

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

1 **A simple, safe, and effective sampling technique for investigating earthworm communities**
2 **in woodland soils: implications for citizen science**

3

4 **Basil V. Iannone III¹, Lauren G. Umek², David H. Wise³, and Liam Heneghan²**

5

6 ¹*Corresponding author.* Email address: bianno2@uic.edu; Phone: 312-355-3231; Fax: 312-413-
7 2435; LEAP IGERT, Department of Biological Sciences (MC 066), University of Illinois at
8 Chicago, 845 W. Taylor St., Chicago, IL 60607, U.S.A.

9

10 ² Environmental Science Program, DePaul University, 1110 W Belden, Chicago, IL 60614,
11 U.S.A.

12

13 ³Institute for Environmental Science and Policy, and Department of Biological Sciences,
14 University of Illinois at Chicago, 845 W. Taylor St., Chicago, IL 60607, U.S.A.

15

16 *Word count (Abstract through Literature Cited): 6544*

17

18

19

20

21

22

23

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

24 **ABSTRACT:** We evaluated the efficacy of a mixture of ground hot mustard and water as a
25 sampling method for earthworms in research projects involving citizen scientists. To do so we
26 conducted a field experiment to determine if sampling earthworms using mustard-powder would
27 reveal similar patterns of earthworm abundance and community composition as relying on the
28 more difficult to prepare, and possibly hazardous, allyl isothiocyanate (AITC) solution.
29 Earthworms were sampled using either mustard or AITC in four pairs of 0.25-m² plots located in
30 each of four woodland sites that were predicted to exhibit a range of earthworm densities. Soil
31 gravimetric water content (GWC) of each plot was quantified as a covariate. For analyses of
32 changes in abundance and community structure, earthworms were classified as belonging to one
33 of five groups based on where they occur in the soil profile, developmental stage, and level of
34 taxonomic identification. The two sampling techniques revealed similar earthworm abundances
35 and community composition across the four sites (all $P_s \geq 0.16$) and across the range in GWC
36 (all $P_s \geq 0.36$). We conclude that using the mustard-water mixture to sample earthworms at our
37 study site appears to be just as effective and reliable as using AITC. The mustard-water mixture,
38 which is easier to prepare and is less hazardous than AITC solution, should therefore be
39 considered as an appropriate tool to be utilized by researchers who collaborate with citizen
40 scientists to help collect the large datasets needed to reveal how woodland management and
41 restoration programs affect earthworms.

42

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

45

46

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

47 **INTRODUCTION**

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

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

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

70 Finding non-destructive, yet reliable, strategies to sample earthworms in natural areas is
71 particularly important for those temperate woodlands of North America where invasions by
72 exotic earthworms have had profound effects on aboveground and belowground community
73 structure and ecosystem functioning (Bohlen and Edwards 1995; Bohlen et al. 2002; Li et al.
74 2002; Bohlen et al. 2004; Suarez et al. 2004; Hale et al. 2005; Frelich et al. 2006; Hale et al.
75 2006; Migge-Kleian et al. 2006; Heneghan et al. 2007). Furthermore, land managers in these
76 regions may want to understand how management actions (e.g. controlled burning, exotic plant
77 removal, ecological restoration, etc.) will influence the abundance and community composition
78 of invasive earthworms. Thanks to taxonomic keys developed for use by non-earthworm experts,
79 such as the one developed by Hale (2007) for identifying earthworms of the Great Lakes region,
80 non- experts can typically identify earthworms to genus or sometimes species with relatively
81 little training. Thus, citizen scientists could play a key role in increasing our understanding of
82 how management activities influence earthworm populations so long as the techniques used to
83 sample earthworms are practical, safe, and reliable.

84 Chemical expulsion, i.e. irrigating the soil with a chemical irritant that causes earthworms
85 to emerge on the surface (Raw 1959; Gunn 1992; Zaborski 2003), is more logistically feasible
86 than hand-sorting, particularly when large numbers of samples are required. Hand-sorting is time
87 consuming, labor intensive, and requires digging up and sifting through soils in order to collect
88 earthworms, which can be destructive and may be prohibited in natural areas (Springett 1981;
89 Gunn 1992). Chemical expulsions, on the other hand, require that water (20-40 L H₂O for each
90 square meter sampled) be transported to sampling locations. This challenge, however, can be met
91 if citizen scientists assist with sampling efforts.

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

92 The effectiveness of chemical expulsion relative to hand-sorting depends on earthworm
93 size, type, and sexual development, as well as the depth in the soil where the worms occur, soil
94 moisture, and soil temperature (Raw 1959; Chan and Munro 2001; Zaborski 2003; Bartlett et al.
95 2006). Nonetheless, differences in efficiency between chemical expulsion and hand sorting are
96 consistent across a range of habitats and soil conditions (Lawrence and Bowers 2002),
97 suggesting that chemical expulsion can be a powerful technique when comparisons between sites
98 of relative, rather than absolute, abundance will answer the scientific question posed. Expulsion
99 methods could be an excellent option for research projects that rely on citizen scientists to
100 sample earthworms, because they are both suitable for among-site comparisons, and generally
101 easier and quicker to administer than hand-sorting.

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

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

114 These standardized AITC solutions are effective for sampling earthworms (Zaborski
115 2003; Pelosi et al. 2009) and are environmentally safe in that they are not known to harm plants
116 and have a low residual period in the soil (Borek et al. 1995). However, the use of AITC
117 solutions presents some unique challenges. First, prior to its addition to water, AITC must be
118 mixed with 100% alcohol (“stock solution”). Secondly, AITC in its concentrated form is caustic,
119 may be fatal if absorbed through the skin, and can irritate the skin, eyes, and respiratory tract (see
120 MSDS and product label for details), requiring that AITC stock solutions be prepared underneath
121 a fume hood while wearing protective clothing and eyewear. This requirement is a major
122 obstacle to researchers and land managers relying on citizen scientists. These drawbacks in
123 preparing AITC raise issues of both safety and practicality, and suggest that mixtures of mustard
124 powder and water should be used in research projects involving citizen scientists if these
125 mixtures are as effective at expelling earthworms as AITC solutions.

126

127 **OBJECTIVE**

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

135

136

137 **METHODS**

138 **Overview of Experimental Design**

139 Earthworms were sampled in four pairs of plots in each of four woodland sites that were
140 predicted to have different earthworm abundances due to different histories of management of
141 the invasive, exotic shrub *Rhamnus cathartica* L. (European buckthorn; hereafter buckthorn).
142 Earthworm abundances are hypothesized to increase in response to buckthorn invasion
143 (Heneghan et al. 2007) and decrease in response to buckthorn removal (Madritch and Lindroth
144 2009). Therefore, by replicating the experiment in these four sites we hoped to be able to
145 compare the effectiveness of the two expulsion methods at different levels of earthworm
146 abundance. The four sites differed as follows: Site 1, currently invaded by buckthorn; Site 2,
147 buckthorn had been removed for 5 yrs; Site 3, buckthorn had been removed for 12 yrs; and Site
148 4, had never been invaded by buckthorn. In each site, four pairs of plots (50 x 50 cm) were
149 located 12.5 m from a center point, one pair at each cardinal direction. The plots within a pair
150 were 2 m apart. One plot from each pair was randomly chosen to be sampled using AITC, the
151 other plot was then sampled using mustard powder.

152

153 **Study Site**

154 This study was conducted at Mary-Mix McDonald Woods, a remnant native woodland located
155 approximately 35 km N of downtown Chicago at the Chicago Botanical Garden (Coordinates:
156 42° 8'59.90"N, 87°46'46.30"W). This woodland was set aside for conservation and is therefore
157 an excellent example of a place where hand-sorting and formalin expulsion would not be
158 appropriate. The mean summer high temperatures at this site are 26-33°C, the mean summer low
159 temperatures are 18°C, and mean annual precipitation amounts are 920 mm (NOAA 2010).

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

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

175

176 **Preparation of the Expulsion Mixtures**

177 The AITC solution and mustard powder mixture were prepared following methods of Zaborksi
178 (2003) and Heneghan and Umek (unpubl. data), respectively. First, stock solutions for both
179 extracts were prepared. AITC (ARCOS ORGANICS; 94%; density 1.017) was diluted with
180 100% ethanol to a 5 g L^{-1} concentration. This AITC stock solution was placed in a lightproof
181 container and refrigerated for 48 hrs prior to use. Because AITC is not readily soluble in water,
182 ethanol acts as an emulsifier when AITC is added to water (see below). In the evenings prior to

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

183 sampling, a separate 125-mL plastic bottle of mustard powder stock solution was prepared for
184 each plot that would be sampled. In each bottle, 38.1 g of dried extra-hot oriental mustard
185 powder (*Brassica juncea* L.) [Frontier Natural Products Co-op (Norway, IA)] was added to 100
186 mL of water (381 g L^{-1}) and shaken until the mixture was paste-like. Just prior to sampling, 100
187 mL of the AITC stock solution or an individualized 100 mL of mustard-powder stock mixture
188 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}
189 mustard. This AITC concentration was shown by Zaborski (2003) to expel the greatest number
190 and biomass of earthworms when compared with AITC concentrations ranging from 0.005 to
191 0.250 g L^{-1} . The mustard powder concentration is recommended by Clapperton et al. (2008).

192

193 **Earthworm sampling and identification**

194 Earthworms were sampled on 21 and 22 October 2008 [daily temperature range of 3 - 14°C,
195 mean daily temperature of 8°C (NOAA 2010)]. At each sampling location a 50 x 50-cm plot
196 marker constructed of 13-cm high lawn edging was hammered 5 cm into the ground using a
197 rubber mallet. We then waited a minimum of 10 minutes before sampling earthworms in order to
198 reduce the impact that hammering might have had on sampling. During this waiting period we
199 collected a 6 x 10-cm soil core from a point located approximately 10 cm from the lower right
200 corner and outside of the plot marker. Gravimetric water content (GWC) of these soil cores were
201 later estimated on a dry-weight basis as described in Robertson et al. (1999). Leaf litter was
202 removed from the soil surface and inspected for the presence of epigeic earthworms. The
203 appropriate expulsion mixture for that plot was then slowly poured over the soil until pooling of
204 the mixture occurred. After the mixture had percolated into the soil, we waited 4 minutes and
205 then poured more mixture over the plot until pooling occurred again. This process was repeated

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

206 until the 5 L of AITC solution or mustard mixture had been used. All earthworms that came to
207 the surface within the boundaries of the plot marker were collected until 10 minutes after the last
208 of the solution or mixture had been emptied onto the plot. Preliminary trials under similar soil
209 conditions revealed that using greater than 5 L resulted in excessive and prolonged pooling,
210 which hindered our ability to collect earthworms.

211 After being collected, earthworms were placed in plastic jars with moist paper towels and
212 taken back to the lab. Paper towels and debris were then removed from each jar; earthworms
213 were rinsed off using tap water, placed back into the empty jars, and placed in a refrigerator at
214 4°C for 72 hrs to allow time for the earthworms to empty their gut contents. Earthworms were
215 then identified, counted, dried at 70°C for 48 hrs, and weighed.

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

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

228 (Bouché 1972); and epi-endogeic species are those that form horizontal borrows in the upper
229 layers of the mineral soil, but consume leaf litter (Terhivuo 1988).

230

231 **Statistical Analyses**

232 To determine whether the two expulsion methods varied in their effectiveness, permutational
233 multivariate analysis of variance [PERMANOVA; (Anderson 2001; McCardle and Anderson
234 2001; Anderson et al. 2008)] was conducted on the number of individuals and biomass of the
235 five earthworm groups sampled from all plots. PERMANOVA tests for differences in
236 community distance measures (e.g. Bray-Curtis or Euclidean) across experimental treatment
237 levels, and like multivariate analysis of variance (MANOVA), uses one test for determining
238 treatment effects on multiple co-varying responses rather than multiple univariate analyses,
239 thereby reducing the likelihood of Type I errors. PERMANOVA, however, calculates p-values
240 using permutations of model residuals or raw data, and therefore, unlike with MANOVA, data
241 need not exhibit multivariate normality. Our PERMANOVA analyses were conducted on
242 Euclidean distance measures and P-values were estimated from 10,000 permutations of model
243 residuals. Euclidean distance was a suitable measure because unlike typical community data, our
244 data set had few zeros (McCune and Grace 2002). Therefore, a zero contributing to the similarity
245 between two sample points, as occurs with the Euclidean distance measure (Gotelli and Ellison
246 2004), was ecologically relevant. In addition to these PERMANOVA analyses, total earthworm
247 numbers and biomass (i.e. summed across the five functional/taxonomic categories) were
248 analyzed using analysis of covariance (ANCOVA). Using two different statistical models
249 increases the likelihood of detecting differences between expulsion methods and finding similar
250 patterns strengthens confidence in the inferences from the statistical tests.

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

251 All models were Type I sequential models that tested for differences between expulsion
252 methods (AITC or mustard; fixed effects) only after accounting for any effects that GWC
253 (continuous covariate) and site (1-4; fixed effect) had on the response variables. All possible
254 interactions between terms were included in the initial models. Interaction terms were removed
255 in a hierarchical manner from PERMANOVA models and pooled with the model's residual error
256 if their P-values were > 0.15 (Crawley 2005; Anderson et al. 2008). Interaction terms for which
257 $P > 0.15$ were similarly removed from ANCOVA models when log-likelihood ratio tests and
258 AIC values for models with and without the term revealed no significant difference or
259 improvement, respectively, from including the term in the model (Zuur et al. 2009). The factor
260 "site" was not treated as a random effect in order to test our prediction that there were differences
261 in earthworm community composition and abundances across the range of sites, and to determine
262 if the effectiveness of expulsion methods varied in relation to changes in earthworm community
263 composition and abundance. GWC was included as a covariate to allow for testing the
264 effectiveness of the expulsion methods across the range of soil moisture levels found on our
265 study sites (0.24 – 0.56). Preliminary analyses following methods of Anderson et al. (2008) and
266 Zuur et al. (2009) revealed that including the fact that sampling plots were paired did not
267 increase the explanatory value of our models nor affect the interpretation of our results ($L_1 \leq$
268 1.053 , $P \geq 0.341$, AIC differences < 2); therefore, samples taken within a site were treated as if
269 the expulsion treatments had been assigned completely at random.

270 As an aid to interpreting PERMANOVA results, principle coordinate ordinations (PCO)
271 (Gower 1966; Anderson et al. 2008) were conducted on Euclidean distance measures calculated
272 from numbers and biomass of each earthworm group. If terms were statistically significant in the
273 PERMANOVA, a test for homogeneity of multivariate dispersions (PERMDISP), i.e.,

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

274 multidimensional variance, was conducted for those terms using 10,000 permutations of model
275 residuals to determine if the significance was a result of differing locations of treatment-level
276 centroids in multivariate space, or the result of differences in dispersion (Anderson 2006).
277 Differences between and among levels of significant model terms were then determined using
278 permutational pair-wise comparisons (Anderson et al. 2008). Differences among levels of
279 significant terms in the ANCOVA were determined using Tukey HSD tests. PERMANOVA,
280 PCO, PERMDISP, and multivariate pairwise comparisons were conducted using
281 PERMANOVA+ © (Anderson et al. 2008). ANCOVA, and Tukey HSD tests were conducted
282 using R (R Development Core Team 2008).

283

284 **RESULTS**

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

296 Both PERMANOVA and ANCOVA analyses revealed that earthworm abundance and
297 community structure do not differ between the two different sampling techniques (Table 1,
298 Figure 1). Principle coordinate ordinations of count and biomass data further supported this
299 finding by showing no clear separation of plots based on expulsion method (Figure 2).
300 Furthermore, the lack of significant interaction terms in the PERMANOVA analyses suggested
301 that expulsion methods were equally efficient across all sites, i.e., across the range of earthworm
302 densities, as well as the range of GWC sampled (Table 1). Similarly, ANCOVA's revealed that
303 the total earthworm count and biomass sampled did not differ between expulsion methods
304 regardless of site or GWC (Table 2; Figure 3).

305 As predicted, community composition varied significantly among sites when defined
306 either in terms of numerical abundance or biomass of the individual earthworm groups
307 (PERMANOVA; Table 1). PERMDISP analyses revealed that this variability can be attributed to
308 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
309 revealed that total earthworm numbers and biomass also varied among the four sites (Table 2;
310 Figure 3). Soil moisture (GWC) was negatively related to community composition [defined by
311 biomass (PERMANOVA, Table 1; $r = -0.28$ in relation to PCO1 of Figure 2)], and total
312 earthworm biomass (ANCOVA, Table 2; $r = -0.45$).

314

315 **DISCUSSION**

316 **Overall Findings**

317 Earthworm abundances (numbers and biomass) as well as earthworm community composition
318 did not differ between plots sampled with AITC or mustard. This pattern was consistent across

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

319 the suspected gradients of earthworm abundance (Figure 3) and GWC found at our study site.
320 These findings are consistent with our hypothesis that the earthworm community composition
321 and abundance revealed by both expulsion methods would be indistinguishable from one
322 another. We therefore confirmed that mustard powder is as effective as AITC in extracting
323 earthworms from woodland soil; and because it is a safer and more practical method than AITC,
324 can be utilized by scientists and land managers who involve citizen scientists in their
325 investigations of earthworms.

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

334

335 **Applications for Research Involving Citizen Scientists**

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

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

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

349 Involving the public in scientific investigations is increasingly recognized as a productive
350 outreach strategy for educating the public about how science is conducted, the role of science in
351 addressing problems faced by society (e.g., climate change, pollution, invasive species, etc.), and
352 general scientific principles (Jenkins 1999; Trumbull et al. 2000; Bonney et al. 2009; Silvertown
353 2009). Involving citizens can also directly benefit researchers. Citizens can facilitate the
354 accumulation of large amounts of data that would be fiscally or logistically impossible for most
355 studies (e.g. Lepczyk 2005). Growing recognition of the value of non-scientists as volunteer
356 labor in turn is a major reason why interested non-scientists are being utilized in more research
357 projects (Silvertown 2009). For example, in another research project, undergraduate students
358 and volunteers helped us sample earthworms, using mustard expulsion, in 90 study plots within a
359 single day. At over 20 minutes a plot this effort would have cost more than 30 hours worth of
360 labor from paid technicians. Another example, on a much larger scale, of how citizen scientists
361 have benefited earthworm studies comes from the Great Lakes Worm Watch (2012). With the
362 help of citizen scientists using mustard expulsions, this group has collected approximately
363 22,000 earthworms of 17 species from 9 states across the Great Lakes region of the United States
364 (Ryan Hueffmeier, personal communication).

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

365 **Further Considerations**

366 Utilization of mustard-powder expulsions may not always be the best strategy for sampling
367 earthworms. For example, AITC is cheaper than mustard powder. We estimate that the mustard-
368 powder expulsion costs \$2.40 m⁻², whereas the AITC solution costs from \$0.90 to \$1.90 m⁻²,
369 depending upon the price of ethanol. Thus, when considering chemical costs alone, using AITC
370 is cheaper than mustard powder. The extra cost of mustard powder, however, is compensated for
371 by the fact that it is easy to purchase, ship, transport (at least in our study area), and mix. These
372 advantages make the use of mustard more viable than AITC for earthworm-sampling research
373 programs that involve citizen scientists lacking access to a laboratory. The extra cost of mustard
374 is yet further justified when considering that its use allows for data collection by volunteers
375 rather than paid technicians.

376 Mustard powder may not be as effective as we found it to be if one is sampling
377 earthworms under quite different soil conditions than ours. We compared mustard powder and
378 AITC in two very similar soil types occurring in one woodland. Both soils consisted of silty
379 loams formed under hardwood forests (USDA 2009). It is unknown if a similar equivalency
380 between mustard and AITC would be found for all types of soils. For example, we observed a
381 possible tendency for the mustard suspension to take longer to percolate into the soil than the
382 AITC solution, although we did not quantify this time difference. While this possible difference
383 in percolation times did not affect the utility of the mustard expulsions under our study
384 conditions, it does suggest that mixtures of mustard powder and water may not be able to
385 percolate into soils with high clay content. However, we are utilizing mustard expulsions in an
386 ongoing study of earthworm abundances in natural areas spread across the Chicago region that

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

387 have a wide range of soils that vary in particle sizes, and we have yet to notice any possible
388 complications due to slow percolation of mustard-water mixtures (Umek et al. unpublished data).

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

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

409

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

410 **ACKNOWLEDGEMENTS**

411 This research was funded by National Science Foundation Grant DGE-0549245, “Landscape
412 Ecological and Anthropogenic Processes,” the Gaylord and Dorothy Donnelley Foundation, and
413 the Gutsell Family Foundation. We would like to thank Jim Steffen of the Chicago Botanical
414 Garden for his assistance and his management of the study site, Mac Callaham of the US Forest
415 Service’s Southern Research Station for taking time to review and provide detailed comments
416 that improved the quality of this document, and two anonymous reviewers for their comments
417 and evaluation of this document. Thanks to Joan Nikki Chaffin, Kathleen Soler, Justin Wexler,
418 and Alexandra Zelles for their assistance in the field.

419

420

421

422

423

424

425

426

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

427 *Basil Iannone is a Ph.D. candidate in the Department of Biological Sciences at the University of*
428 *Illinois at Chicago, and a fellow in both the NSF-funded Landscape, Ecological, and*
429 *Anthropogenic Processes IGERT program, and the Institute for Environmental Science and*
430 *Policy. His research focuses on the effects of interactions among individual species, community*
431 *composition, and ecosystem-level processes on community change over time, particularly in the*
432 *context of biological invasions and ecological restorations. bianno2@uic.edu*

433
434 *Lauren Umek is a Project Coordinator in the Environmental Science Department at DePaul*
435 *University. Her research interests include plant-soil interactions with plant invasions and*
436 *ecological restoration. LUMEK@depaul.edu*

437
438 *David Wise is Associate Director of the Institute for Environmental Science and Policy, and*
439 *Professor of Ecology and Evolution in the Department of Biological Sciences at the University of*
440 *Illinois at Chicago. His research interests include the structure and dynamics of detritus-based*
441 *terrestrial food webs; and the dynamics, both ecological and human, of conserving and restoring*
442 *natural areas in heavily populated metropolitan landscapes. dhwis@uic.edu*

443
444 *Liam Heneghan is a professor of Environmental Science at DePaul University. His is co-chair of*
445 *the Chicago Wilderness Science team. His research interests are in incorporating insights from*
446 *soil ecology into restoration practice. LHENEGHA@depaul.edu*

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

447 **LITERATURE CITED**

- 448 Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance.
449 Austral Ecology 26:32-46.
- 450 Anderson, M.J. 2006. Distance-based tests for homogeneity of multivariate dispersions.
451 Biometrics 62:245-253.
- 452 Anderson, M.J., R.N. Gorley, and K.R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to
453 Software and Statistical Methods. PRIMER-E Ltd, Plymouth, UK.
- 454 Baker, G., P. Carter, V. Barrett, J. Hirth, P. Mele, and C. Gourley. 2002. Does the deep-
455 burrowing earthworm, *Aporrectodea longa*, compete with resident earthworm
456 communities when introduced to pastures in south-eastern Australia? European Journal of
457 Soil Biology 38:39-42.
- 458 Bartlett, M.D., M.J.I. Briones, R. Neilson, O. Schmidt, D. Spurgeon, and R.E. Creamer. 2010. A
459 critical review of current methods in earthworm ecology: From individuals to
460 populations. European Journal of Soil Biology 46:67-73.
- 461 Bartlett, M.D., J.A. Harris, I.T. James, and K. Ritz. 2006. Inefficiency of mustard extraction
462 technique for assessing size and structure of earthworm communities in UK pasture. Soil
463 Biology & Biochemistry 38:2990-2992.
- 464 Bohlen, P.J., and C.A. Edwards. 1995. Earthworm effects on N dynamics and soil respiration in
465 microcosms receiving organic and inorganic nutrients. Soil Biology & Biochemistry
466 27:341-348.

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

- 467 Bohlen, P.J., C.A. Edwards, Q. Zhang, R.W. Parmelee, and M. Allen. 2002. Indirect effects of
468 earthworms on microbial assimilation of labile carbon. *Applied Soil Ecology* 20:255-261.
- 469 Bohlen, P.J., P.M. Groffman, T.J. Fahey, M.C. Fisk, E. Suarez, D.M. Pelletier, and R.T. Fahey.
470 2004. Ecosystem consequences of exotic earthworm invasion of north temperate forests.
471 *Ecosystems* 7:1-12.
- 472 Bonney, R., C.B. Cooper, J. Dickinson, S. Kelling, T. Phillips, K.V. Rosenberg, and J. Shirk.
473 2009. Citizen science: a developing tool for expanding science knowledge and scientific
474 literacy. *Bioscience* 59:977-984.
- 475 Borek, V., M.J. Morra, P.D. Brown, and J.P. McCaffrey. 1995. Transformation of the
476 glucosinolate-derived allelochemicals allyl isothiocyanate and allylnitrile in soil. *Journal*
477 *of Agricultural and Food Chemistry* 43:1935-1940.
- 478 Bouché, M.B. 1972. *Lombriciens de France: Écologie Systématique*. Institut National de la
479 Recherche Agronomique, Paris, France.
- 480 Butt, K.R. 1998. Interactions between selected earthworm species: A preliminary, laboratory-
481 based study. *Applied Soil Ecology* 9:75-79.
- 482 Callaham, M.A., and P.F. Hendrix. 1997. Relative abundance and seasonal activity of
483 earthworms (Lumbricidae and Megascolecidae) as determined by hand-sorting and
484 formalin extraction in forest soils on the southern Appalachian Piedmont. *Soil Biology &*
485 *Biochemistry* 29:317-321.

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

- 486 Chan, K.Y., and K. Munro. 2001. Evaluating mustard extracts for earthworm sampling.
487 *Pedobiologia* 45:272-278.
- 488 Clapperton, M.J., G.H. Baker, and C.A. Fox. 2008. Earthworms. Pages 427-444 in M. R. Carter
489 and E. G. Gregorich, eds. *Soil Sampling and Methods of Analysis*. Taylor & Francis
490 Group, LLC, Boca Raton, FL.
- 491 Cohn, J.P. 2008. Citizen science: can volunteers do real research? *Bioscience* 58:192-197.
- 492 Coja, T., K. Zehetner, A. Bruckner, A. Watzinger, and E. Meyer. 2008. Efficacy and side effects
493 of five sampling methods for soil earthworms (Annelida, Lumbricidae). *Ecotoxicology*
494 and *Environmental Safety* 71:552-565.
- 495 Crawley, M.J. 2005. *Statistics: An Introduction using R*. John Wiley & Sons, Ltd, Chichester,
496 England.
- 497 Curry, J.P. 2004. Factors affecting the abundance of earthworms in soils. Pages 91 - 113 in C. A.
498 Edwards, ed. *Earthworm Ecology*. CRC Press, Boca Raton, London, New York,
499 Washington, D.C.
- 500 Dalby, P.R., G.H. Baker, and S.E. Smith. 1998. Competition and cocoon consumption by the
501 earthworm *Aporrectodea longa*. *Applied Soil Ecology* 10:127-136.
- 502 Edwards, C.A., and J.R. Lofty. 1982. The effect of direct drilling and minimal cultivation on
503 earthworm populations. *Journal of Applied Ecology* 19:723-734.

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

- 504 Eichinger, E., A. Bruckner, and M. Stemmer. 2007. Earthworm expulsion by formalin has severe
505 and lasting side effects on soil biota and plants. *Ecotoxicology and Environmental Safety*
506 67:260-266.
- 507 Frelich, L.E., C.M. Hale, S. Scheu, A.R. Holdsworth, L. Heneghan, P.J. Bohlen, and P.B. Reich.
508 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests.
509 *Biological Invasions* 8:1235-1245.
- 510 Gotelli, N.J., and A.M. Ellison. 2004. *A Primer of Ecological Statistics*. Sinauer Associates, Inc.,
511 Sunderland, MA.
- 512 Gower, J.C. 1966. Some distance properties of latent root and vector methods used in
513 multivariate analysis. *Biometrika* 53:325-338.
- 514 Great Lakes Worm Watch. 2012. URL: <http://www.greatlakeswormwatch.org>.
- 515 Gunn, A. 1992. The use of mustard to estimate earthworm populations. *Pedobiologia* 36:65-67.
- 516 Hale, C.M. 2007. *Earthworms of the Great Lakes*. Kollath and Stensaas Publishing, Duluth, MN.
- 517 Hale, C.M., L.E. Frelich, and P.B. Reich. 2005. Exotic European earthworm invasion dynamics
518 in northern hardwood forests of Minnesota, USA. *Ecological Applications* 15:848-860.
- 519 Hale, C.M., L.E. Frelich, and P.B. Reich. 2006. Changes in hardwood forest understory plant
520 communities in response to European earthworm invasions. *Ecology* 87:1637-1649.
- 521 Heneghan, L., J. Steffen, and K. Fagen. 2007. Interactions of an introduced shrub and introduced
522 earthworms in an Illinois urban woodland: impact on leaf litter decomposition.
523 *Pedobiologia* 50:543-551.

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

- 524 Jenkins, E.W. 1999. School science, citizenship, and the public understanding of science.
525 International Journal of Science Education 21:703-710.
- 526 King, R.A., A.L. Tibble, and W.O.C. Symondson. 2008. Opening a can of worms:
527 unprecedented sympatric cryptic diversity within British lumbricid earthworms.
528 Molecular Ecology 17:4684-4698.
- 529 Kushad, M.M., A.F. Brown, A.C. Kurilich, J.A. Juvik, B.P. Klein, M.A. Wallig, and E.H.
530 Jeffery. 1999. Variation of glucosinolates in vegetable crops of *Brassica oleracea*.
531 Journal of Agricultural and Food Chemistry 47:1541-1548.
- 532 Lawrence, A.P., and M.A. Bowers. 2002. A test of the 'hot' mustard extraction method of
533 sampling earthworms. Soil Biology & Biochemistry 34:549-552.
- 534 Lepczyk, C.A. 2005. Integrating published data and citizen science to describe bird diversity
535 across a landscape. Journal of Applied Ecology 42:672-677.
- 536 Li, X.Y., M.C. Fisk, T.J. Fahey, and P.J. Bohlen. 2002. Influence of earthworm invasion on soil
537 microbial biomass and activity in a northern hardwood forest. Soil Biology &
538 Biochemistry 34:1929-1937.
- 539 Lowe, C.N., and K.R. Butt. 2008. *Allolobophora chlorotica* (Savigny, 1826): evidence for
540 classification as two separate species. Pedobiologia 52:81-84.
- 541 Madritch, M.D., and R.L. Lindroth. 2009. Removal of invasive shrubs reduces exotic earthworm
542 populations. Biological Invasions 11:663-671.

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

- 543 McCardle, B.H., and M.J. Anderson. 2001. Fitting multivariate models to community data: A
544 comment on distance-based redundancy analysis. *Ecology* 82:290-297.
- 545 McCune, B., and J.B. Grace. 2002. *Analysis of Ecological Communities*. MjM Software Design,
546 Gleneden Beach, OR.
- 547 Migge-Kleian, S., M.A. McLean, J.C. Maerz, and L. Heneghan. 2006. The influence of invasive
548 earthworms on indigenous fauna in ecosystems previously uninhabited by earthworms.
549 *Biological Invasions* 8:1275-1285.
- 550 NOAA. 2010. National Weather Service Forecast Office Website. National Oceanic &
551 Atmospheric Administration, U.S. Department of Commerce,
552 URL:<http://www.weather.gov>.
- 553 Pelosi, C., M. Bertrand, Y. Capowiez, H. Boizard, and J. Roger-Estrade. 2009. Earthworm
554 collection from agricultural fields: comparisons of selected expellants in
555 presence/absence of hand-sorting. *European Journal of Soil Biology* 45:176-183.
- 556 R Development Core Team. 2008. R: A language and environment for statistical computing. R
557 Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org>.
- 558 Raw, F. 1959. Estimating earthworm populations by using formalin. *Nature* 184:1661-1662.
- 559 Robertson, G.P., D.C. Coleman, C.S. Bledsoe, and P. Sollins. 1999. *Standard Soil Methods for*
560 *Long-Term Ecological Research*. Oxford University Press, New York, NY.
- 561 Satchell, J.E. 1967. Colour dimorphism in *Allolobophora chlorotica* Sav. (Lumbricidae). *Journal*
562 *of Animal Ecology* 36:623-630.

Cite as: Iannone et al. (2012; In press). Natural Areas Journal.

- 563 Schwert, D.P. 1990. Oligochaeta: Lumbricidae. Pages 341-356 in D. L. Dindal, ed. Soil Biology
564 Guide. John Wiley and Sons, New York, NY.
- 565 Silvertown, J. 2009. A new dawn for citizen science. Trends in Ecology & Evolution 24:467-
566 471.
- 567 Springett, J.A. 1981. A new method for extracting earthworms from soil cores, with a
568 comparison of four commonly used methods for estimating earthworm populations.
569 Pedobiologia 21:217-222.
- 570 Suarez, E.R., D.M. Pelletier, T.J. Fahey, P.M. Groffman, P.J. Bohlen, and M.C. Fisk. 2004.
571 Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate
572 forests. Ecosystems 7:28-44.
- 573 Temple-Smith, M.G., T.J. Kingson, T.L. Furlonge, and T.B. Garnsey. 1993. The effect of the
574 introduction of the earthworms *Aporrectodea caliginosa* and *Aporrectodea longa* on
575 pasture production in Tasmania. Page 373 Proceedings of the Seventh Australian
576 Agronomy Conference, Adellaide, Australia.
- 577 Terhivuo, J. 1988. The finnish Lumbricidae (Oligochaeta: Lumbricidae). Annales Zoologici
578 Fennici 25:229-247.
- 579 Thielemann, U. 1986. The octet-method for sampling earthworm populations. Pedobiologia
580 29:296-302.
- 581 Trumbull, D.J., R. Bonney, D. Bascom, and A. Cabral. 2000. Thinking scientifically during
582 participation in a citizen-science project. Science Education 84:265-275.

Cite as: Iannone et al. (2012; In press). *Natural Areas Journal*.

583 USDA. 2009. United States Department of Agriculture, Natural Resources Conservation Service
584 Web Soil Survey. URL: <http://websoilsurvey.nrcs.usda.gov/app>.

585 Valckx, J., G. Govers, M. Hermy, and B. Muys. 2011. Optimizing earthworm sampling in
586 ecosystems. Pages 19-38 in A. Karaca, ed. *Biology of Earthworms*. Springer, Heidelberg,
587 Germany, Dordrecht, Netherlands, London, England, New York, NY.

588 Zaborski, E.R. 2003. Allyl isothiocyanate: an alternative chemical expellant for sampling
589 earthworms. *Applied Soil Ecology* 22:87-95.

590 Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G.M. Smith. 2009. *Mixed Effects Models*
591 *and Extensions in Ecology with R*. Springer Science and Business Media, New York,
592 NY.

593

594

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.

(A) Earthworm numbers

<u>Model Term</u>	<u>Unique permutations</u>	<u>DF</u>	<u>MS</u>	<u>Pseudo F</u>	<u>P-value</u>
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

<u>Model Term</u>	<u>Unique permutations</u>	<u>DF</u>	<u>MS</u>	<u>Pseudo F</u>	<u>P-value</u>
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

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

<i>Model Term</i>	<i>DF</i>	<i>MS</i>	<i>F-ratio</i>	<i>P-value</i>
GWC	1	8861	3.8093	0.0618
Site	3	14462	6.2169	0.0025
EM	1	835	0.3590	0.5543
Residuals	26	2326		
Total	31			

Removed Terms

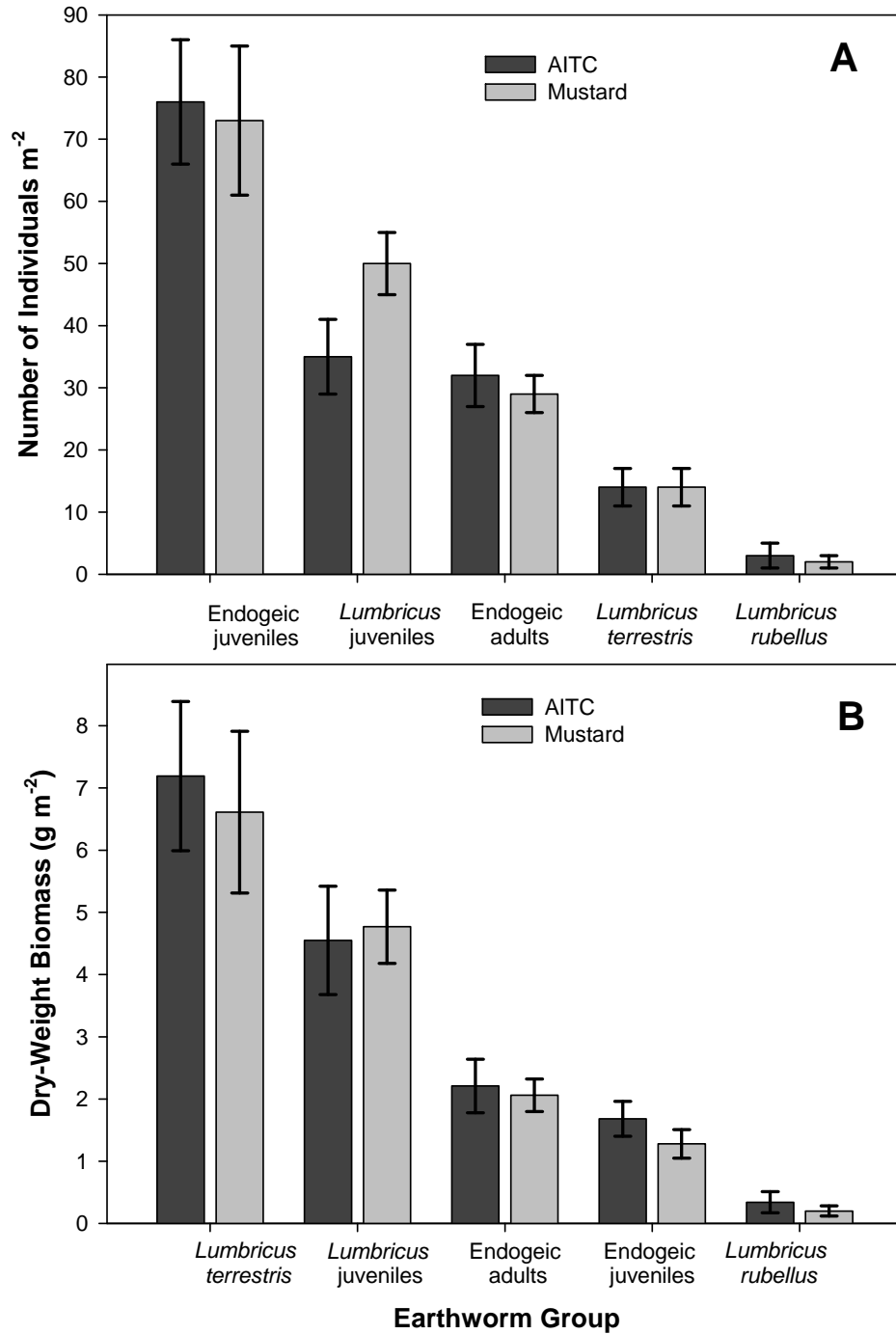
GWC x Site	3	2052	0.8689	0.4714
GWC x EM	1	463	0.1890	0.6680
Site x EM	3	1760	0.6885	0.5702
GWC x Site x EM	3	1175	0.4171	0.7431

(B) Total earthworm biomass

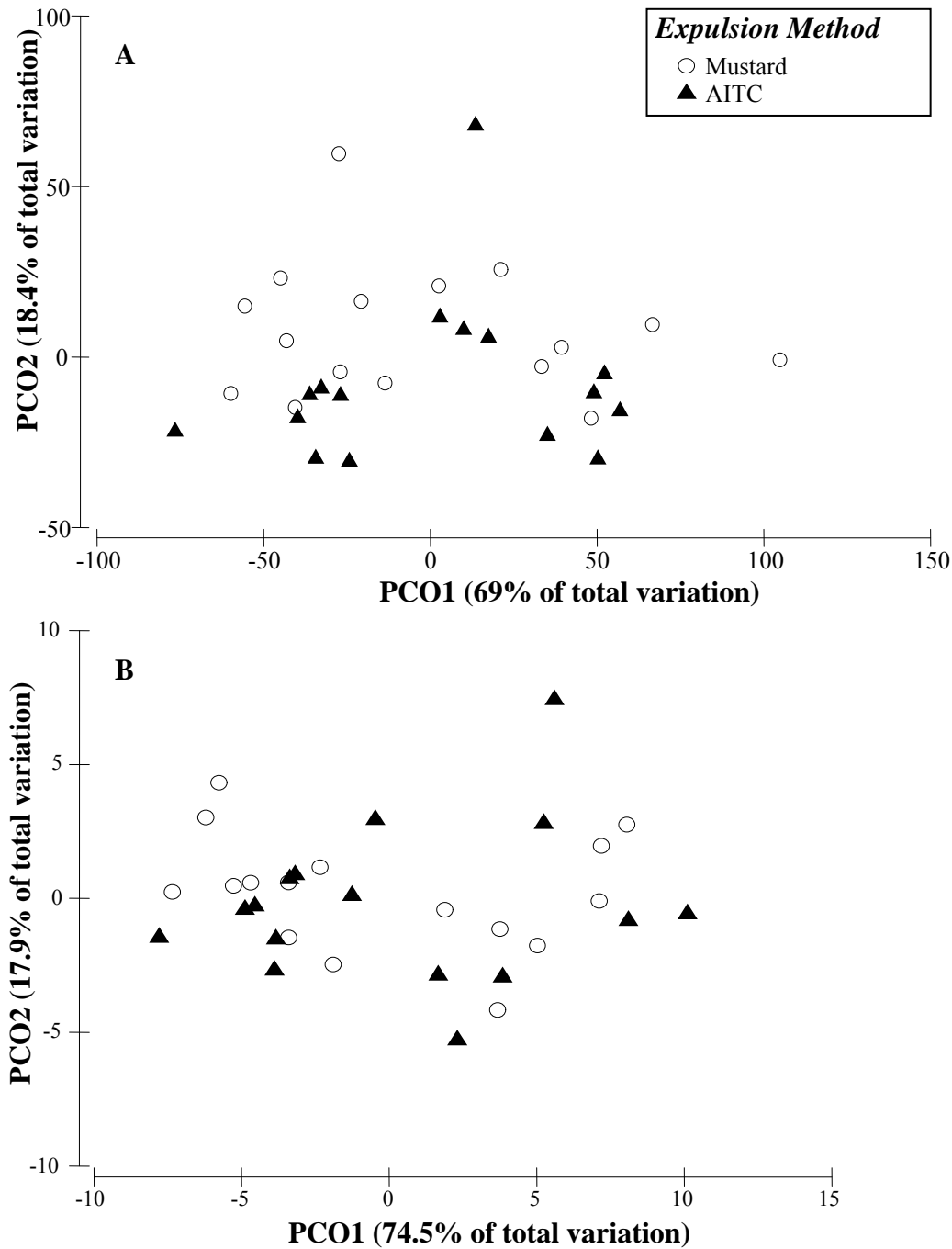
<i>Model Term</i>	<i>DF</i>	<i>MS</i>	<i>F-ratio</i>	<i>P-value</i>
GWC	1	252.21	17.7394	0.0003
Site	3	215.70	15.1709	< 0.0001
EM	1	4.46	0.3133	0.5804
Residuals	26	14.22		
Total	31			

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



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



597

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

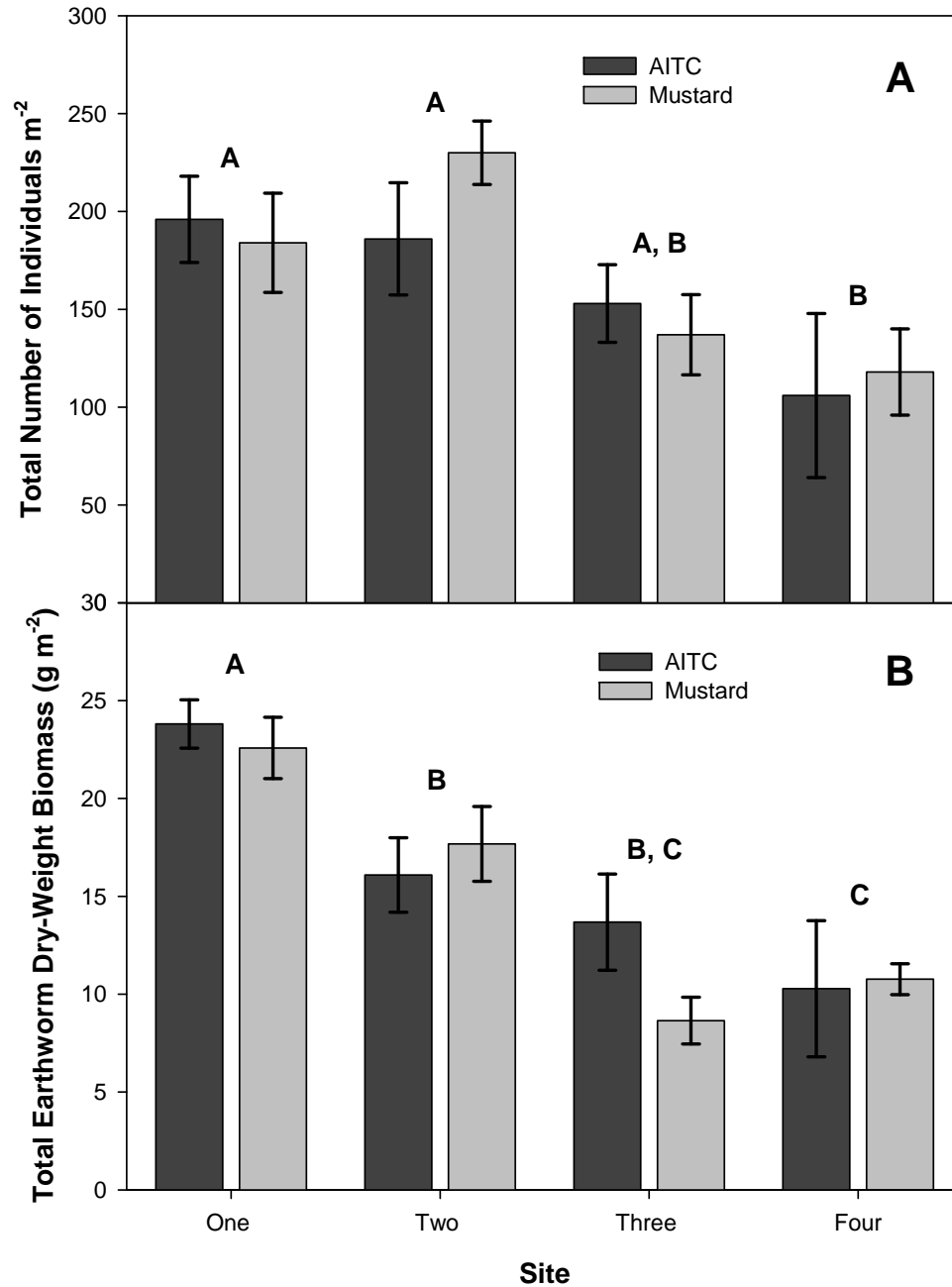


Figure 3. Total earthworm (A) numbers and (B) biomass sampled across sites using AITC or mustard powder expulsion ($n = 4$ for each site by expulsion treatment combination, i.e., bar). Site 1, currently invaded by buckthorn; Site 2, buckthorn removed for 5 yrs; Site 3, buckthorn removed for 12 yrs; and 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 \pm SE.