Snyder et al., 2011). Many earthworm species have spread beyond their origins, and have caused such effects on native organisms (Hendrix et al., 2006, 2008). Thus, developing management strategies for non-native earthworms is a growing matter of concern for ecosystem conservation.

Since most native earthworm species in North America (particularly *Diplocardia* species) live in the soil (James and Cunningham, 1989; Jordan et al., 2000; Callaham et al., 2001; Callaham personal observation), many non-native earthworms living in the litter layer or soil surface were able to invade and establish in North America (Hendrix and Bohlen, 2002). Sixty of 246 species reported in North America are non-native (Reynolds and Wetzel, 2008). It is thought that *Amynthas* species (family Megascolecidae) originally from Asia were brought to North America via soil with Asian plants, and spread with horticultural trade and as fishing bait in the United States (Gates, 1958, 1982). *Amynthas agrestis* (Fig. S1, Goto and Hatai, 1899) is one wide-spread Asian invasive species, of which the first report in the US was in 1939 in Maryland (Gates, 1982), and has expanded its distribution range to include not only disturbed areas, but also relatively undisturbed forests in 17 states in the eastern United States (Callaham et al., 2003b; Reynolds and Wetzel, 2008). *Amynthas agrestis* shows strong invasiveness, as its density is sometimes 10 times greater than the other non-native species, it negatively affects native millipedes and other non-native earthworms by changing soil microbial community composition (Callaham et al., 2003b; Zhang et al., 2010; Snyder et al., 2011). Thus, it appears that this species may have potential to continue to expand its distribution range in the future, and this suggests that development of control strategies will be of critical importance to managing these invasions.

Epigeic *Amynthas* species including *A. agrestis* live primarily in the litter layer (Ishizuka, 2001), and have an annual life cycle: they produce cocoons in late summer and early autumn, overwinter in the cocoon stage, cocoons hatch in early spring, and adults mature in summer (Uchida, 2004; Uchida and Kaneko, 2004). Callaham et al. (2003b) collected earthworms using pitfall traps in the Southern Appalachian Mountains of northern Georgia during summer and autumn, and showed that the first adults of *A. agrestis* were trapped in late August. Some short-term feeding experiments were also conducted for *A. agrestis* (Richardson et al., 2009; Zhang et al., 2010). However, their basic ecological traits such as feeding preferences and reproductive attributes are still not well understood. To develop effective management methods for invasive species, it is critical to understand their life history over the course of an entire generation.

Prescribed fire has long been a management tool in forested ecosystems across North America (Ryan et al., 2013), and has been used to manage invasive plant species (e.g., Ditomaso et al., 2006). In southeastern US forests, prescribed fire is used primarily as a method of reducing hazardous fuel, for removing woody plant competition from the forest understory, and as a means of improving habitat for desirable wildlife species, but very few studies have examined the effects of such management on soil macroinvertebrates (Callaham et al., 2012). In the only other study we know of where interactions between fire and earthworms have been examined, Callaham et al. (2003a) showed that prescribed burning in tallgrass prairies decreased the relative abundance of introduced species, but the effects of fire on invasive earthworms in forested systems has not yet been evaluated.

Our objectives for this study were to 1) Determine how food type and availability influences reproductive output in the lab, 2) Clarify feeding preferences of *A. agrestis* from juvenile to adult stages in a natural field setting, and 3) Determine whether prescribed fire can reduce populations of *A. agrestis* in a field mesocosm study. We hypothesized that heat from the fire might cause direct mortality to adults of *A. agrestis*, and we further hypothesized that consumption of litter in fires would result in decreased food availability for *A. agrestis*, possibly indirectly affecting their populations in burned forests.

2. Materials and methods

2.1. Feeding experiment

We collected *A. agrestis* from the litter layer at Chattahoochee National Forest, Georgia, USA (34.91°N, 83.62°W). We incubated earthworms in plastic pots (diameter at the bottom: 8.8 cm, depth: 14 cm) at room temperature beginning August 4, 2011. To examine the relative importance of different food resources for their survival and reproduction, we set seven different treatments of food provision with five replicates for each treatment using standard topsoil mix (Backyard to Nature, Colbert, GA), and litter and fragmented litter which were collected from the same site where earthworms were collected. Litter which passed a 4.75 mm standard soil sieve was defined as fragmented litter, and was considered to represent the partially decomposed litter in the F/H-layer of a forest floor. The treatments were as follows: 1) Litter only (hereafter "L"); 2) Fragmented litter only ("F"); 3) Litter plus Fragmented litter ("LF"); 4) Soil only ("S"); 5) Litter plus Soil ("LS"); 6) Fragmented litter plus Soil ("FS"); and 7) Litter plus Fragmented litter plus Soil ("LFS"). We added 175 g soil, 7.5 g fragmented litter and 7.5 g litter per pot. We placed two individuals of similar size and weight (either clitellate adult or large pre-clitellate juvenile) of *A. agrestis* into each pot. Pots were watered weekly with a mister. To insure optimal conditions for reproduction and growth, we placed new soil, litter and fragmented litter in these pots every 4 weeks. When we changed these materials, we measured earthworm masses, and collected cocoons by wet sieving the pot contents through a 1.0 mm standard soil sieve. Collected cocoons were incubated with wet paper tissues in petri dishes (diameter: 8.7 cm) at room temperature for about 4 months, and then dissected to determine their viability. We checked these dishes weekly, and added water as necessary to keep the moisture level consistent. One cocoon collected from LS treatment on January 19 was lost, and it was excluded during data analysis. A multinomial model (function multinom in nnet library) was fitted to cocoon viability using the data from each pot. A likelihood ratio test between the model including treatments as a parameter and the model without treatments was performed for statistical analysis to compare cocoon viability among feeding treatments using R3.0.2 (R Development Core Team., 2013).

We measured the C and N content of the original litter, fragmented litter and soil used in the construction of the experimental units. When pots were reconstructed every 4 weeks, we also sampled soil and litter after incubation and measured C and N content (September 1–January 19, 6 sample dates in total). In contrast to litter ("L" material), it was not possible to measure the chemistry of fragmented litter ("F" material) after incubation, because most of this food source was consumed by earthworms over the 4 week incubation. Samples were oven dried at 80 °C for >45 h prior to analysis. The C and N content of soil and litter were measured using a Perkin–Elmer series II CHNS/O analyzer 2400 (USA). We then calculated the difference in %C and %N content from the initial contents after 4 weeks of incubation with earthworms.

2.2. Stable isotope ratios from field sampling

We collected earthworms at the west side of Great Smoky Mountains National Park (GSMNP), near the Chilhowee reservoir in Tennessee (35.54–35.56°N, 83.99–84.01°W) every 3–4 weeks

(April 22, May 13, June 3 and 27, July 26, August 22, September 15, 2011) to estimate the main food resource in the field. It is known that *A. agrestis* dominates in the forest area at this site (Snyder et al., 2011). We collected *Amynthas* species using setae arrangement and prostomium type as characters to separate *Amynthas* juveniles from other juvenile specimens collected (Reynolds, 1978; Schwert, 1990). Adult specimens collected included individuals of *A. agrestis* as well as *Amynthas corticis*. *A corticis* is an endogeic species living in the litter and shallow soil layer (Ishizuka, 2001; Uchida and Kaneko, 2004). In an investigation of *Amynthas* spp life history and behavior in their native range in Japan, Uchida and Kaneko (2004) showed that the epigeic species including *A. agrestis* overwintered in the cocoon stage, hatched in April and May, and became reproductive adults in June and July, with a single generation per year. In contrast, the endogeic species including *A. corticis* overwintered in both juvenile and adult stages, with adults maturing in May. Their cocoons hatched without overwintering, and thus, juveniles emerged in summer and autumn. Considering these life history patterns, we reasoned that *Amynthas* juveniles collected in April and May in our study were *A. agrestis*, and *Amynthas* juveniles collected in July, August and September should have been *A. corticis*. Therefore, because all *Amynthas* samples collected in April and May were small juveniles, we provisionally identified them as *A. agrestis* juveniles. We also collected litter, fragmented litter in F/H-layer and soil at the 0–5 cm depth and 5–10 cm depth with three or four replications sampled on each sampling date.

Collected earthworms were dissected, and their gut contents were removed in the laboratory as in Hendrix et al. (1999). They were then freeze-dried, and ground inside a vial with a glass stirring rod for analysis. For some specimens, we processed only the posterior segments in order to preserve anterior segments for morphological identification later. We compared posterior and anterior segments in some specimens, and found no differences for both $\delta^{13}C$ and $\delta^{15}N$ among them ($n = 6$, $P = 0.75$ for $\delta^{13}C$; $P = 0.20$ for $\delta^{15}N$ by generalized linear model (GLM) analysis: distribution = Gaussian, link function = identity). Litter and soil samples were oven dried at 65 °C for >72 h, and ground in a ball-mill for analysis.

We used a Thermo-Finnigan Delta V, Magnetic Sector Isotope Ratio Mass Spectrometer (Bremen, Germany) coupled to a Carlo Erba NA1500 CHN Combustion Analyzer (Milan, Italy) via the Thermo Conflo III Interface (Bremen, Germany) to measure the $\delta^{13}C$ and $\delta^{15}N$ values of earthworms, litter, fragmented litter and soil. Isotope ratios were expressed as parts per thousand (‰) relative to the international standard: $\delta^{13}C, \delta^{15}N = (R_{sample} - R_{standard})/R_{standard} \times 1,000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Isotopic signatures of C and N were expressed relative to Pee Dee belemnite and atmospheric nitrogen. A secondary standard of bovine liver (NBS 1577b) was repeated every 12 samples throughout each run with a standard error of 0.011‰ and 0.016‰ across all runs for $\delta^{13}C$ and $\delta^{15}N$, respectively. We conducted GLM analysis and the Tukey post hoc test (function glht in library multcomp) to compare the differences of $\delta^{13}C$ and $\delta^{15}N$ among food candidates (litter, fragmented litter, 0–5 cm soil and 5–10 cm soil) using R3.0.2 (R Development Core Team, 2013). In the GLM analysis, gaussian distribution and identity link function were employed, and food candidates and sampling season were included as explanatory variables.

We applied a Bayesian mixing model to $\delta^{13}C$ and $\delta^{15}N$ using MixSIR version 1.0.4 (Moore and Semmens, 2008) to estimate the relative proportion of the contribution of each food candidate (litter, fragmented litter, 0–5 cm soil and 5–10 cm soil) for the food use of earthworms. We used average fractionation values (isotopic shift from diet to consumer) and the standard deviations (SD) of fractionation values calculated from the values for earthworms reported in the literature (Spain et al., 1990; Martin et al., 1992; Spain and Le Feuvre, 1997; Neilson et al., 2000; Uchida et al., 2004). MixSIR were run in 100,000,000 iterations. The median and 90% Bayesian credible interval of the contribution of each food candidate were estimated using the mixing model.

2.3. Fire experiment

For the experiment, we used four existing experimental beds (1.3 × 6.2 m) constructed of concrete blocks. These four beds were divided into eight experimental plots (1.3 × 3.0 m) using a central divider of concrete blocks and plastic sheets (Fig. S1) at Whitehall Forest, University of Georgia, Athens (33.89°N, 83.36°W). These experimental beds had previously been filled with soil to a height of ~60 cm. Soils in the beds were composed of coarse river sand with small silt and clay components (>90% sand), and low organic matter content derived from fallow vegetation (mostly weedy grass species). Experimental beds were prepared by removing all vegetation that had established during several years of disuse. This involved pulling vegetation by hand, and then removing the top 10 cm of soil in the beds, in order to remove coarse roots and seeds.

We collected litter, fragmented litter and *A. agrestis* at Chattahoochee National Forest, west of Clayton, Georgia (34.91°N, 83.62°W). One hundred individuals of *A. agrestis* were introduced to each of eight experimental plots (800 individuals in total) on July 8, 2011. We added 24.8 kg wet litter per plot (dry weight: 10.28 kg, C content: 42.89% ± 0.26 (SE), N content: 1.37% ± 0.02). In order to simulate forest floor conditions under full canopy, we shaded these beds with plywood. To prevent desiccation, we watered the beds to saturation every day or every other day for the duration of the experiment, except we allowed 3 days drying prior to experimental fires. Eight weeks after *A. agrestis* introduction, we burned one of two experimental plots in each bed (four burned plots and four unburned plots) on September 13 and 14. Experimental fires were timed such that they would occur in the autumn, when adults and cocoons would be most susceptible to mortality due to heat exposure.

Surface radiant heat release was measured with an FLIR Inc. SC660 digital thermal imaging system. Thermal images were captured at 1 Hz for the duration of the fires. The imager was coupled to a 0.5X lens to increase the field of view so the entire burned plot was able to be captured. The SC660 records temperature in each of 307,200 pixels per image (640 by 480 pixels) with a sensitivity of 0.06 °C and accuracy of ±2%. The temperature of fire surface was measured until smoldering combustion ended. We also calculated total amount of energy during experimental fires from the fire surface temperature.

In order to measure soil heating, we installed six 16 AWG type T thermocouples, three buried at 1 cm and three buried at 5 cm below the mineral substrate in each burned plot. The sensors were spaced evenly along the long axis of the plot and sampled at 1 min intervals using a Campbell Scientific Inc. (Logan, UT) CR23X datalogger programmed and wired for differential temperature measurements. Sensors were unplugged in Plot 4 after flaming phase combustion was complete, but before smoldering combustion had ended. The soil heating was declining asymptotically, though this resulted in some loss of information.

One day after the fires, we counted live earthworms in each plot (burned and unburned) by turning over one half of the remaining litter and the surface soil. At this time we also collected litter and soil samples using a square sampler (9.5 × 9.8 × 10.0 cm depth) with 6 replicate samples taken per plot after burning. We wet sieved these through a 1.0 mm standard soil sieve to collect cocoons, and then litter was oven dried at 90 °C for >100 h to estimate dry weights. The viability of cocoons was examined with the same method described above for the feeding experiment.

We conducted GLM analysis (function glm) for each variable (dried litter weight after burning, number of earthworms, number of cocoons, viability of cocoons) to compare them between burned and unburned plots using R3.0.2 (R Development Core Team, 2013). Gaussian distribution and identity link function were employed in each GLM analysis. We conducted GLM analysis using the average value for dried litter weight, number of cocoons, viability of cocoons in each plot.

3. Results

3.1. Feeding experiment

We conducted the feeding experiment for 196 days until February 16. Though a few individuals were still alive in the lab, all adults would be dead by February under normal field conditions, considering the life history of this species (Uchida, 2004; Uchida and Kaneko, 2004), and thus we terminated the experiment. Under two feeding treatments (L and F), all individuals died within 100 days (Fig. 1a). All the individuals in the pots with LF treatment died within 140 days. For all feeding treatments that included soil, at least some individuals survived throughout the experiment.

The weights of earthworms decreased gradually in all feeding treatments (Fig. 1b). The earthworms under three treatments that did not include soil (L, F and LF) lost weight rapidly. In the other four treatments in which some of the individuals survived through the experiment, the earthworms reared with only soil were the lightest through the experiment, whereas earthworms in the remaining treatments (FS, LS and LFS) showed weight loss patterns that were similar to one another.

Earthworms under the three feeding treatments without soil (10 individuals in each treatment) did not produce cocoons (Fig. 1c). Earthworms reared with only soil (10 individuals) produced only five cocoons in total. In the three treatments with combinations of litter and/or fragmented litter, and soil (LS, LF and LFS; 10 individuals in each treatment), earthworms produced cocoons throughout the experiment, except on the last sampling date (February 16). Earthworms in the LFS treatment produced the highest number of cocoons throughout the experimental period except the final sampling (148 cocoons in total). The earthworms under the LS and FS treatments produced 60 and 79 cocoons in total, respectively. Most cocoons contained a single hatchling, but 5 out of 195 total cocoons contained two hatchlings. Viabilities were from 60 to 70% under the four feeding treatments in which cocoons were collected, and there was no difference in cocoon viability among feeding treatments ($\chi^2 = 4.55$, $P = 0.21$; Fig. 1d).

The C and N content of litter, fragmented litter and soil used for feeding experiments were 47.3% ± 0.09 ($n = 10$), 45.74% ± 0.13 ($n = 5$) and 6.80% ± 0.11 ($n = 10$) in carbon, and 1.33% ± 0.03 ($n = 10$), 1.52% ± 0.02 ($n = 5$) and 0.26% ± 0.00 ($n = 10$) in nitrogen, respectively. The C content of the litter decreased by 3–10% in the LS treatment, and by 1–6% in the LFS treatment from initial C content after 4 week incubation (Fig. S2a). The C content of the soil didn't increase after incubation in most of samples, though that of the litter decreased. When the earthworms were reared with only soil (S treatment), C content in soil decreased after 4 week incubation from the initial condition in all the sampling time, suggesting that earthworms either acquired carbon from soil or stimulated microbial respiration, or both. The N content of the litter changed from 1.33% to 0.91% ± 0.04 under the LS treatment, and to 1.10% ± 0.04 under the LFS treatment from initial N content after 4 week incubation (Fig. S2b). The N content increased in the soil under the treatments which include F material (FS and LFS), whereas N content didn't change under the other treatments (S and LS).

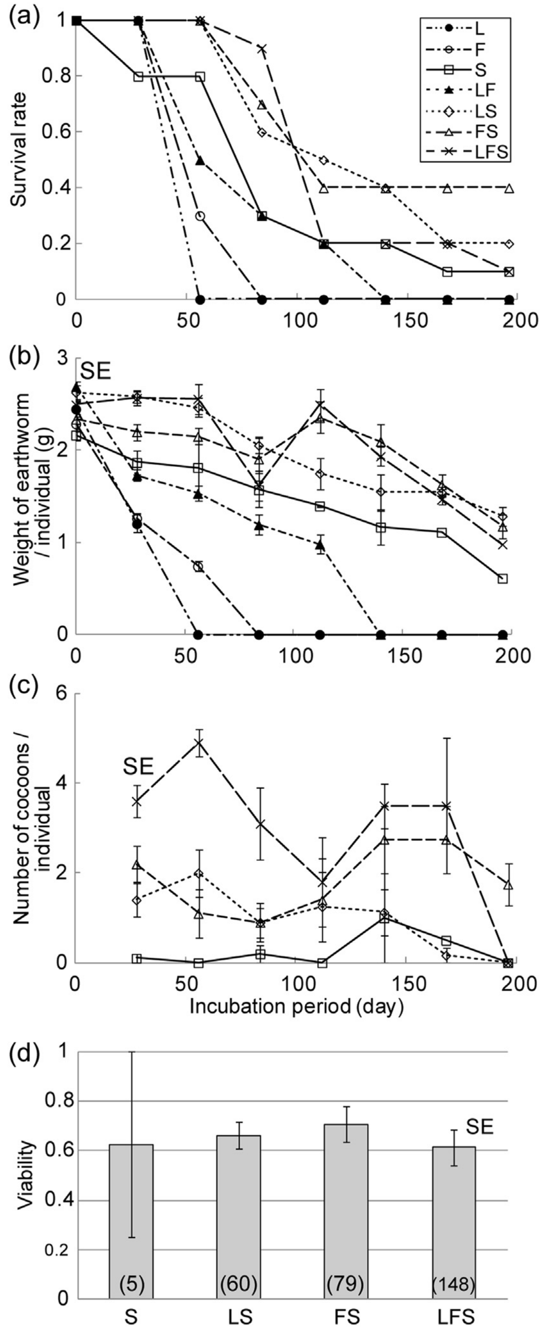

Fig. 1. (a) Survival rates, (b) weights of earthworms and (c) numbers of cocoons per individual related to incubation period, and (d) viabilities under each feeding treatment. The averages and the standard errors in (b), (c) and (d) were calculated based on the data in each pot. Values in parentheses in (d) indicate the total number of cocoons produced in pots under a particular treatment. Earthworms produced no cocoons in three feeding treatments (L, F and LF).

3.2. Stable isotope ratios from field sampling

Both $\delta^{13}C$ and $\delta^{15}N$ signatures were significantly different among litter, fragmented litter, 0–5 cm depth soil and 5–10 cm depth soil (Table S1). They were lowest in litter, and fragmented litter also showed low isotope ratios, whereas soil collected from 0 to 5 cm depth and 5–10 cm depth showed higher isotope ratios (Fig. 2).

We used fractionation values of 4.11 ± 1.37 (SD) for $\delta^{13}C$ and 2.73 ± 1.80 for $\delta^{15}N$ calculated from literature in the mixing model

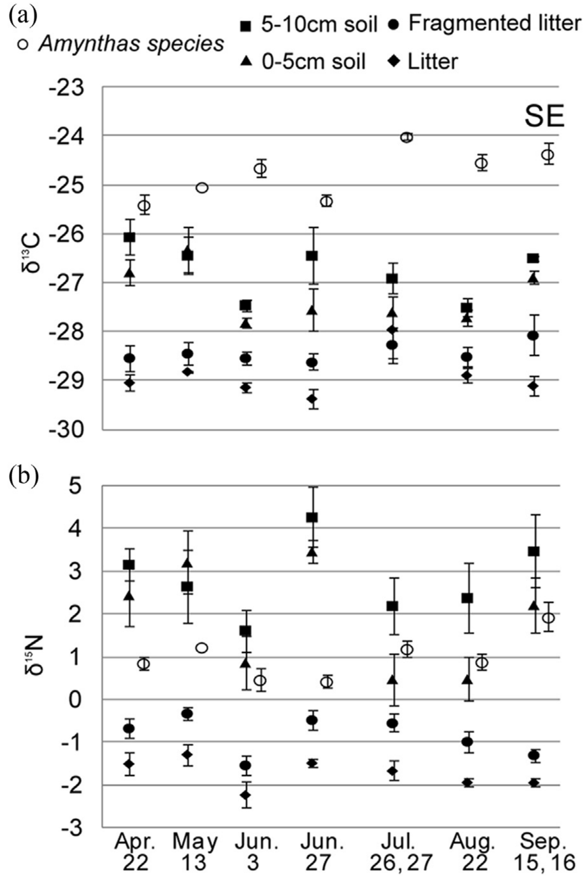

Fig. 2. (a) δ^{13}C and (b) δ^{15}N of litter, fragmented litter, soil and earthworms collected in the field. *Amynthas* species includes *A. agrestis* and *A. corticis*.

analyses (see Methods). *Amynthas* species were composed of *A. agrestis* and *A. corticis*. The mixing model indicated that *Amynthas* species, regardless of developmental stage, primarily fed on litter and fragmented litter through the season (Table 1).

3.3. Fire experiment

The maximum and average temperatures at the fire surface during burning were from 244 to 1036 °C and from 223 to 454 °C, respectively (Fig. S3). However, it was much cooler under the soil surface (Table 2). The maximum temperature increase at the 1 cm depth was 10.9–50.5 °C (average increase of 27.3 °C ± 4.6 (SE)), while the increase at the 5 cm depth was 5.6–10.3 °C (average increase of 7.7 °C ± 0.6). Total energy in each plot was from 5.03 to 14.51 MJ (Table 2, Fig. S4).

Litter mass was significantly reduced by burning ($t = -2.95$, $P < 0.05$, Fig. 3a). There were no differences in the numbers of earthworms or cocoons between burned and unburned plots ($t = -1.01$, $P = 0.35$; $t = -0.24$, $P = 0.82$, respectively; Fig. 3b, c). Cocoon viability was significantly lower in burned than in unburned plots ($t = -4.39$, $P < 0.01$; Fig. 3d). Cocoon viability in unburned plots from the fire experiment was similar to those observed in the feeding experiment.

Table 2
Average starting and maximum temperatures below the soil surface (1 cm and 5 cm; ±SE) and total energy calculated from the fire surface temperature in each plot during experimental fires. Values represent average temperature and standard error of three sensors in each plot, at each depth.

Depth	Starting temperature (°C)		Maximum temperature (°C)		Total energy (MJ)
	1 cm	5 cm	1 cm	5 cm	
Burned plot 1	24.7 ± 0.4	24.3 ± 0.3	39.4 ± 5.5	29.9 ± 1.5	5.03
Burned plot 2	24.8 ± 0.2	24.5 ± 0.5	35.7 ± 3.8	30.0 ± 1.4	6.53
Burned plot 3	25.4 ± 0.9	24.5 ± 0.7	58.3 ± 9.9	34.0 ± 1.4	14.51
Burned plot 4	24.8 ± 0.2	24.5 ± 0.5	75.4 ± 20.9	34.8 ± 1.7	12.22

4. Discussion

In our experimental plots, soil temperature increased only slightly at the 5 cm depth (from 24.3 to 24.5 °C up to 29.9–34.8 °C). Richardson et al. (2009) conducted an experiment to examine survival rates of *A. agrestis* under different temperature and moisture conditions, and showed that *A. agrestis* did not survive after 28 days at 35 °C, but did survive with high activity under 25 °C as long as moisture levels were adequate. Therefore, if earthworms can temporarily escape from the heat of the fire by burrowing into the soil to a depth of 5 cm or more, they can survive the direct impacts of fire. On the other hand, cocoons seem to be more vulnerable to heat than adults and juveniles, and this is likely due to their placement at or near the surface of the soil at the time of production. The moisture content of litter and soil can affect the spread of the fire and the heat transfer to the soil. In our experiment, we provided ample moisture to keep the earthworms alive, and thus the effects of fire on the earthworms and cocoons may be underestimated. Although the effects of fire are highly dependent upon topographic features, fuel moisture and weather conditions, the behavior of actual prescribed fires is generally more intense than what we achieved in our experimental fires, and thus we expect that these fires would be more effective at earthworm control.

Our fire experiment indicated that prescribed fire can be an effective management tool for invasive earthworms. In Japan, which is the original distribution area of *A. agrestis*, birds (Yui, 1988), moles (Imaizumi, 1979) and ground beetles (Sota, 1985a, b) are known as the major predators for earthworms. Some members of the detrital food web in, for example, the GSMNP are known to feed on *A. agrestis* (D. Straube et al., unpublished results). Furthermore, some vertebrate predators may have come to primarily consume the high density prey, *A. agrestis*, rapidly by learning. Such predation pressure should also contribute to the population decline of invasive earthworms.

Table 1
Estimates of food contribution by source as calculated using MixSIR (values are median and 90% Bayesian credible intervals). Contributions greater than 50% are shown in ***bold Italic***, and those greater than 30% are shown in *Italic*.

	April 22		May 13		June 3		June 27		July 26, 27		August 22		September 15, 16	
	n = 18		n = 1		n = 12		n = 16		n = 12		n = 14		n = 9	
Litter	***0.75***	(0.56–0.90)	*0.46*	(0.05–0.79)	***0.54***	(0.29–0.74)	***0.76***	(0.59–0.90)	*0.42*	(0.22–0.62)	***0.54***	(0.35–0.71)	*0.48*	(0.24–0.68)
Fragmented litter	0.21	(0.04–0.41)	0.29	(0.02–0.75)	*0.34*	(0.08–0.61)	0.20	(0.04–0.39)	*0.38*	(0.14–0.60)	*0.31*	(0.09–0.53)	0.29	(0.06–0.58)
0–5 cm soil	0.02	(0.00–0.07)	0.08	(0.01–0.31)	0.06	(0.01–0.19)	0.02	(0.00–0.07)	0.14	(0.02–0.33)	0.10	(0.01–0.24)	0.12	(0.01–0.28)
5–10 cm soil	0.01	(0.00–0.06)	0.09	(0.01–0.37)	0.05	(0.00–0.15)	0.01	(0.00–0.05)	0.04	(0.00–0.14)	0.04	(0.00–0.13)	0.09	(0.01–0.22)

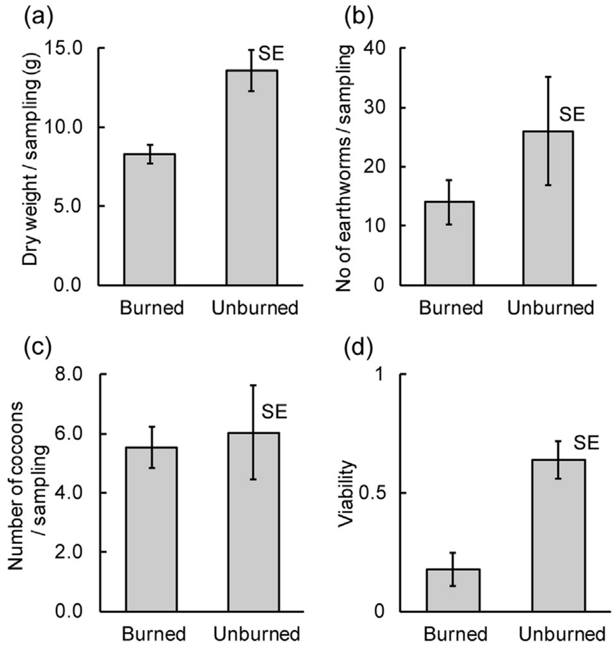

Fig. 3. (a) Dry weights of litter, (b) numbers of earthworms, (c) numbers of cocoons and (d) viabilities in burned and unburned plots. Averages and standard errors were calculated based on the data in each plot.

Our feeding experiment showed that the *A. agrestis* earthworms lost less weight, and produced more cocoons under three feeding treatments including soil and some form of organic matter (LS, FS, and LFS). The mixing model using stable isotope ratios also showed *A. agrestis* to be mainly feeding on litter and fragmented litter through the season in the field. These results indicate that the litter and fragmented litter are important food resource for *A. agrestis*. Prescribed fire can lead to a decline in invasive earthworm populations because of depriving the remaining adults' food resources after the fire as well as high cocoon mortality (no adults surviving to the next generation according to this study and Uchida and Kaneko, 2004). Furthermore, the mixing model showed that juveniles of *A. agrestis* in April and May are also feeding on litter and fragmented litter. Thus, prescribed fire also increases the mortality of juveniles in the next season by decreasing the availability of their preferred food resource.

The C and N content of soil generally did not increase in our feeding experiment, even when C and N of litter was strongly decreased. Though earthworms contributed to the decomposition of litter, they didn't directly contribute to the increase of C and N in the soil in our experiment, probably because C and N are used for respiration, biomass production, metabolic needs, and reproductive needs, and for the respiration of microbes. The decrease of C and N from the litter was lower under the LFS treatment than those under the LS treatment, because earthworms were able to acquire carbon and nitrogen from not only litter, but also fragmented litter. The C and N in soil increased under the feeding treatments including F material (FS, LFS), and this tendency was more pronounced for N content. Because fragmented litter is richer in nitrogen than litter (fragmented litter: 1.52%, litter: 1.33%), fragmented litter may be a more important nitrogen source for the decomposers such as earthworms that tend to have a shortage of nitrogen in their foods, though the nitrogen in more decomposed litter may contain more recalcitrant molecules such as lignin. The subsequent nutrient flows to soil may be more likely to happen through mixing of materials in the pots via burrowing and casting.

In our feeding experiment, under the S treatment (which provided the lowest nutritional resource), earthworms survived the entire experiment (196 days), but they lost mass, and produced only a few cocoons. However, in the treatments that did not contain any soil, all earthworms died within 140 days. These results suggest that the soil *per se* is not essential as a food resource, but that soil is nevertheless required to provide a habitat, perhaps serving as an important buffer to environmental changes. Another possible essential role of mineral soil may be that the earthworms need at least a few mineral particles (e.g., sand-sized particles) to ingest along with their food so that this food can be efficiently processed in the gut, as the gizzards of these worms function to grind the food prior to passage through the gut where nutrients are extracted and assimilated. Further experimental work will be required to fully understand the feeding and nutrition of these earthworms.

Our results from the fire experiment suggest that fire may not decrease the number of earthworms directly, but can greatly decrease the viability of their cocoons. Therefore, fire management may contribute to population decline in the next generation, and ultimately allow control of the invasive species over time. Thus, we recommend that when management of invasive earthworms is a concern, prescribed fires should be conducted after cocoons have been produced (i.e., October) to maximize the effect of prescribed fire.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.12.011.

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