Assessment of an Electrical Method of Earthworm Extraction in a Northern Temperate Forest

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Abstract

Invasion of north temperate forests by exotic earthworm species is an emerging and important area of study in forest ecology and management. Quantitative methods for extraction of earthworms from forest soils with minimal disturbance are required by these studies. This study compares the efficacy of one such method, the electrical sampling method originally described by Bohlen, to the widely used hand sorting method. The method was not effective enough to create earthworm free plots, but reduction of earthworm biomass was quite high under favorable soil moisture conditions. This method achieved high levels of extraction of *Lumbricus* species and moderate levels of extraction of *Aporrectodea* species. It is concluded that under favorable conditions, electrical extraction is a viable alternative to hand sorting in estimation of earthworm populations and in reduction of earthworm biomass, especially that of *Lumbricus* species.

Introduction

Exotic earthworms have been invading previously earthworm-free northern temperate forests since European settlement (Reynolds 1994). The introduction of these major detrivores had caused a myriad of changes in the forest systems, including the loss of much of the organic horizon (Gundale 2002, Hale 2004a), an increase in nutrient availability in soils (Burtelow et al 1998, Bohlen et. al 2004, Suarez et al 2004), and a reduction of understory plant diversity (Gundale 2002, Hale 2004a). Further research into the dynamics of this invasion will require the quantitative extraction of earthworms through an effective, rapid, and ideally non-invasive method.

The literature has chiefly used three general techniques for sampling earthworms: digging and hand sorting, chemical extraction, and electrical extraction. Hand sorting is highly thorough and reliable, but it is laborious,

destroys soil structure, and specimens less than 2 cm may escape collection (Reynolds 1977). Liquid chemical expellants are a more time-efficient alternative to hand-sorting, and can achieve high levels of extraction (Lawerence and Bowers 2002, Zaborski 2003). The use of "hot" mustard solution has largely replaced the use of formalin as the major chemical expellant due to the possible carcinogenic properties of formalin. Hot mustard extraction can be more effective than hand-sorting for some earthworm species, but largely ineffective for others (Lawerence and Bowers 2002). Use of this method is also limited to flat or gently sloping land, adds unnatural chemicals to the soil, and is highly effective only warm and moderately moist but unsaturated soils (Reynolds 1977).

Electrical extraction is a less frequently used method, possibly because recent reviews of earthworm collection techniques have focused on the drawbacks and potential dangers associated with early electrical techniques such as Satchell (1955) (Schmidt 2000). The major advantage of this technique is minimal soil disturbance, and recent design improvements have allowed for highly effective earthworm removal in agroecosystems (Bohlen et al 1995, Schmidt 2000). However, the effectiveness of the electrical method is limited during periods of low soil moisture and earthworm activity, and is dependent on physical and chemical properties of the soil (Reynolds 1977, Schmidt 2000).

In this study I will examine the effectiveness of an electroshocking apparatus for extraction of invasive earthworms in a northern hardwood forest at

the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan. Three genera of earthworms with characteristic habitat and feeding preferences have been found on the UNDERC property (Lehman 2005). *Dendrobaena octaedra* is a small epigeic (litter dwelling) species, *Aporrectodea spp.* are small to medium-sized endogeic (horizontally burrowing) species, and *Lumbricus spp*, which is dominated by the large anecic (vertically burrowing) species *L. terrestris* at UNDERC (Lehman 2005). I will determine the overall earthworm density at the site through hand sorting, and determine the proportion of earthworms removed through electroshocking. I will also determine the functional area of the electroshocking apparatus, as well as investigate re-colonization of the shocked plots. I expected electroshocking to be most effective for extracting medium to large sized earthworms (2-10 g) in the anecic functional group because their vertical burrows will allow them to reach the surface more readily than horizontally burrowing species. Also, close placement of the electrodes is expected to more effectively extract earthworms than placement further apart. The shocked plots are expected to recover to their original earthworm abundance within 21 days, as earthworms have been shown to naturally migrate 5-10 meters per year (James 2004, Hale et al 2004a). Additionally, dependence of electroshocking efficacy on soil moisture will be qualitatively analyzed.

Methods

Site Characterization

This study was performed from June 6 to July 21, 2006 at UNDERC. The study forest was composed chiefly of sugar maple (*Acer saccharum*), with quaking aspen (*Populus tremuloides*) and yellow birch (*Betula alleghaniasis*) as secondary components. The entire study site was located at least 10 m from any roads, and terrain was generally flat with a low grade slope. Soil moisture can be implied to have declined during the study, as it declined at 8 sites located on the UNDERC property throughout the study period (Sean Cullen, personal communication).

Earthworm Sampling

I tested the efficacy of earthworm extraction by electroshocking 16 0.25 m^2 plots. The electroshocking apparatus was constructed based on the original design described in Bohlen et al 1995. The set-up consists of two electrodes connected to a 120V AC generator. The electrodes are constructed of four steel rods that are connected in series and are bolted into a 4" x 4" x 30" plank of wood. Each rod projects 30 cm into the soil. I cleared 1 m^2 plots of detritus and plant material, then drove the electrodes into the soil at a distance of 30, 50, or 70 cm. I connected the electrodes to the generator, which was subsequently run for 20 min. I then rotated the electrodes 90° and ran the generator for 20 additional minutes. The voltage was about 90V at the center of each plot, and wasn't significantly different between distances. I collected all earthworms emerging in

the 0.25 m^2 plot preserved them in 70% ethanol. I removed any earthworms emerging outside of the plot to prevent immediate re-colonization of the study plot. Electroshocking was performed twice, on June 6 and June 26-27.

I dug the study plots and three control plots to a depth of 27 cm and hand-sorted the soil with a sieve to recover any remaining earthworms, which were also preserved in 70% ethanol. I allowed 6 of the study plots to recover 20-21 days before digging in order to test for re-colonization rates of the shocked plots. I keyed the earthworms to genus (Reynolds 1977), measured their length, and determined their ash free biomass using allometric equations given in Hale et al (2004b).

Statistical Analysis

All statistical procedures were performed with SYSTAT version 11 (SYSTAT Software Inc, Point Richmond, CA). ANOVA was used to determine whether significant differences existed between the biomass of earthworms extracted with the digging and hand-sorting method and those extracted with the electroshocking apparatus. Another ANOVA was used to determine whether significant differences existed between the biomasses of earthworms extracted by the electroshocking apparatus at the three distances. Linear regression was used to analyze the dependence of the proportion of total earthworm biomass at each site extracted by the digging/hand sorting method on the number of days elapsed from shocking until digging. Additionally, the possible effects of soil moisture on

electroshocking efficacy was investigated by using an ANOVA to determine if the proportion of earthworm biomass extracted with shocking was dependent on the date of shocking.

Results

I found three genera of earthworms, in accord with Lehman (2005): *Dendrobaena octaedra* in 7 plots; *Aporrectodea spp.* in all plots; and *Lumbricus spp.* in all plots. *D. octaedra* was excluded from statistical analyses because it was found at few sites and in low abundance (n=11). Overall earthworm biomass was estimated for the hand sorting method, electroshocking, and electroshocking followed by hand sorting. Shocking extracted 6.36 g AFDW m^{-2}, hand sorting extracted 9.77 g AFDW m^{-2}, and shocking plus hand sorting yielded 11.31 g AFDW m^{-2}, but the shocking and hand sorting methods were only significantly different for extraction of *Aporrectodea spp.* (Fig 1, $F=4.80$, $p=0.043$).

I tested dependence of electroshocking efficacy on electrode separation distance and found a trend toward higher extracted biomass when the electrodes were 50 cm apart ($F=3.53$, $p=0.059$), and that the 70cm and 30cm treatments were not significantly different (Fig 2).

Time elapsed between shocking period and hand sorting period was found to have no significant effect on the biomass of earthworms recovered by the hand sorting method ($r^2=0.198$, $p=0.083$), but there was a general trend toward higher proportion of biomass from hand sorting after more time had elapsed (Fig 3).

The dependence of electroshocking efficacy on the date shocked was tested in an attempt to investigate the effect of soil moisture on electroshocking. I found that electroshocking was more effective on June 6 than June 26-27 for both earthworm species (Fig 4, $F=4.75$, $p=0.047$).

I compared the efficacy of earthworm extraction by shocking under the best conditions (50cm, highest soil moisture) with the extraction achieved by hand sorting and found that both methods were very similar (Fig 5, $F=0.001$, $p=0.97$).

Discussion

The overall earthworm biomass for the study forest was found to be in the low range of that reported from other northern hardwood forests, as these estimates ranged from 0.5-31.9 g/m^2 (Shakir and Dindal 1997, Whalen 2004, Coderre et al 1995). This result was not unexpected, as UNDERC forests have shown less extreme rates of litter removal and mixing of soil horizons than other invaded forests, suggesting a smaller earthworm population (Dave Costello, personal communication). Furthermore, the study was undertaken chiefly during a warm and dry period, and earthworm populations are known to vary seasonally due to the changes in soil moisture and temperature (Rozen 1988). Additionally, the efficacy of one electroshocking method has been shown to be highly reduced during periods of low soil moisture, which is largely due to earthworm aestivation (Schmidt 2000).

Biomass estimates of *Aporrectodea spp.* from the electroshocking technique were lower than those from hand sorting. The horizontal burrowing habit of this genera could explain this result, as electrical irritation could lead to the earthworms simply burrowing away from the area rather than burrowing to the surface. *Lumbricus spp.*, a largely anecic group, did surface enough in response to electroshocking to relatively accurately estimate biomass. Schmidt (2000) found a similar difference in the efficacy of extraction of the *Lumbricu spp.* versus the *Aporrectodea spp.* by electroshocking.

The electroshocking apparatus tended to be most effective at earthworm extraction from the plots at a distance of 50 cm, and further replication may improve the significance of this result. As the study plots measure 50 cm x 50 cm, 50 cm is the closest the electrodes can be that still allows a current to run directly through the entire plot. The 70 cm distance is less effective because the electroshocking current is likely reduced with greater electrode distance. The 30 cm distance was also less effective, but this is due to portions of the plot not being in the area of direct current. However, smaller plot sizes would likely obtain the highest extraction efficacy if the electrodes were placed at the edges of the plots.

Significant recolonization of shocked plots was not observed. The lack of a significant relationship between the proportion of earthworm biomass recovered by hand sorting and time elapsed could be explained by the low biomass recovered by shocking. The reduction in the overall biomass would be too small

notice any significant recovery of earthworm populations, especially when seasonal variations in earthworm populations are considered. If future studies are conducted over a wider time range, and especially during the most active periods of spring and fall, recolonization would be more easily measured.

While I collected no quantitative data on soil moisture at the study site, electroshocking was qualitatively observed to be a more effective method of extraction when soil moisture was high. This result is supported by several previous studies (Bohlen et al 1995, Schmidt 2000).

Overall, electroshocking was found to be as effective as hand sorting for extraction of *Lumbricus spp.* under certain favorable conditions. *Apporectodea spp.* are not as effectively removed, but their density and biomass could still potentially be estimated by the electrical method. While not an effective method for creation of completely earthworm free plots, the electrical method can effectively estimate earthworm populations by arguably the least destructive method. The method could easily be applied to situations in which chemical extraction is not a viable option, for example on slopes, in saturated soils, or on lands in which minimal disturbance is required. (Schmidt 2000).

Future studies of the efficacy of earthworm extraction methods should be conducted over a longer time period than one season, and should increase their replication to achieve more significant results. Soil moisture should be quantitatively measured, as its effect on earthworm activity can potentially reduce

extraction levels. Extraction efficacy in different soil types should also be investigated, in an attempt to find a universally effective method.

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References Cited

Bohlen, P.J., R.W. Parmalee, J.M. Blair, C.A. Edwards, and B.R. Stinner. 1995. Efficacy of Methods for Manipulating Earthworm Populations in Large Scale Field Experiments in Agroecosystems. Soil Biology and Biochemistry 27:993-997.

Bohlen, P. J., D. Pelletier, P.M. Groffman, T.J. Fahey, and M.C. Fisk. 2004. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:1-12.

Burtelow, A.E., P.J. Bohlen, and P.M. Groffman. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Applied Soil Ecology 9:197-202.

Coderre, D., D. Gagnon, S. Tousignant, and G. Bessette. 1995. Earthworm populations in healthy and declinging sugar maple forests. Pedobiologica 39:86-89.

Gundale, M.J. 2002. The influence of exotic earthworms on soil organic horizon and the rare ferm *Botrychium mormo*. Conservation Biology 16:1555-1573.

Hale, C.M. 2004a. Ecological consequences of exotic invaders: interaction involving European earthworms and native plant communities in hardwood forests. Ph.D. dissertation. University of Minnesota, St. Paul.

Hale, C.M., P.B. Reich, and L.E. Frelich. 2004b. Allometric Equations for Estimation of Ash-Free Dry Mass from Length Measurements for Selected European Earthworm Species (Lumbricidae) in the Western Great Lakes Region. American Midland Naturalist 151:179-185.

James, S.W. 2004. Planetary processes and their interactions with earthworm distributions and ecology. Pages 53-73 in C.A. Edwards, editor. Earthworm Ecology 2nd ed. CRC Press, Boca Raton, Florida.

Lawerence A.P. and M.A. Bowers. 2002. A Test of the 'Hot' Mustard Extraction Method of Sampling Earthworms. Soil Biology & Biochemistry 34:549-552.

Lehman F. 2005. Earthworm Species Composition and Distribution in the Upper Peninsula of Michigan. UNDERC Practicum in Environmental Field Biology report.

Reynolds J.W. 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum Miscellaneous Publication, Toronto, Ontario, Canada.

Reynolds J.W. 1994. The distribution of the earthworms (Oligochaeta) of Indiana: a case for the post-Quaternary introduction theory for megadrile migration in North America. Megadrilogica 5:13-32.

Rozen, A. 1988. The annual cycle in populations of earthworms (Lumbricidae Oligochaeta) in three types of oak-hornbeam of the Niepolomicka Forest. Pedobiologica 31:169-178.

Satchell J.E. 1955. An electrical method of sampling earthworm populations. Pedabiologica 27:15-19.

Schmidt O. 2001. Appraisal of the Electrical Octet Method for Estimating Earthworm Populations in Arable Land. Annals of Applied Biology 138:231-241.

Shakir, S.H. and D.L. Dindal. 1997. Density and biomass of earthworms in forest and herbaceous microecosystems in central New York, North America. Soil Biology and Biochemistry 29: 275-285.

Suarez, E.R., D. Pelletier, T.J. Fahey, P.M. Groffman, P.J. Bohlen, and M.C. Fisk. 2004. Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. Ecosystems 7:28-44.

Whalen, J.K. 2004. Spatial and temporal distribution of earthworm patches in corn field, hayfield and forest systems in southwestern Quebec, Canada. Applied Soil Ecology 27:143-151.

Zaborski E.R. 2003. Allyl Isothiocyanate: an Alternative Chemical Expellant for Sampling Earthworms. Applied Soil Ecology 22:87-95.

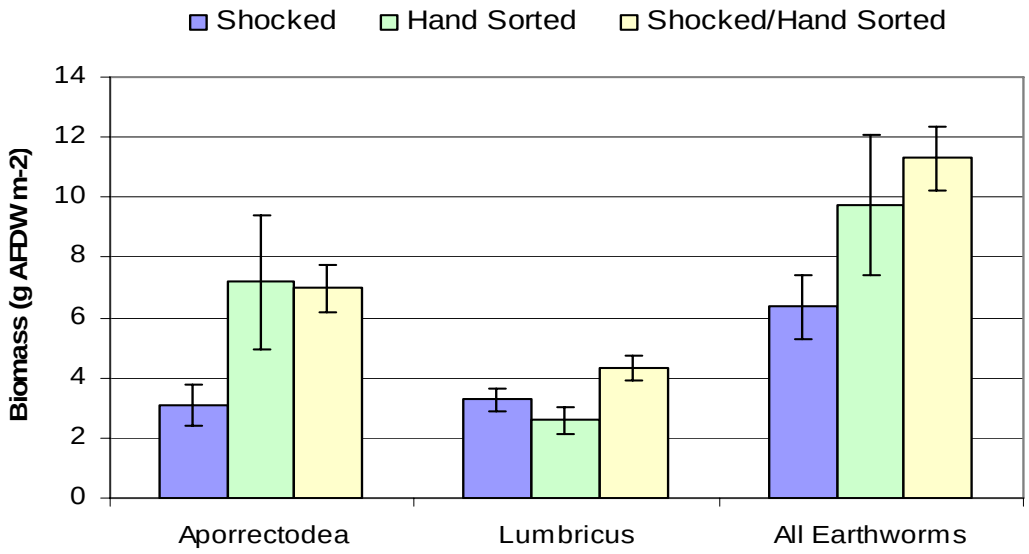

Fig 1. Earthworm biomass and species composition estimates for the three extraction techniques. Estimates of *Aporrectodea* biomass for shocking and hand sorted methods were significantly different. Errors bars represent standard error.

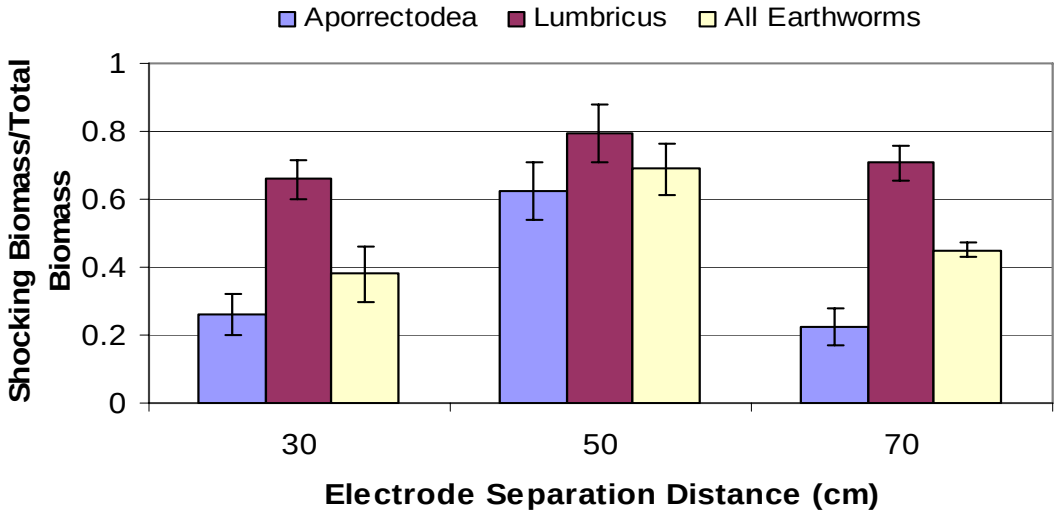

Fig 2. Effect of electrode separation distance on extraction efficacy. 50cm had a higher proportion of earthworm biomass extracted with electroshocking for all earthworm species, but differences were not statistically significant. Error bars represent one standard error.

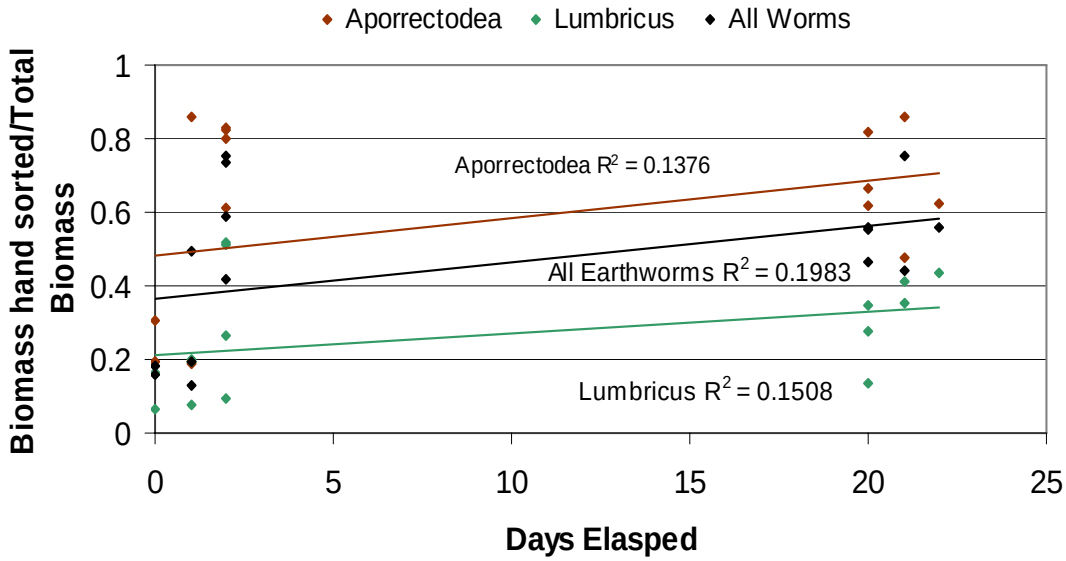

Fig 3. Recovery of earthworm populations in electroshocked plots. Trends of recovery for both earthworm genera and all earthworms were not significant.

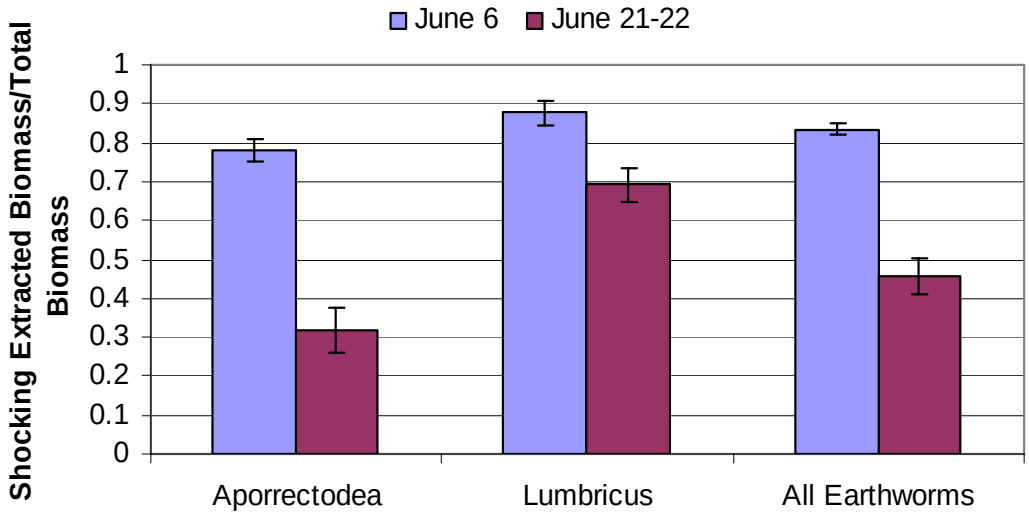

Fig 4. Dependence of the efficacy of electroshocking for earthworm removal on the date electroshocked. June 6 was characterized by higher soil moisture. Error bars represent one standard error.

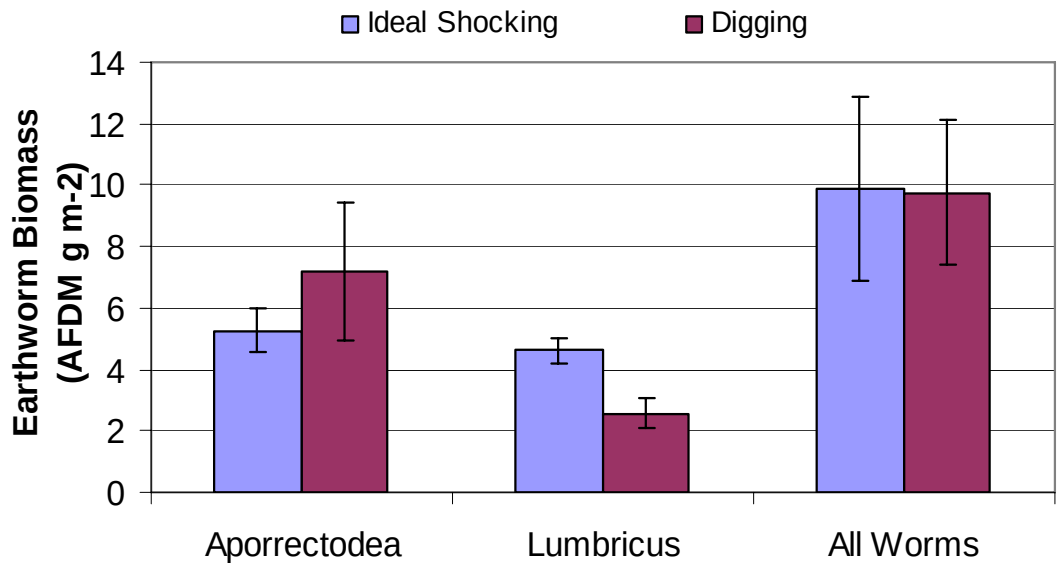

Fig. 5. Earthworm biomass extracted with shocking under ideal conditions versus hand sorting. Overall biomass estimates were very similar.