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Source: Natural Areas Journal, 32(3):283-292. 2012.
Published By: Natural Areas Association
DOI: http://dx.doi.org/10.3375/043.032.0305
URL: http://www.bioone.org/doi/full/10.3375/043.032.0305

RESEARCH ARTICLE

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A Simple, Safe, and Effective Sampling Technique for Investigating Earthworm Communities in Woodland Soils: Implications for Citizen Science

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Natural Areas Journal 32:283–292

ABSTRACT: We evaluated the efficacy of a mixture of ground hot mustard and water as a sampling method for earthworms (Lumbricina) in research projects involving citizen scientists. To do so we conducted a field experiment to determine if sampling earthworms using mustard-powder would reveal similar patterns of earthworm abundance and community composition as relying on the more difficult to prepare, and possibly hazardous, allyl isothiocyanate (AITC) solution. Earthworms were sampled using either mustard or AITC in four pairs of 0.25-m^2 plots located in each of four woodland sites that were predicted to exhibit a range of earthworm densities. Soil gravimetric water content (GWC) of each plot was quantified as a covariate. For analyses of changes in abundance and community structure, earthworms were classified as belonging to one of five groups based on where they occur in the soil profile, developmental stage, and level of taxonomic identification. The two sampling techniques revealed similar earthworm abundances and community composition across the four sites (all $Ps \geq 0.16$) and across the range in GWC (all $Ps \geq 0.36$). We conclude that using the mustard-water mixture to sample earthworms at our study site appears to be just as effective and reliable as using AITC. The mustard-water mixture, which is easier to prepare and is less hazardous than AITC solution, should, therefore, be considered as an appropriate tool to be utilized by researchers who collaborate with citizen scientists to help collect the large datasets needed to reveal how woodland management and restoration programs affect earthworms.

Index terms: allyl isothiocyanate, hot-mustard powder, *Rhamnus cathartica* L., vermifuge, woodland management

INTRODUCTION

This study compares the efficacy of sampling earthworms (Lumbricina) with ground hot mustard mixed with water (Gunn 1992) to a more standardized, yet difficult to prepare and possibly hazardous, solution of allyl isothiocyanate (AITC) (Zaborski 2003). The rationale for the study is to determine if ground mustard can be used as a safe and non-destructive sampling method by scientists and land managers who collaborate with citizen scientists (sensu, Cohn 2008; Silvertown 2009) in their research on earthworms in natural areas. Sampling techniques such as formalin, hand-sorting, and electro-shocking (Raw 1959; Thielemann 1986; Bartlett et al. 2010) were not investigated because they either pose serious health risks or cause prolonged, negative impacts to plant and soil communities (Gunn 1992; Eichinger et al. 2007; Coja et al. 2008), and are, therefore, inappropriate for use by citizen scientists or for sampling natural areas.

This study, in two major ways, expands upon prior studies that compared mustard with AITC (Pelosi et al. 2009; Valckx et al. 2011) or other earthworm-sampling methods (Raw 1959; Springett 1981; Gunn 1992; Chan and Munro 2001; Lawrence and Bowers 2002; Zaborski 2003; Bartlett et al. 2006). First, this study was conducted in a native woodland rather than a human-created environment (e.g., pasture, field, turf lawn). Secondly, we searched for possible differences between mustard and AITC in revealing earthworm community structure in addition to measuring responses of separate taxa. The previously cited studies measured either total earthworm abundance or the abundances of selected taxa of earthworms, but ignore possible differences in uncovering earthworm community structure. These earlier approaches implicitly assume that the abundance of one earthworm taxon does not affect the other; or that different groups respond similarly, which may not be safe assumptions (Edwards and Lofty 1982; Temple-Smith et al. 1993; Butt 1998; Dalby et al. 1998; Baker et al. 2002; Curry 2004).

Finding non-destructive, yet reliable, strategies to sample earthworms in natural areas is particularly important for those temperate woodlands of North America where invasions by exotic earthworms have had profound effects on aboveground and belowground community structure and ecosystem functioning (Bohlen and Edwards 1995; Bohlen et al. 2002; Li et al. 2002; Bohlen et al. 2004; Suarez et al. 2004; Hale et al. 2005; Frelich et al. 2006; Hale et al. 2006; Migge-Kleian et al. 2006; Heneghan et al. 2007). Furthermore, land managers in these regions may want to understand how management actions (e.g., controlled burning, exotic plant removal, ecological restoration, etc.) will influence the abundance and community composition of invasive earthworms. Thanks to taxonomic keys developed for use by non-earthworm experts, such as the one developed by Hale (2007) for identifying earthworms of the Great Lakes region, non-experts can typically identify earthworms to genus or sometimes species with relatively little training. Thus, citizen scientists could play a key role in increasing our understanding of how management activities influence earthworm populations so long as the techniques used to sample earthworms are practical, safe, and reliable.

Chemical expulsion, [i.e., irrigating the soil with a chemical irritant that causes earthworms to emerge on the surface (Raw 1959; Gunn 1992; Zaborski 2003)], is more logistically feasible than hand-sorting, particularly when large numbers of samples are required. Hand-sorting is time consuming, labor intensive, and requires digging up and sifting through soils in order to collect earthworms, which can be destructive and may be prohibited in natural areas (Springett 1981; Gunn 1992). Chemical expulsions, on the other hand, require that water (20–40 L H_2O for each square meter sampled) be transported to sampling locations. This challenge, however, can be met if citizen scientists assist with sampling efforts.

The effectiveness of chemical expulsion relative to hand-sorting depends on earthworm size, type, and sexual development, as well as the depth in the soil where the worms occur, soil moisture, and soil temperature (Raw 1959; Chan and Munro 2001; Zaborski 2003; Bartlett et al. 2006). Nonetheless, differences in efficiency between chemical expulsion and hand-sorting are consistent across a range of habitats and soil conditions (Lawrence and Bowers 2002), suggesting that chemical expulsion can be a powerful technique when comparisons between sites of relative, rather than absolute, abundance will answer the scientific question posed. Expulsion methods could be an excellent option for research projects that rely on citizen scientists to sample earthworms, because they are both suitable for among-site comparisons, and generally easier and quicker to administer than hand-sorting.

Several types of expulsion chemicals have been evaluated for sampling earthworms. Formalin, which can be effective (Raw 1959), is not suitable for use by citizen scientists or for sampling areas of conservation concern because it is a known carcinogen [see material safety data sheet (MSDS) for details], and is toxic to plants and soil organisms (Gunn 1992; Eichinger et al. 2007). A mixture of mustard powder and water is a non-toxic alternative to formalin that has been reported to be effective under a wide range of environmental conditions (Gunn 1992; Lawrence and Bowers 2002; Heneghan et al. 2007). Despite the safety and environmental benefits of using mustard powder over formalin, there are concerns about how to standardize mustard-powder expulsions (Zaborski 2003). One method of standardization has been to use solutions of the chemical found in the powder made from mustard seed, AITC, which is a substance that irritates the mucus membrane of earthworms, causing them to move to the soil surface (Zaborski 2003).

These standardized AITC solutions are effective for sampling earthworms (Zaborski 2003; Pelosi et al. 2009) and are environmentally safe in that they are not known to harm plants and have a low residual period in the soil (Borek et al. 1995). However, the use of AITC solutions presents some unique challenges. First, prior to its addition to water, AITC must be mixed with 100% alcohol ("stock solution"). Secondly, AITC in its concentrated form is caustic, may be fatal if absorbed through the skin, and can irritate the skin, eyes, and respiratory tract (see MSDS and product label for details), requiring that AITC stock solutions be prepared underneath a fume hood while wearing protective clothing and eyewear. This requirement is a major obstacle to researchers and land managers relying on citizen scientists. These drawbacks in preparing AITC raise issues of both safety and practicality, and suggest that mixtures of mustard powder and water should be used in research projects involving citizen scientists if these mixtures are as effective at expelling earthworms as AITC solutions.

OBJECTIVE

Our objective was to test the hypothesis that a mixture of mustard powder and water is as effective as a solution of AITC at expelling earthworms from woodland soils, and in doing so, establish that mustard powder can be used as a reliable sampling technique in research projects with citizen scientists. We further hypothesized that mustard powder and AITC expulsions would be equally effective across a suspected gradient of earthworm abundance. In particular, we hypothesized that patterns of abundance (count and biomass) and community composition revealed by both expulsion methods would be indistinguishable from one another.

METHODS

Overview of Experimental Design

Earthworms were sampled in four pairs of plots in each of four woodland sites that were predicted to have different earthworm abundances due to different histories of management of the invasive, exotic shrub *Rhamnus cathartica* L. (European buckthorn; hereafter buckthorn). Earthworm abundances are hypothesized to increase in response to buckthorn invasion (Heneghan et al. 2007) and decrease in response to buckthorn removal (Madritch and Lindroth 2009). Therefore, by replicating the experiment in these four sites, we hoped to be able to compare the effectiveness of the two expulsion methods at different levels of earthworm abundance. The four sites differed as follows: Site 1) currently invaded by buckthorn; Site 2) buckthorn had been removed for 5 yrs; Site 3) buckthorn had been removed for 12 yrs; and Site 4) had never been invaded by buckthorn. In each site, four pairs of plots (50 cm x 50 cm) were located 12.5 m from a center point, one pair at each cardinal direction. The plots within a pair were 2 m apart. One plot from each pair was randomly chosen to be sampled using AITC; the other plot was then sampled using mustard powder.

Study Site

This study was conducted at Mary-Mix McDonald Woods, a remnant native woodland located approximately 35 km N of downtown Chicago at the Chicago Botanical Garden (Coordinates: 42°8'59.90"N, 87°46'46.30"W). This woodland was set aside for conservation and is, therefore, an excellent example of a place where hand-sorting and formalin expulsion would not be appropriate. The mean summer high temperatures at this site are 26 – 33 ºC, the mean summer low temperatures are 18 °C, and mean annual precipitation amounts are 920 mm (NOAA 2010).

The canopies of the areas from which buckthorn was still present or had been removed, Sites 1 – 3, were dominated by *Quercus alba* L. (white oak), with *Quercus rubra* L. (red oak) and *Fraxinus* species (ash) as sub-dominants. The canopy of the uninvaded area (Site 4) was dominated by *Q. rubra*, and contained *Q. alba* and *Acer sacharum* Marshall (sugar maple) as sub-dominants. The shrub layer of Site 1 was dominated by a dense thicket of buckthorn (approximately 4 m high; vegetative cover ≥ 95%). Shrub layers were not present in the two sites from which buckthorn had been removed (Sites 2 and 3). The shrub layer of Site 4 had *Hamamelus virginianus* L. (witch hazel), *Prunus* species (cherry), and *Tilia americana* L. (basswood) at low abundances. Except for a few sedges and shade-tolerant forbs, understory vegetation was absent in Site 1, which was dominated by the buckthorn thicket. The understory of Sites 2 and 3 had smaller (≤ 1 m tall) buckthorn due to re-invasion, along with mostly shade-intolerant grasses and forbs. The understory of Site 4 consisted of a high cover of shade-tolerant and shade-intolerant forbs, with some grasses present. Soils in Sites 1 – 3 were Ozaukee silt loam (mesic Oxyaquic Hapludalfs), whereas Frankfort silt loam (mesic Udollic Epiaqualfs) characterized Site 4 (USDA 2009).

Preparation of the Expulsion Mixtures

The AITC solution and mustard powder mixture were prepared following methods of Zaborksi (2003) and Heneghan and Umek (unpubl. data), respectively. First, stock solutions for both extracts were prepared. AITC (ARCOS ORGANICS; 94%; density 1.017) was diluted with 100% ethanol to a 5 g L^{-1} concentration. This AITC stock solution was placed in a lightproof container and refrigerated for 48 hrs prior to use. Because AITC is not readily soluble in water, ethanol acts as an emulsifier when AITC is added to water (see below). In the evenings prior to sampling, a separate 125 mL plastic bottle of mustard powder stock solution was prepared for each plot that would be sampled. In each bottle, 38.1 g of dried extra-hot oriental mustard powder (*Brassica juncea* L.) [Frontier Natural Products Co-op (Norway, IA)] was added to 100 mL of water (381 g L^{-1}) and shaken until the mixture was paste-like. Just prior to sampling, 100 mL of the AITC stock solution or an individualized mustard-powder stock mixture (100 mL water plus 38.1 g dried mustard powder) were added to 5 L of water, resulting in a final concentration of 0.10 g L^{-1} AITC or 7.47 g L^{-1} mustard. This AITC concentration was shown by Zaborski (2003) to expel the greatest number and biomass of earthworms when compared with AITC concentrations ranging from 0.005 to 0.250 g L^{-1}. The mustard powder concentration is recommended by Clapperton et al. (2008).

Earthworm sampling and identification

Earthworms were sampled on 21 and 22 October 2008 [daily temperature range of 3 – 14 ºC, mean daily temperature of 8 ºC (NOAA 2010)]. At each sampling location, a 50-cm x 50-cm plot marker constructed of 13 cm high lawn edging was hammered 5 cm into the ground using a rubber mallet. We then waited a minimum of 10 minutes before sampling earthworms in order to reduce the impact that hammering might have had on sampling. During this waiting period, we collected a 6-cm x 10-cm soil core from a point located approximately 10 cm from the lower right corner and outside of the plot marker. Gravimetric water content (GWC) of these soil cores were later estimated on a dry-weight basis as described in Robertson et al. (1999). Leaf litter was removed from the soil surface and inspected for the presence of epigeic earthworms. The appropriate expulsion mixture for that plot was then slowly poured over the soil until pooling of the mixture occurred. After the mixture had percolated into the soil, we waited 4 minutes and then poured more mixture over the plot until pooling occurred again. This process was repeated until the 5 L of AITC solution or mustard mixture had been used. All earthworms that came to the surface within the boundaries of the plot marker were collected until 10 minutes after the last of the solution or mixture had been emptied onto the plot. Preliminary trials under similar soil conditions revealed that using greater than 5 L resulted in excessive and prolonged pooling, which hindered our ability to collect earthworms.

After being collected, earthworms were placed in plastic jars with moist paper towels and taken back to the lab. Paper towels and debris were then removed from each jar; earthworms were rinsed off using tap water, placed back into the empty jars, and placed in a refrigerator at 4 °C for 72 hrs to allow time for the earthworms to empty their gut contents. Earthworms were then identified, counted, dried at 70 °C for 48 hrs, and weighed.

Earthworm identification followed Hale (2007) and Schwert (1990). Both keys resulted in the same classification for all specimens. For analysis of changes in abundance and community structure, earthworms were placed in one of five functional/taxonomic groups based on where they occur in the soil profile, developmental stage, and certainty of the identification: *Lumbricus terrestris* L. adults (anecic), *Lumbricus rubellus* Hoffmeister (epi-endogeic), *Lumbricus* juveniles (both anecic and epi-endogeic – includes both *L. terrestris* and *L. rubellus*), endogeic adults, and endogeic juveniles. *Lumbricus terrestris* and *L. rubellus* juveniles were grouped together because definitive differentiation between these species is not possible until sexual organs are developed (Schwert 1990). Anecic species are those that form and live in deep vertical borrows, but feed on leaf litter at the soil's surface; endogeic species are those that both form largely horizontal burrows and feed within the upper layers of the mineral soil; epigeic species are those that live in and feed on the decaying leaf litter that lies on the soil's surface (Bouché 1972); and epi-endogeic species are those that form horizontal borrows in the upper layers of the mineral soil, but consume leaf litter (Terhivuo 1988).

Statistical Analyses

To determine whether the two expulsion methods varied in their effectiveness, permutational multivariate analysis of variance [PERMANOVA; (Anderson 2001; McCardle and Anderson 2001; Anderson et al. 2008)] was conducted on the number of individuals and biomass of the five earthworm groups sampled from all plots. PERMANOVA tests for differences in community distance measures (e.g., Bray-Curtis or Euclidean) across experimental treatment levels, and like multivariate analysis of variance (MANOVA), uses one test for determining treatment effects on multiple co-varying responses rather than multiple univariate analyses, thereby reducing the likelihood of Type I errors. PERMANOVA, however, calculates P-values using permutations of model residuals or raw data, and, therefore, unlike MANOVA, data need not exhibit multivariate normality. Our PERMANOVA analyses were conducted on Euclidean distance measures and P-values were estimated from 10,000 permutations of model residuals. Euclidean distance was a suitable measure because, unlike typical community data, our data set had few zeros (McCune and Grace 2002). Therefore, a zero contributing to the similarity between two sample points, as occurs with the Euclidean distance measure (Gotelli and Ellison 2004), was ecologically relevant. In addition to these PERMANOVA analyses, total earthworm numbers and biomass (i.e., summed across the five functional/taxonomic categories) were analyzed using analysis of covariance (ANCOVA). Using two different statistical models increases the likelihood of detecting differences between expulsion methods, and finding similar patterns strengthens confidence in the inferences from the statistical tests.

All models were Type I sequential models that tested for differences between expulsion methods (AITC or mustard; fixed effects) only after accounting for any effects that GWC (continuous covariate) and site (1 – 4; fixed effect) had on the response variables. All possible interactions between terms were included in the initial models. Interaction terms were removed in a hierarchical manner from PERMANOVA models and pooled with the model's residual error if their P-values were > 0.15 (Crawley 2005; Anderson et al. 2008). Interaction terms for which $P > 0.15$ were similarly removed from ANCOVA models when log-likelihood ratio tests and AIC values for models with and without the term revealed no significant difference or improvement, respectively, from including the term in the model (Zuur et al. 2009). The factor "site" was not treated as a random effect in order to test our prediction that there were differences in earthworm community composition and abundances across the range of sites, and to determine if the effectiveness of expulsion methods varied in relation to changes in earthworm community composition and abundance. GWC was included as a covariate to allow for testing the effectiveness of the expulsion methods across the range of soil moisture levels found on our study sites (0.24 – 0.56). Preliminary analyses following methods of Anderson et al. (2008) and Zuur et al. (2009) revealed that including the fact that sampling plots were paired did not increase the explanatory value of our models nor affect the interpretation of our results ($L_1 \leq 1.053$, $P \geq 0.341$, AIC differences < 2); therefore, samples taken within a site were treated as if the expulsion treatments had been assigned completely at random.

As an aid to interpreting PERMANOVA results, principle coordinate ordinations (PCO) (Gower 1966; Anderson et al. 2008) were conducted on Euclidean distance measures calculated from numbers and biomass of each earthworm group. If terms were statistically significant in the PERMANOVA, a test for homogeneity of multivariate dispersions [(PERMDISP) (i.e., multidimensional variance)] was conducted for those terms using 10,000 permutations of model residuals to determine if the significance was a result of differing locations of treatment-level centroids in multivariate space or the result of differences in dispersion (Anderson 2006). Differences between and among levels of significant model terms were then determined using permutational pairwise comparisons (Anderson et al. 2008). Differences among levels of significant terms in the ANCOVA were determined using Tukey HSD tests. PERMANOVA, PCO, PERMDISP, and multivariate pairwise comparisons were conducted using PERMANOVA+ © (Anderson et al. 2008). ANCOVA, and Tukey HSD tests were conducted using R (R Development Core Team 2008).

RESULTS

Earthworm abundance (number of individuals, categories pooled) averaged across all sites was 164 ± 11 m^{-2}, and total earthworm biomass was 15.4 ± 1.1 g m^{-2}. All earthworms sampled were exotic to the study area (Schwert 1990). *Lumbricus terrestris* was the only anecic species found. All endogeic specimens belonged to the genus *Aporrectodea* except for one individual of *Allolobophora chlorotica* Savigny. Because no juveniles were found with green pigment, which is characteristic of *A. chlorotica* (Schwert 1990), and all endogeic adults but one belonged to the genus *Aporrectodea*, it is likely that all endogeic juveniles also belonged to the genus *Aporrectodea*. While a pink morph of *A. chlorotica* exist (Satchell 1967), possibly representing separate species (King et al. 2008; Lowe and Butt 2008), we found no evidence from the literature that it is present in the Chicago region. *Lumbricus rubellus* was the only epi-endogeic earthworm species sampled in our study. No epigeic worms were found.

Both PERMANOVA and ANCOVA analyses revealed that earthworm abundance and community structure do not differ between the two different sampling techniques (Table 1, Figure 1). Principle coordinate ordinations of count and biomass data further supported this finding by showing no clear separation of plots based on expulsion method (Figure 2). Furthermore, the lack of significant interaction terms in the PERMANOVA analyses suggested that expulsion methods were equally efficient across all sites (i.e., across the range of

Table 1. PERMANOVA results for earthworm (A) numbers and (B) biomass. The reduced model is presented (i.e., P values refer to the model with all non-significant interaction terms removed). Values for removed terms are also given. GWC = soil gravimetric water content, EM = expulsion method. Terms are presented in the order by which they were included in the model.

Model Term	Unique permutations	DF	MS	Pseudo F	P-value
(A) Earthworm numbers					
GWC	9946	1	4265.4	2.0633	0.1266
Site	9935	3	9119.4	4.4112	0.0016
EM	9947	1	2047.2	0.9903	0.3642
Residuals		26	2067.3		
Total		31			
Removed Terms					
GWC x Site	9964	3	1751.8	0.8308	0.5459
GWC x EM	9940	1	946.3	0.4378	0.6760
Site x EM	9943	3	1930.7	0.8785	0.5175
GWC x Site x EM	9949	3	2283.9	1.0469	0.4061
(B) Earthworm biomass					
GWC	9955	1	149.55	8.9475	0.0011
Site	9920	3	179.45	10.7360	0.0001
EM	9947	1	2.563	0.1533	0.9366
Residuals		26	16.72		
Total		31			
Removed Terms					
GWC x Site	9935	3	17.069	1.0240	0.4118
GWC x EM	9935	1	15.427	0.9224	0.4135
Site x EM	9935	3	15.879	0.9420	0.4726
GWC x Site x EM	9955	3	18.319	1.1045	0.3673

earthworm densities), as well as the range of GWC sampled (Table 1). Similarly, ANCOVA's revealed that the total earthworm count and biomass sampled did not differ between expulsion methods regardless of site or GWC (Table 2; Figure 3).

As predicted, community composition varied significantly among sites when defined either in terms of numerical abundance or biomass of the individual earthworm groups (PERMANOVA; Table 1). PERMDISP analyses revealed that this variability can be attributed to differences in centroid location rather than to differences in dispersion (count results: Pseudo $F_{3, 28} = 0.394$; P = 0.82; biomass results: Pseudo $F_{3, 28} = 0.365$; P = 0.81). ANCOVA analyses revealed that total earthworm numbers and biomass also varied among the four sites (Table 2; Figure 3). Soil moisture (GWC) was negatively related to community composition [defined by biomass (PERMANOVA, Table 1; r = -0.28 in relation to PCO1 of Figure 2B)] and total earthworm biomass (ANCOVA, Table 2; r = -0.45).

DISCUSSION

Overall Findings

Earthworm abundances (numbers and biomass) as well as earthworm community composition did not differ between plots sampled with AITC or mustard. This pattern was consistent across the suspected gradients of earthworm abundance (Figure 3) and GWC found at our study site. These findings are consistent with our hypothesis that the earthworm community composition and abundance revealed by both expulsion methods would be indistinguishable from one another. We, therefore, confirmed that mustard powder is as effective as AITC in extracting earthworms from woodland soil; and because it is a safer and more practical method than AITC, can be utilized by scientists and land managers who involve citizen scientists in their investigations of earthworms.

The only differences found in total earthworm abundance and community composition were related to site and GWC. The variability in earthworm community composition and abundance observed across the levels of site coincide with patterns of increased earthworm abundance observed in buckthorn-invaded woodlands relative to buckthorn-free woodlands (Heneghan et al. 2007) and patterns of decreased earthworm abundance observed in response to buckthorn removal (Madritch and Lindroth 2009). The finding of relevance to our study's objectives, however, is that mustard-powder and AITC expulsions were equally effective across a range of earthworm densities and soil moistures in the woodland soils we sampled.

Applications for Research Involving Citizen Scientists

Mustard-powder expulsion of earthworms meets all three criteria for usefulness in research involving citizen scientists – ease, safety, and reliability. Like other expulsion methods (e.g., AITC or formalin), irrigating the soil with a mixture of water and mustard-powder to collect earthworms requires much less time, and is much less destructive of the soil subsystem, than digging followed by hand-sorting. AITC is both safe to handle and environmentally safe when it is diluted to the concentration necessary for sampling earthworms, which is less than concentrations found in many Brassicaceae crop species (Kushad et al.

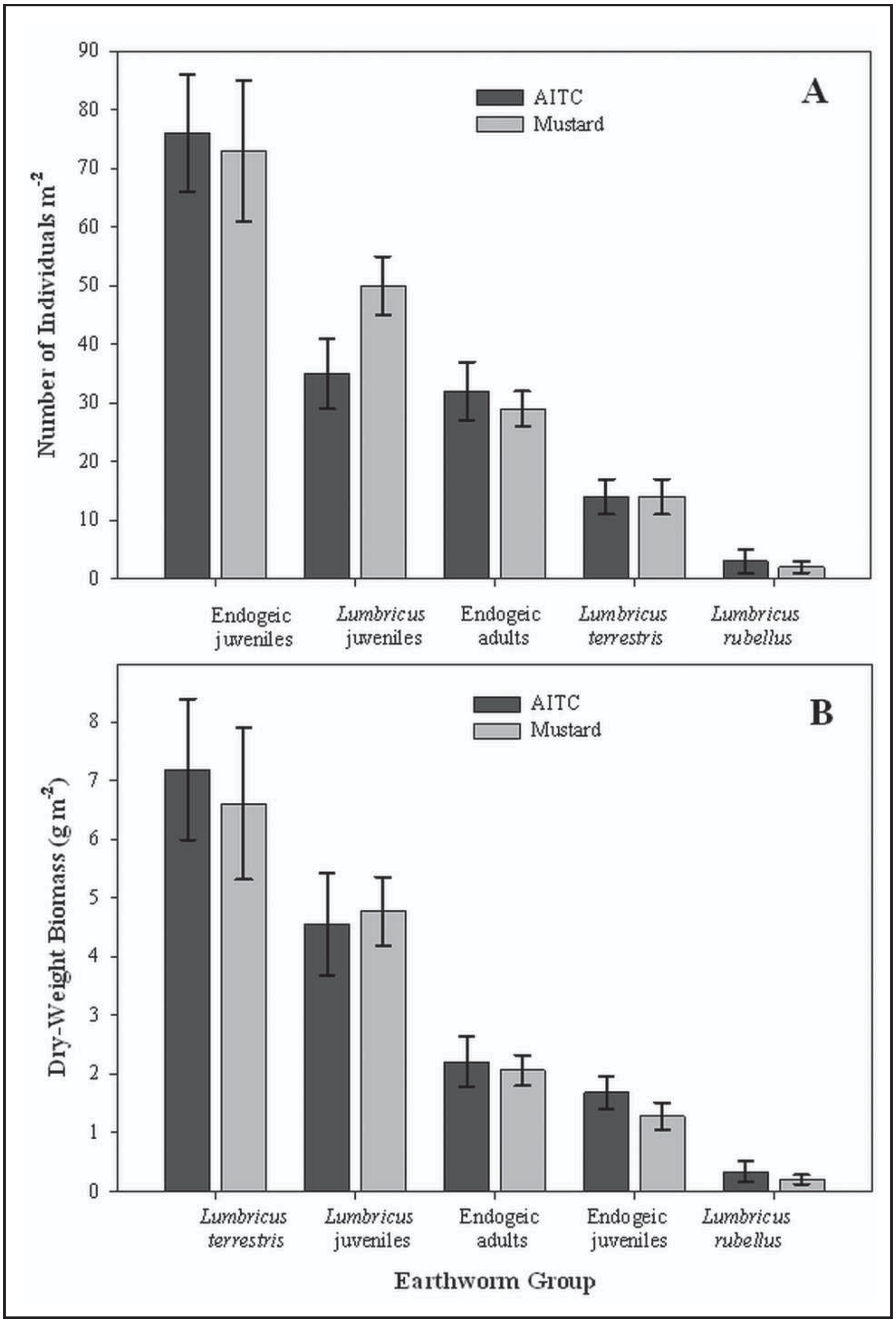

Figure 1. The (A) numbers and (B) biomass collected of each earthworm group using AITC or mustard powder expulsions. Means ± SE.

1999). However, the logistical difficulties associated with preparing the concentrated AITC stock solution (e.g., health issues, need to use a fume hood, and need to dilute with 100% alcohol) make the use of AITC unsuitable for broader use. Mustard powder, on the other hand, simply needs to be mixed with water, making it a practical and safe alternative to AITC. Finally, our results indicate that mustard-powder and AITC yielded similar conclusions about earthworm communities across four woodland sites differing in earthworm abundance and soil moisture.

Involving the public in scientific investigations is increasingly recognized as a productive outreach strategy for educating the public about how science is conducted, the role of science in addressing problems faced by society (e.g., climate change, pollution, invasive species, etc.), and general scientific principles (Jenkins 1999; Trumbull et al. 2000; Bonney et al. 2009; Silvertown 2009). Involving citizens can also directly benefit researchers. Citizens can facilitate the accumulation of large amounts of data that would be fiscally or logistically impossible for most studies (e.g., Lepczyk 2005). Growing recognition of the value of non-scientists as volunteer labor in turn is a major reason why interested non-scientists are being utilized in more research projects (Silvertown 2009). For example, in another research project, undergraduate students and volunteers helped us sample earthworms, using mustard expulsion, in 90 study plots within a single day. At over 20 minutes a plot, this effort would have cost more than 30 hours worth of labor from paid technicians. Another example, on a much larger scale, of how citizen scientists have benefited earthworm studies comes from the Great Lakes Worm Watch (2012). With the help of citizen scientists using mustard expulsions, this group has collected approximately 22,000 earthworms of 17 species from 9 states across the Great Lakes region of the United States (Ryan Hueffmeier, Program Coordinator of the Great Lakes Worm Watch, pers. comm.).

Further Considerations

Utilization of mustard-powder expulsions may not always be the best strategy for sampling earthworms. For example, AITC is cheaper than mustard powder. We estimate that the mustard-powder expulsion costs \$2.40 m^{-2}, whereas the AITC solution costs from \$0.90 to \$1.90 m^{-2}, depending upon the price of ethanol. Thus, when considering chemical costs alone, using AITC is cheaper than mustard powder. The extra cost of mustard powder, however, is compensated for by the fact that it is easy to purchase, ship, transport (at least in our study area), and mix. These advantages make the use of mustard more viable than AITC for earthworm-sampling research programs that involve citizen scientists lacking access to a laboratory. The extra

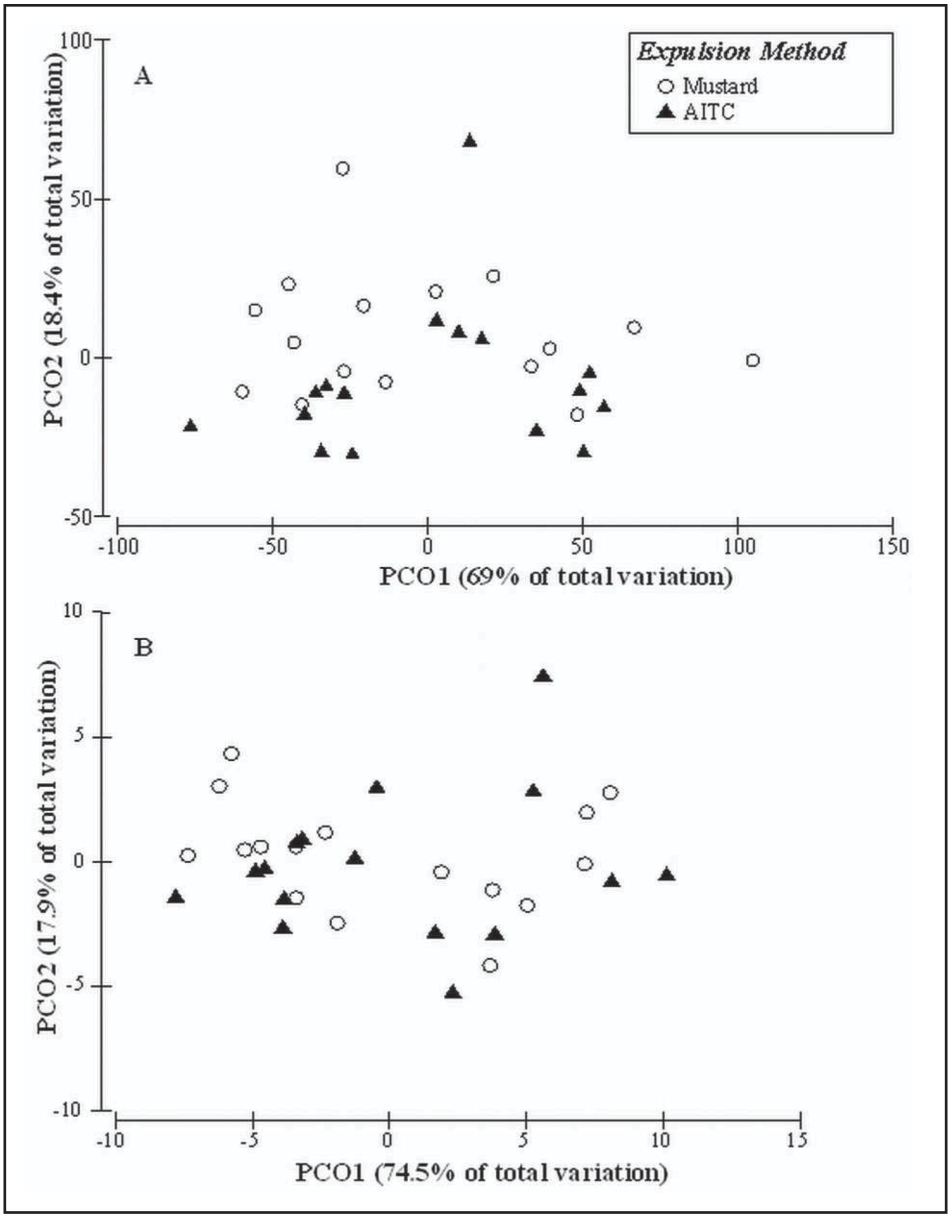

Figure 2. Principle coordinate ordination (PCO) of Euclidean distances between plots based upon (A) numbers and (B) biomass of the five functional/taxonomic earthworm groups, showing a lack of separation of plots based upon expulsion method.

cost of mustard is yet further justified when considering that its use allows for data collection by volunteers rather than paid technicians.

Mustard powder may not be as effective as we found it to be if one is sampling earthworms under quite different soil conditions than ours. We compared mustard powder and AITC in two very similar soil types occurring in one woodland. Both soils consisted of silty loams formed under hardwood forests (USDA 2009). It is unknown if a similar equivalency between mustard and AITC would be found for all types of soils. For example, we observed a possible tendency for the mustard suspension to take longer to percolate into the soil than the AITC solution, although we did not quantify this time difference. While this possible difference in percolation times did not affect the utility of the mustard expulsions under our study conditions, it does suggest that mixtures of mustard powder and water may not be able to percolate into soils with high clay content. However, we are utilizing mustard expulsions in an ongoing study of earthworm abundances in natural areas spread across the Chicago region that have a wide range of soils that vary in particle sizes, and we have yet to notice any possible complications due to slow percolation of mustard-water mixtures (L.G. Umek et al., unpubl. data).

Other caveats relate to seasonality and variation between mustards. The comparison between mustard and AITC expulsions made in our study was done during one season. Because the effectiveness of expulsion methods can vary seasonally (Callaham and Hendrix 1997), the similarity in effectiveness between mustard and AITC that we observed may not be seasonally universal. Since earthworm expulsions using mustard are difficult to standardize due to variability among and even within mustard species (Zaborski 2003; Pelosi et al. 2009), researchers will, for consistency, want to use the same mustard product in their study. If necessary, researchers may also want to conduct trials comparing different types of mustard to AITC. In doing so, one can find out which kinds of mustard produce similar results to AITC and, therefore, facilitate cross-study comparisons. The experimental design (comparison of paired plots) coupled to the multivariate statistical analyses used in this study can act as a repeatable framework for future studies evaluating these among-mustard comparisons, as well as future research into possible differences between mustard and AITC due to soil type or sampling season.

These caveats have most relevance for the research team that relies solely on professional scientists or paid technicians and that is not sampling in natural areas or preserves where the more destructive methods may be prohibited. For research projects that depend upon active collaborations with large teams of citizen scientists for all phases of earthworm sampling, a mixture of mustard powder and water is clearly a viable alternative approach for woodland soils and, perhaps, also for many other soil types.

Table 2. ANCOVA results for earthworm (A) numbers and (B) biomass. The reduced model is presented (i.e., P values refer to the model with all non-significant interaction terms removed).Values for removed terms are also given. GWC = soil gravimetric water content, EM = expulsion method. Terms are presented in the order by which they were included in the model.

(A) Total Earthworm numbers				
Model Term	DF	MS	F-ratio	P-value
GWC	1	8861	3.8093	0.0618
Site	3	14462	6.2169	0.0025
EM	1	835	0.359	0.5543
Residuals	26	2326		
Total	31			
Removed Terms				
GWC x Site	3	2052	0.8689	0.4714
GWC x EM	1	463	0.1890	0.6680
Site x EM	3	1760	0.6885	0.5702
GWC x Site x EM	3	1175	0.4171	0.7431
(B) Total Earthworm biomass				
Model Term	DF	MS	F-ratio	P-value
GWC	1	252.21	17.7394	0.0003
Site	3	215.70	15.1709	< 0.0001
EM	1	4.46	0.3133	0.5804
Residuals	26	14.22		
Total	31			
Removed Terms				
GWC x Site	3	12.64	0.8762	0.4678
GWC x EM	1	2.14	0.1432	0.7088
Site x EM	3	25.43	1.9077	0.1626
GWC x Site x EM	3	8.41	0.5895	0.6310

ACKNOWLEDGMENTS

This research was funded by National Science Foundation Grant DGE-0549245, "Landscape Ecological and Anthropogenic Processes," the Gaylord and Dorothy Donnelley Foundation, and the Gutgsell Family Foundation. We thank Jim Steffen of the Chicago Botanical Garden for his assistance and his management of the study site, Mac Callaham of the U.S. Forest Service's Southern Research Station for taking time to review and provide detailed comments that improved the quality of this document, and two anonymous reviewers for their comments and evaluation of this document. Thanks to Joan Nikki Chaffin, Kathleen Soler, Justin Wexler, and Alexandra Zelles for their assistance in the field.

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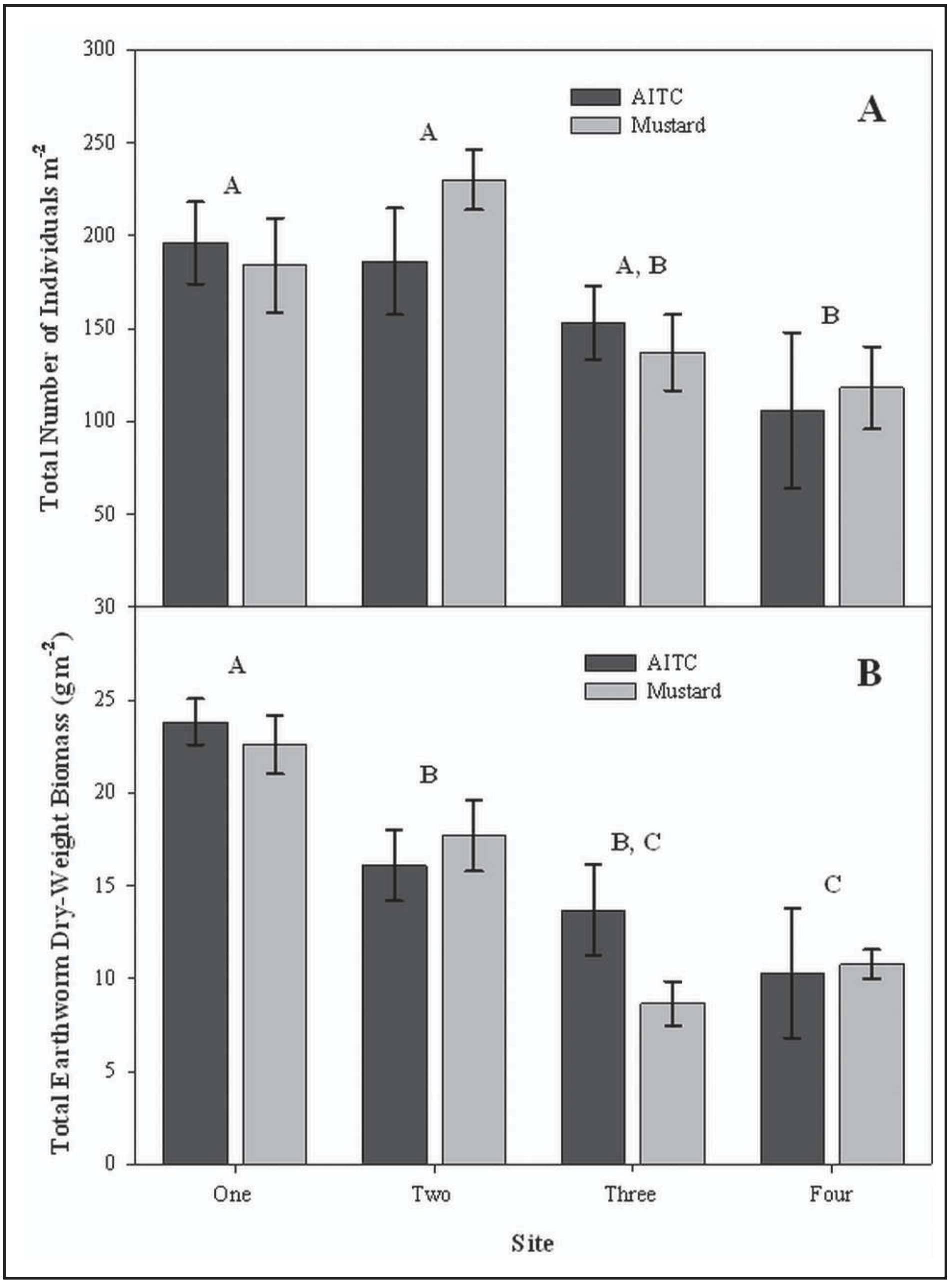

Figure 3. Total earthworm (A) numbers and (B) biomass sampled across sites using AITC or mustard powder expulsion (n = 4 for each site by expulsion treatment combination, i.e., bar). Site 1, currently invaded by buckthorn; Site 2, buckthorn removed for 5 yrs; Site 3, buckthorn removed for 12 yrs; and Site 4, never invaded by buckthorn. Sites that have different letters above their pair of bars are significantly different from one another (result of Tukey HSD test). Means ± SE.

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