2009 Project Abstract
For the Period Ending December 30, 2012

PROJECT TITLE: Prevention and Early Detection of Asian Earthworms and Reducing the Spread of European Earthworms
PROJECT MANAGER: Cindy Hale
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: http://www.nrri.umn.edu/staff/chale.asp

APPROPRIATION AMOUNT: $150,000

Overall Project Outcome and Results

We used a multi-pronged approach to quantify of the relative importance of different vectors of spread for invasive earthworms, make management and regulatory recommendations and create mechanisms for public engagement and dissemination of our project results through the Great Lakes Worm Watch website and diverse stakeholders. Internet sales of earthworms and earthworm related products posed large risks for the introduction of new earthworm species and continued spread of those already in the state. Of 38 earthworm products sampled, 87% were either contaminated with other earthworm species or provided inaccurate identification. Assessment of soil transported via ATV's and logging equipment demonstrated that this is also a high risk vector for spread of earthworms across the landscape, suggesting that equipment hygiene, land management activities and policies should address this risk. Preliminary recommendations for organizations with regulatory oversight for invasive earthworms (i.e. MN-DNR, MDA and MPCA) include the implementation of required trainings on invasive earthworms for commercial operations involved in any enterprise using or selling earthworm or earthworm products (i.e. fishing bait, composting, etc.). Recommended trainings would be, similar to those already required of minnow bait operations. Finally, substantial efforts were completed to train, inform and actively engage diverse stakeholders in efforts to document invasive earthworm and their relative impacts across the state/region and to identify earthworm-free and minimally impacted areas worthy of protection. As a result of this project we added 716 survey points and 9,697 specimens to our database and worked directly with 40 groups and over 1300 individuals (e.g. citizens, college students-teachers, K-12 students-teachers, natural resource managers, and researchers) in 10 different states (Minnesota, Wisconsin, Ohio, New York, Massachusetts, Virginia, Pennsylvania, Alaska, Kentucky, Michigan). Five peer-reviewed publications, a second edition of the book "**Earthworms of the Great Lakes**", and two online maps were produced and disseminate our results.

Project Results Use and Dissemination

The project has allowed us to greatly enhance and expand the quality and quantity of resources provided through the Great Lakes Worm Watch website

<http://www.greatlakeswormwatch.org>. In addition to the many people we interact with directly there are thousands that access our website resources annually.
In 2012, Great Lakes Worm Watch established and now maintains a Facebook page. We use the platform, linked to our website, to communicate research, outreach and educational opportunities http://www.facebook.com/pages/Great-Lakes-Worm-Watch/123279661062852.

Additionally, this project has resulted in five peer-reviewed publications; information has been presented at 20 professional seminars/conferences and approximately 40 trainings to natural resource professionals, students, and the public; media coverage in over 40 different stories; and participated in numerous other public outreach activities such as exhibits at conferences and fairs.

Environment and Natural Resources Trust Fund 2009 Work Program Final Report

Date of Report: March18, 2013
Date of Next Progress Report: Final Report
Date of Work Program Approval: June 16, 2009
Project Completion Date: December 30, 2012

I. PROJECT TITLE: Prevention and Early Detection of Asian Earthworms and Reducing the Spread of European Earthworms

Project Manager: Cindy Hale
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Location: Statewide

Total Trust Fund Project Budget:	**Trust Fund Appropriation**	**$**	**150,000**
	Minus Amount Spent:	**$**	**150,000**
	Equal Balance:	**$**	**0**

Legal Citation: M.L. 2009, Chp. 143, Sec. 2, Subd. 6e

Appropriation Language:

$150,000 is from the trust fund to the Board of Regents of the University of Minnesota Natural Resources Research Institute for a risk assessment of the methods of spreading, testing of management recommendations, and identification of key areas for action in the state to reduce the impacts of invasive earthworms on hardwood forest productivity. This appropriation is available until June 30, 2012, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. and III. FINAL PROJECT SUMMARY:

We used a multi-pronged approach to quantify of the relative importance of different vectors of spread for invasive earthworms, make management and regulatory recommendations and create mechanisms for public engagement and dissemination of our project results through the Great Lakes Worm Watch website and diverse stakeholders. Internet sales of earthworms and earthworm related products posed large risks for the introduction of new earthworm species and continued spread of those already in the state. Of 38 earthworm products sampled, 87% were either contaminated with other earthworm species or provided inaccurate identification.

Assessment of soil transported via ATV's and logging equipment demonstrated that this is also a high risk vector for spread of earthworms across the landscape, suggesting that equipment hygiene, land management activities and policies should address this risk. Preliminary recommendations for organizations with regulatory oversight for invasive earthworms (i.e. MN-DNR, MDA and MPCA) include the implementation of required trainings on invasive earthworms for commercial operations involved in any enterprise using or selling earthworm or earthworm products (i.e. fishing bait, composting, etc.). Recommended trainings would be, similar to those already required of minnow bait operations. Finally, substantial efforts were completed to train, inform and actively engage diverse stakeholders in efforts to document invasive earthworm and their relative impacts across the state/region and to identify earthworm-free and minimally impacted areas worthy of protection. As a result of this project we added 716 survey points and 9,697 specimens to our database and worked directly with 40 groups and over 1300 individuals (e.g. citizens, college students-teachers, K-12 students-teachers, natural resource managers, and researchers) in 10 different states (Minnesota, Wisconsin, Ohio, New York, Massachusetts, Virginia, Pennsylvania, Alaska, Kentucky, Michigan). Five peer-reviewed publications, a second edition of the book "**Earthworms of the Great Lakes**", and two online maps were produced and disseminate our results.

IV. OUTLINE OF PROJECT RESULTS*:*

Result 1: Risk-Assessment of Vectors of Earthworm Introduction

Description:

In contrast to the traditional approach of species-based risk assessments, we propose to identify, describe and quantify the potential vectors of in-state spread of established earthworm species and of interstate transport and introduction of non-established earthworm species (i.e. intentional and unintentional transport of earthworms through compost, mulch, soils and fishing bait), including field-based measures of earthworm species and relative abundance present in each vector.

This will be done in a 2 step process beginning in 2009 and completed in 2010. Preliminary sampling of various in-state and interstate vectors will be conducted in summer and fall 2009 to provide an initial indication of the relative importance of different vectors and to identify any obstacles that need to be overcome in order to adequately assess their level of risk. From the preliminary sampling and analysis in 2009, more comprehensive and/or targeted sampling of the most important vectors of earthworms spread will be conducted in spring, summer & fall 2010.

A manuscript will be submitted for publication in a peer-reviewed professional journal, such as Biological Invasions, summarizing and reporting the research conducted under result 1 of this project. The delay between the 4th update and final report of this result provides adequate time for the peer-review process to be completed and final publication of the submitted manuscript.

Summary Budget Information for Result 1:	**Trust Fund Budget:**	**$ 43,268**
	Amount Spent:	**$ 43,268**
	Balance:	**$ 0**

Deliverable	Completion Date	Budget
1. *Preliminary descriptions and risk assessment of in-state and interstate vectors of earthworm spread.*	December 30, 2009	Staff $7972 Data storage $300 Supplies $400 Travel $2656 **Total: $11,328**
2. *Final descriptions and risk assessment of vectors of earthworm spread*.	December 30, 2010; Revised to June 30, 2012	Staff $19,457 Supplies $400 Travel $5391 **Total: $25,248**
3. *Analysis of data and preparation of a report submitted to MDA, DNR and other governmental agencies charged with managing or regulation invasive species to help promote the health of our forests. This will also be submitted for publication in a professional peer-reviewed journal (i.e. Biological Invasions)*	June 30, 2011; Revised to June 30, 2012	Staff $ 6,692 **Total: $6,692**

Final Report Summary:
Two documents (Appendix 1 and 2) provide the three deliverables listed above.

A draft manuscript titled "**Internet Sales as Vectors of Non-native Earthworm Introductions in the western Great Lakes Region.**" (Appendix 1) includes an introduction and literature review of the primary vectors of intra- and inter-state transport of non-native earthworms in the Great Lakes region of North America. Our assessment included all non-native earthworms to the western Great lakes region (e.g. European species that have already been established in the state), but particularly focused on Asian species in the genus *Amynthas* because they are not yet established in the state and have shown to pose severe threats to native forests in eastern states where invasions of this species are more prevalent. Internet sales of earthworm and vermicomposting (compost) were identified as a potentially important vector that had not been assessed. A combination of protocol based internet searches, targeted interviews, site inspections and purchasing earthworms from internet vendors across the country was used to quantitatively and qualitatively describe the level and nature of internet sales and vermicomposting as potential vectors for the introduction and spread of non-native earthworms in our region. This manuscript is currently in draft form. It will be submitted to internal review to selected agencies and stakeholders (i.e. MN Department of Agriculture, MN Department Natural Resources, APHIS, The Nature Conservancy, and others). Following their review a final revision will be completed and the manuscript will be submitted to a peer-reviewed scientific journal for publication (i.e. Biological Invasions). Tables X and Y below provide a summary of some of the most relevant data from the draft manuscript.

An informational brochure titled "**ABC's of Composting with Earthworms Safely"** (Appendix 2) is the final version of a document described in earlier reports as **"Best Management Practices for Vermicomposting"**. Following external review and a final revision, it was determined that the new title better captures the contents of the document. The document is a full color, double sided, tri-fold brochure appropriate for distribution at displays and educational events. It is also freely available for download from the Great Lakes Worm Watch website. It is intended for broad use by stakeholders, educators, vendors and the public as a resource to identify the primary ways in which vermicomposting can be a vector for the introduction and spread of non-native earthworms and what precautions can be taken to limit or prevent spread via vermicomposting. The "**ABC's of Composting with Earthworms Safely"** is downloadable from our website <http://www.nrri.umn.edu/worms/downloads/team/vermicompostingBMP.pdf>.

The manuscript and brochure will serve as resources for a variety of stakeholders to understand the nature and level of threats posed by non-native earthworms as a group of invasive species. Given the difficulty of identifying many of these species speaks to the need to address the invasion of earthworms from a non-species specific perspective. Further, greater understanding of the potential for internet sales and vermicomposting to be important vectors leading to the introduction and spread of non-native earthworms will aid in the development and implementation of policies, regulations and educational outreach designed to limit future introduction in Minnesota and Great Lakes region of North America.

Additional revenues were provided to augment this effort through a Federal Grant: "**Reducing human-mediated spread of non-native earthworms in vulnerable northern hardwood forests**", CSREES USDA-AFRI Biology of Weedy and Invasive Species in Agroecosystems. Lead primary investigator David Andow and co-investigators, Terry Hurley, George Host, and Cindy Hale. Funded in 2010, 3 year project, award amount $491,000

Results from the federal grant that contributed to Result 1 include independent internship projects that assessed selected potential vectors of non-native earthworm introduction and spread, including:

Christianson, Drew. 2010. **Effects of fishing tournaments on earthworm introductions in Minnesota's Laurentian region**. Presented at the 2010 Minnesota-Wisconsin Invasive Species Conference, November 8-10, 2010, St. Paul, MN. Abstract Booklet page 6.

Northbird, David. 2010. **Demand for earthworm bait**. Presented at the 2010 Minnesota-Wisconsin Invasive Species Conference, November 8-10, 2010, St. Paul, MN. Abstract Booklet page 62.

Result 2: Testing Effectiveness of Management Recommendations
Description:

Management recommendations resulting from previous work in 2008 and further developed through the information provided by Result 1 of this project (i.e. equipment hygiene, public land-use restrictions, bait labeling or restrictions, etc.) will be field tested to determine the cost-benefit and relative effectiveness of different recommendations to actually limit the spread/introduction of different earthworm species.

Rebecca Knowles of the Leech lake Band of Ojibwe Department of Resource Management will provide primary coordination and management these activities including, recruitment, training and supervision of undergraduate interns from the UMD, State colleges and Tribal colleges. Informed by a previous project in 2008 and our preliminary result in 2009 from Result 1, the project partners will collaborate to identify, describe and prioritize a list of management recommendations they want to explicitly test. Sampling methods and protocols will be developed for each and field testing/sampling will be conducted in 2010. For example, if we want to test the effectiveness of logging equipment hygiene on limiting the spread of earthworms; we may collect samples of soil from equipment treads and underbodies and inspect them for earthworms and earthworm egg cocoons before and after implementation of hygiene protocols. This will allow us to quantify the cost vs. benefit based on the actual effects of the management recommendation on earthworm and egg cocoon presence, absence and relative abundance.

A manuscript will be submitted for publication as a General Technical Report summarizing and reporting the research conducted under result 2 of this project. The delay between the 4th update and final report of this result provides adequate time for the peer-review process to be completed and final publication of the submitted manuscript.

Summary Budget Information for Result 2: **Trust Fund Budget: $ 44,046**
Amount Spent: $ 44,046
Balance: $ 0

Deliverable	**Completion Date**	**Budget**
1. *Identify & describe the specific management recommendations we will field test for effectiveness*	February 30, 2010; Revised to June 30, 2011	Staff $ 5,351 **Total: 5,351**
2. *Develop sampling protocols for each management recommendation to be tested*	June 30, 2010; Revised to June 30, 2011	Staff $ 8,107 **Total: 8,107**
3. *Conduct field testing/sampling for each management recommendation to be tested*	December 30, 2010; Revised to August 30, 2011	Staff $ 11,378 Undergraduates$ 6,867 Data storage $300 Supplies $ 800 Travel $8,047 **Total: 27,392**
4. *General Technical Report: results of testing regional management recommendations*	June 30, 2011; Revised to June 30, 2012	Staff $ 2,946 Publication $ 250 **Total: 3,196**

Final Report Summary:
The draft manuscript (Appendix 3) provides the four deliverables listed above.

It will be finalized for publication in the coming months and is intended for submission as a US. Forest Service General Technical Report titled **"Non-native Earthworms Transported on Treads of ATVs and Logging Equipment in Northern Hardwood Forests of Minnesota, USA"**.
Forests of glaciated regions of North America evolved over thousands of years in the absence of earthworms. Multiple species of European and Asian earthworms now exist in northern forests across the Great Lakes region. Abundant and ecologically diverse communities of non-native earthworms are altering the health and functioning of northern hardwood forests in Minnesota. Humans are a primary source of introduction and spread of these invasive species. This study quantified the relative risk of earthworm spread resulting from soil transport via of all-terrain vehicles (ATVs) and logging equipment in sugar maple (*Acer saccharum*) dominated hardwood forests of the Chippewa National Forest, Minnesota, USA. Soil collected from tires and underbodies of ATVs and logging vehicles were found to contain significant numbers of live earthworms and viable earthworm cocoons. It was concluded that ATV travel and logging activity may be an important vector of continued introductions and spread of invasive earthworms in our region. The greatest threat comes from the transport of earthworm invaded soil picked up on treads and vehicle bodies which move further into the forest from invasion fronts or that are subsequently transported to other sites. We recommend a combination of operator education, equipment hygiene, and land-use and management policies to limit the introduction and spread of non-native earthworms through soil transport by off-road vehicles and logging equipment.

Result 3: Regulatory Responses to Early Detection of Asian Earthworms
Description:

In cooperation with governmental agencies (including but not limited to DNR, and USFS) and based on results 1 & 2, a plan for regulatory responses will be developed to respond to early detection of earthworm species not already established in the state (i.e. *Amynthas* species) including possible control or eradication measures and monitoring for incipient invasions of new species. These will be summarized in a General Technical Report. The delay between the 4th update and final report of this result provides adequate time for the agency-review and adoption to be completed before the final report.

The project manager and projects partners will also collaborate to present the outcomes of results 1, 2 & 3 at national ecological and/or land managers conferences. Our results will serve as a model for others across the country who are also facing the threat of invasive earthworms.

Summary Budget Information for Result 3: **Trust Fund Budget: $ 2,462**
Amount Spent: $ 2,462
Balance: $ 0

Deliverable	**Completion Date**	**Budget**
1. *Technical Report: cooperative regulatory response procedures*	October 30, 2011	$ 1470

2. *Meet with 2-3 regulatory agencies in the development of the technical report.*	December 30, 2011	$ 992

Final Report Summary:

I fall of 2012 we began interagency discussions related to the development of a "**Draft Strategic Plan - Earthworm Regulatory Recommendations**" (Appendix 4). The discussions includes staff from MN Department of Natural Resources (DNR), MN-Department of Agriculture (MDA), MN Pollution Control Agency (MPCA), APHIS, and other interested parties.

Since earthworms are a group of organisms that are terrestrial but have a substantial aquatic pathway for introduction the line between the roles of MDA (terrestrial invasive species) and DNR (aquatic invasive species) can be unclear. However, these agencies have a history of working collaboratively in such situations and anticipate doing so with invasive earthworms. It appears that an opportunity exists to collaboratively develop recommendations for limiting the continued introduction of earthworms involving DNR, MDA and interested NGO's like The Nature Conservancy that may be interested in supporting legislative action in some form.

One suggestion to be pursued would be to develop a training protocol related to earthworms as part of the current licensing requirements for bait minnows. The DNR's approach with minnows thus far is to focus on identifying species that are allowed to be sold, and those that are not, and then providing training and licensing to minimize the threats. The biggest issue for minnows, as for earthworms, in not identifying species that should be allowed, but rather, developing a licensing and training system that sufficiently ensures reasonable compliance. See Appendix 4 with a description and links for what is currently done for minnows.

We feel we have the knowledge to develop an appropriate list of allowed earthworm species based on those that are already well established in the state (i.e. *Lumbricus terrestris, L. rubellus*, *Aporrectodea* spp.). The biggest issue will be to create/provide training so bait sellers know what to look for in earthworm fishing bait to ensure/minimize contamination of non-allowed species. A licensing structure similar to the one they currently have for minnows could also be successful for earthworms. Ideally the sellers of earthworms for fishing bait would be required to receive education on appropriate species and rearing conditions to prevent/minimize contamination of non-allowed species. Resources already developed by Great Lakes Worm Watch could easily be modified to be appropriate for such a training system.

There was broad consensus that education would be a preferable route to legislative action similar to Hawaii at this point (they have banned the importation of any earthworm species). However, continued monitoring for emerging species, not already established in Minnesota would be appropriate. One possible exception might be related to currently unregulated internet sales which have shown to be a potential vector for many species of earthworms that could pose serious threats to our region (see manuscript from Result 1 above).

Discussions and collaborative efforts will continue.

Results from the federal grant (listed under Result 1) that contributed to Result 3 include independent internship projects that assessed the regulatory environment relative to earthworms as an invasive species, including:

Kallestad, Jenna and David A Andow. 2010. **Current regulatory policy for invasive earthworms in Minnesota.** Presented at the 2010 Minnesota-Wisconsin Invasive Species Conference, November 8-10, 2010, St. Paul, MN. Abstract Booklet page 62.

Result 4: Identify Priority Areas for Protection

Description:

A comprehensive and coordinated 3 year effort involving research and educational institutions, governmental agencies, non-governmental organizations and citizen science will inform and involve diverse stakeholders to identify earthworm-free and minimally invaded areas of the state/region in order to prioritize protection efforts and provide rapid detection and response for new species introductions. This component is critical for agencies and project partners to effectively move forward with actions recommended in Results 1-3.

Summary Budget Information for Result 4: **Trust Fund Budget: $ 60,224**
Amount Spent: $ 60,224
Balance: $ 0

Deliverable	**Completion Date**	**Budget**
1. *Updates of Great Lakes Worm Watch and National Institute for Invasive Species Science earthworm survey protocols and online data collection system customized for various potential users/stakeholders.*	November 30, 2009	Staff $ 21,050 **Total: $21,050**
2. *Host a minimum of 36 training workshops (10-14 annually) and regular web casts with collaborators and stakeholder groups to actively support citizen-based earthworm survey activities throughout the state*	December 30, 2011	Staff $ 23,250 Undergraduates$ 6,891 Data storage $300 Supplies $ 150 Travel $2,169 Express mail $150 **Total: $ 32,910**
3.a. *GIS data layer indicating earthworm-free, minimally invaded, moderately invaded and heavily invaded areas of the state* **3.b.***GIS data layer of the known/estimated distributions of all earthworm species documented in the state*	June 30, 2012	Staff $ 4,547 Lab fees $ 800 **Total: $ 5,347**
4. *peer-review publication*	June 30, 2012	Staff $ *667* Publication $ 250 **Total: $917**

Final Report Summary:
LCCMR fund have helped us to secure and leverage additional funding for continued research and outreach. Results from the federal grant (listed under Result 1) that contributed to Result 4 include independent internship projects that are quantifying spread rates of earthworms, assessing the relationship between fishing pressure and earthworms invasions, and methods for identifying vernal pool habitats (see below).

Bray, Kelly P., Ryan Hueffmeier, Gerry Sjerven, George Host, and David Andow. 2012 **Quantifying the Spread of Invasive Earthworms in Minnesota's Northern Forests.** Presented at the Upper Midwest Invasive Species Conference October 29-31, 2012, La Crosse, Wisconsin.

Palokangas, Claire, Laura Christensen, Ryan Hueffmeier, George Host, and David Andow. **Relation Between Fishing Pressure And Earthworm Impacts Near Boat Landings.** Presented at the Upper Midwest Invasive Species Conference October 29-31, 2012, La Crosse, Wisconsin.

Driskell, Stephanie, Jennifer Olker, Ryan Hueffmeier, Cindy Hale. 2012. **Identifying and Evaluating Vernal Pool Habitats Spanning a Continuum of Earthworm Invasion Status**. Presented at the Upper Midwest Invasive Species Conference October 29-31, 2012, La Crosse, Wisconsin.

The latter was used to secure federal funding from NOAA, through the Minnesota Lake Superior Coastal program ($47,765) for a small study titled **"Evaluating vital, small forested wetlands in the Superior National Forest".** It is also being used to pursue funding through the National Science Foundation to for compressive assessment of the nature and mechanisms by which invasive earthworms may be negatively impacting vernal pools across the region which serve as the primary habitat for many invertebrate and vertebrate species at the base of the terrestrial food web.

Additional research that was stimulated by this LCCMR project includes:

Larson, E.R., K.F. Kipfmueller, C.M. Hale, L.E. Frelich, and P.B. Reich. (2010) Tree Rings Detect Earthworm Invasions and their Effects in Northern Hardwood Forests. Biological Invasions **12(5):**1053-1067.

Loss, S R, R M Hueffmeier, C M Hale, G E Host, G Sjerven, and L E Frelich. 2013. A visual method for rapidly assessing earthworm invasions in northern hardwood forests. *Natural Areas Journal*, 33(1):21-30.

Loss, S.R., and R.B. Blair. 2011. Reduced density and nest survival of ground-nesting songbirds relative to earthworm invasions in northern hardwood forests. *Conservation Biology* 5: 983-992.

Loss, S.R., G.J. Niemi and R.B. Blair. 2012. Invasions of non-native earthworms related to population declines of ground-nesting songbirds across a regional extent in northern hardwood forests of North America. Landscape Ecology 27(5): 683-696.

Deliverable #1 - Updates of Great Lakes Worm Watch online resources customized for various potential users/stakeholders

Updates to Great Lakes Worm Watch website survey protocols and online data collection system have been ongoing throughout this 3 year project. Most recently, three training videos for conducting earthworm surveys for participants taking part in the Great Lakes Worm Watch project were added, including:

- Video 1: **Step 1 "mixing the mustard solution."** http://www.youtube.com/watch?v=Yt3nE0LnF9E&feature=plcp
- Video 2: **Step 2 "Setting up the sample grid."** http://www.youtube.com/watch?v=3Non5ZB---4
- Video 3: **Step 3 "extracting the worms!"** http://www.youtube.com/watch?v=7StCMZE936c&feature=youtu.be
- Video 4: **Step 4 "Sampling Design"** http://youtu.be/k1xrxMMbLMc

In concert with previous updates including …

- Interactive online training tutorials, under the "Conduct your Own Surveys" section of the website, were developed to assist participants with the collection of quantitative earthworm data. The tutorials can be viewed as a whole or viewed in sections that participants can quick reference such as: Choosing a sampling location, generating geographic location data, how to use a GPS unit, sampling earthworms, how to send specimens to GLWW and ways that they would be able to analyze the data. http://www.greatlakeswormwatch.org/team/conduct.html
- Updated/adapted lesson plans for K-12 age groups (aligned to state and national standards) and non-formal environmental educators including
    - Creating and using "Earthworm Observatories": http://www.greatlakeswormwatch.org/educator/activities_observatory.html
    - The "Invasion of Exotic Earthworms" Activity http://www.greatlakeswormwatch.org/educator/activities_invasion.html

- A new "Frequently Asked Questions" FAQ sheet developed from the hundreds of email questions we get each year. http://www.greatlakeswormwatch.org/team/action.html

- Final development and implementation of internal protocols for handling earthworms survey data and voucher specimens submitted so that they are adequately quality checked and participants get feedback on the data they submit so as to encourage continued contributions to the project.

    Our originally proposed collaboration with National Institute for Invasive Species Science (NIISS) to develop a national database for the submission of earthworm data proved to be too difficult to implement. Species identification of earthworms are difficult for untrained personnel and did not fit well into the organizational and taxonomic structure used by NIISS. Their program proved too cumbersome for most of our citizen science participants to use and after several failed attempts. We designed comprehensive and detailed data collection and submission protocols now on our Great Lakes Worm Watch website http://www.greatlakeswormwatch.org/team/conduct.html. While the collaboration did not yield the intended database, it served to help us identify the technical and educational issues we needed to address. Their expertise helped us to develop a program that works very well for our program and goals.

A second edition "Earthworms of the Great Lakes" was completed in fall 2012 (copies will be forwarded when we get them from the printer). This edition included:

- Descriptions of four new species, probable introduction via the vermicomposting trade.
- A summary of the IERAT (Invasive Earthworm Rapid Assessment Tool) and how to host land manager trainings.
- New research results on the impacts of invasive earthworms on ground nesting songbirds (i.e. Ovenbirds), salamanders and insects.
- The ABC's of vermicomposting.
- Updates to the sections on ecological groups, earthworm anatomy and biology
- Completed revised and re-designed key to earthworm identification making it much more user friendly.

Deliverable #2 –Host a minimum of 36 training workshops (10-14 annually) and regular web casts with collaborators and stakeholder groups to actively support citizen-based earthworm survey activities throughout the state.

Throughout this project we hosted a total of 40 training or workshops directly reaching 1758 people (Table 1 below). Clearly the impacts go well beyond these direct contacts however, such indirect impacts are very difficult to quantify. That said, we know for the level of interest and unsolicited contacts that we reach well in excess of thousands of individuals annually. We have directly collaborated with individuals and groups in 10 states (Wisconsin, Ohio, New York, Massachusetts, Virginia, Pennsylvania, Alaska, Kentucky, Michigan) to provide services, earthworm education, training and monitoring efforts in their regions. Thirteen of these groups, from 5 states (Minnesota, Wisconsin, Ohio, New York, Massachusetts) submitted earthworm survey data which was added to our archives. See the Dissemination section of the report for a detailed list of outreach activities during the grant period.

Table 1. Summary of trainings, workshops and outreach activities conducted.

Audiences reached via trainings and workshops	Numbers of trainings, workshops conducted	Numbers of people directly reached.
Land Managers-Researchers	10	138
Public	9	495
College Students	12	130
K-12 teachers	3	25
K-12 students	6	575
Total:	**40**	**1363**

Deliverable #3 – **3.a.** GIS data layer indicating earthworm-free, minimally invaded, moderately invaded and heavily invaded areas of the state; **3.b.**GIS data layer of the known/estimated distributions of all earthworm species documented in the state

During this study (2009-12) we had data submitted and verified from 716 sample points or sites from 9 states, including a total of 9,697 earthworm specimens. Summaries of this data by earthworm species and state are provided in tables 2 and 3 below. This included substantial collaborative efforts and our complete database now includes 3,427 sample points with 20,065 verified specimens.

The data collected includes a combination site assessment level data collected using the Invasive Earthworm Rapid Assessment Tool (IERAT) and species specific earthworm data. The IERAT data (Appendix 5) illustrates the areas of Minnesota with different levels of earthworm invasion and associated ecological impacts (category 1= *earthworm-free* through category 5 = *heavily impacted*). Currently an estimated 20% of the landscape is fully earthworm-free and ~50% is minimally impacted. These areas are identified and should be targeted for priority protection and implementation of land-use practices and policies to prevent future introductions roe further spread of earthworms in the areas. This data will be delivered free, online using ESRI powered ArcGIS online*. This interactive is in its beta version titled "***Great Lakes Worm Watch IERAT Classification"*** <http://bit.ly/YstIcE>. It will be finalized and linked to the "Research – Data" section of the Great Lakes Worm watch website when fully tested on the coming weeks.

Earthworm species specific data for Minnesota is also mapped and publicly available the ESRI powered ArcGIS online* interactive beta map titled "***Great Lakes Worm Watch Minnesota Earthworm Species*** <http://bit.ly/YiIlgV>. It contains all quantitative species data recorded in Minnesota to date (2000-2012). Each point on the map represents one of 716+ unique sample plots. Zooming in the interactive map makes clearer many overlapping points and by clicking on a given point all earthworm species present at that sample plot are listed. Great Lakes Worm Watch will continue to add data to this interactive map as a platform for making this data publically available. In total there are 3,427 unique sample points consisting of 20,065 specimens across 10 states (Alaska, Illinois, Indiana, Massachusetts, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin) represent 16 total earthworm species that will be made available through this map.
**ArcGIS Online is a complete, cloud-based, collaborative content management system that lets organizations manage their geographic information in a secure and configurable environment.*

Table 2. Earthworm species and the number of specimens identified and archived by Great Lakes Worm Watch during the LCCMR grant period 2009-2012.

List of species identified	Number of specimens archived
Allolobophora chlorotica	10
Amynthas spp.	410
Aporrectodea spp.	2652

Aporrectodea caliginosa	19
Aporrectodea caliginosa complex	215
Aporrectodea longa	17
Aporrectodea rosea	79
Aporrectodea trapezoides	3
Aporrectodea tuberculata	83
Dendrobaena octaedra	1449
Dendrodrilus rubidus	72
Eisenia eiseni	2
Eisenia fetida	46
Lumbricus spp.	3683
Lumbricus rubellus	402
Lumbricus terrestris	239
Octolasion spp.	227
Octolasion cyaneum	7
Octolasion tyrtaeum	82
TOTAL	**9,697**

Table 3. The number of earthworm specimens identified and archived by Great Lakes Worm Watch for each state for which data was submitted during the LCCMR grant period 2009-2012.

States that submitted data	**Number of specimens archived**
Alaska	2
Indiana	57
Massachusetts	663
Michigan	176
Minnesota	6008
New York	39
Ohio	1469
Pennsylvania	15
Wisconsin	1268
TOTAL	**9,697**

Deliverable #4 - peer-review publication

The peer-reviewed publication titled ““**Earthworm Invasions in Northern Hardwood Forests: a Rapid Assessment Method**” was published in Natural Areas Journal in January 2013 (Appendix 6).

Non-native earthworms have invaded hardwood forests of boreal and north temperate North America, with substantial effects to soil, plants, and ground-dwelling vertebrates. Quantifying these invasions is necessary for understanding the scope of earthworm impacts and for identifying remaining earthworm-free areas in which to target conservation and management activities. Current earthworm sampling methods are effort intensive and/or environmentally damaging, which prevents efficient quantification of invasions at high resolution and across broad spatial scales. A 5-level ranking system of visual classification (earthworm free – heavily earthworm invaded), based on multiple soil and forest floor characteristics was developed and provides an efficient and effective approach for rapidly assessing the distribution and relative severity of earthworm invasions in hardwood forests of the Great Lakes region.

The complete IERAT effort was made possible through the LCCMR funds and additional funds provided by:

2008-2010. MN Coastal Program Grant – "Exotic earthworm invasions: integrated research and education to achieve natural resource protection in North Shore State parks", grant award $46,065.

2009. Grand Portage National Monument, National Park Service – "Grand Portage National Monument- baseline earthworm survey", 1 year project, award amount $2,875.

V. TOTAL TRUST FUND PROJECT BUDGET:

Personnel: $ 107,510

1) Cindy Hale, project manager- year 1=10%, year 2= 15%, year 3= 12% annual salary & fringe (project total $26,574)
2) George Host, experimental design & project support – 1% annual salary & fringe for 3 years (project total $3,889)
3) Gerry Sjerven, GIS specialist - 3% annual salary & fringe for 3 years (project total $6,777)
4) Jane Reed, website design - 2% annual salary & fringe for 2 years (project total $1,871)
5) Ryan Hueffmeier, NRRI junior scientist - 25% in years 1+3 and 50% in year 2 annual salary & fringe (project total $47,289)
6) Undergraduate lab/field staff (Caitlin Leach, Gretchen Anderson) - 25% academic year salary & fringe and 50% summer salary & fringe over 3 years (project total $21,110)

Contracts:
Becky Knowles - subcontract with Leech Lake Band of Ojibwe, primary coordination of field testing of management recommendations (result 2) – 30% annual salary & fringe for 1 year (project total $19,135).

Equipment/Tools/Supplies:

1) Office equipment & computers - project specific data storage (project total $900)

2) Field/Lab Supplies: earthworm sampling and preservation supplies and materials; educational materials (project total $1750)

Overall project total = $ 2,650

Acquisition, including easements: $ 0

Travel:
Travel expenses over 3 years to include lodging, meals as needed, mileage, and camping expenses in remote locations for field work, estimated a total of 91 days travel at per diem rates (lodging $70/day + M&IE $39/day = $109/day), 125 days of NRRI vehicle use ($10/day) and 16,172miles @$0.50/mile (project total $19,255)

Other:
Includes lab fees -NRRI GIS lab user fees (yrs 1&2 $300 each and yr 3 $200); postage for mailing sampling supplies to collaborators ($50 in each year); publication costs for peer-reviewed journal article ($250 x 2 publications) (project total $1,450

TOTAL TRUST FUND PROJECT BUDGET: $ 150,000

Explanation of Capital Expenditures Greater Than $3,500: none

VI. PROJECT STRATEGY:

A. Project Partners:

Partners who will receive funds through this project include:

1) Rebecca Knowles **Leech Lake Band of Ojibwe Division of Resource Management,** Ecologist – will receive $19,135
2) George Host, **The Natural Resources Research Institute, University of Minnesota Duluth GIS lab**, Senior Research Scientist and GIS lab coordinator – will receive $3,889

Partners who will NOT receive funds include:

1) Andy Holdsworth, **MN Department of Natural Resources,** Science Policy Division
2) Ann Pierce, **MN Department of Natural Resources,** Terrestrial invasive species coordinator
3) Jim Barott, **Chippewa National Forest,** Soil Ecologist
4) David Andow, **University of Minnesota,** Distinguished McKnight University Professor, Department of Entomology
5) Jim Graham, **The National Institute of Invasive Species Science, U.S.G.S. & Colorado State University**, Fort Collins Colorado –Research Scientist;
6) Catherine Jarnevich, **The National Institute of Invasive Species Science, U.S.G.S. & Colorado State University**, programmer

B. Project Impact and Long-term Strategy:

The results of this project will fill large gaps in knowledge about 1) the risks associated with different vectors of spread for invasive earthworms such as fishing bait, vermicomposting and the movement of soils, mulch and compost; 2) what different management practices, regulatory responses and educational efforts can do to prevent further spread, 3) and what areas of the state should have the highest priority for protection. With the risk assessments in hand we can readily move towards developing highly effective strategies for limiting the spread of established earthworms and preventing introductions of species not yet present in the state, thereby protecting native forests resources from future aesthetic, biologic and economic impacts. Specifically, native plant populations, tree seedling regeneration, habitat for forest birds, amphibians and small mammals will be protected. We also expect to help limit the spread of many of the most destructive invasive plant species such as buckthorn and garlic mustard which appear to be facilitated by earthworm invasions. The results will be applicable to the state as a whole, since earthworm invasions are occurring statewide, and specifically to the hardwood forested ecosystems where large impacts have already been documented. In addition, we will provide the first coordinated data collection effort in the prairie regions and conifer dominated forests of MN. These results will be broadly applicable to the previously earthworm-free, cold-temperate regions of North America and the Eastern Deciduous Forest Biome of North America, where invasive earthworm invasions are occurring. The technology and information infrastructure created through this project will be available for free on the Great Lakes Worm Watch website and can use by others to support local and regional efforts to limit the introduction, spread and ecological impacts of invasive earthworms. It will also lay the foundation for the development of an accessible and comprehensive system to involve professionals and citizens in long-term monitoring and rapid response to invasive species invasions.

C. Other Funds Proposed Spent during the Project Period:
2008-2010. MN Coastal Program Grant – "Exotic earthworm invasions: integrated research and education to achieve natural resource protection in North Shore State parks", grant award $46,065.

2009. University of Minnesota - Undergraduate Research Opportunties Grant – "Testing Educational Effectiveness of an "*Invasive Earthworm Disposal*" Message in our North Shore, State Parks", student - Nicole Vander Heiden, grant award $1,700 (see "AttachmentC.doc")

2009. Grand Portage National Monument, National Park Service – "Grand Portage National Monument- baseline earthworm survey", 1 year project, award amount $2,875.

2010-2013. USDA – CREES Invasive Species grant. (WoBL) "Reducing human-mediated spread of non-native earthworms in vulnerable northern hardwood forests.", CSREES USDA-AFRI Biology of Weedy and Invasive Species in Agroecosystems. Co-PI's include David Andow (lead PI), Terry Hurley, George Host, Cindy Hale and Rebecca Knowles. Grant award $491,000

D. Spending History:

2007-08. National Forest Foundation -"Regional Assessment And Proposed Actions To Address Non-Native Earthworm Invasion Threats To Northern Forests Of The Great Lakes Region.", award amount $4,999

No previous trust fund dollars have been spend on this project.

VII. DISSEMINATION:

The project has allowed us to greatly enhance and expand the quality and quantity of resources provided through the Great Lakes Worm Watch website <http://www.greatlakeswormwatch.org>. In addition to the many people we interact with directly there are thousands that access our website resources annually.
In 2012, Great Lakes Worm Watch established and now maintains a Facebook page with 175+ "Likes". We use the platform, linked to our website, to communicate research, outreach and educational opportunities <http://www.facebook.com/pages/Great-Lakes-Worm-Watch/123279661062852>.

A list/summary of dissemination activities is provided below:

Peer reviewed publications:

Larson, E.R., K.F. Kipfmueller, C.M. Hale, L.E. Frelich, and P.B. Reich. (2010) Tree Rings Detect Earthworm Invasions and their Effects in Northern Hardwood Forests. Biological Invasions **12(5):**1053-1067.

Loss, S R, R M Hueffmeier, C M Hale, G E Host, G Sjerven, and L E Frelich. 2013. A visual method for rapidly assessing earthworm invasions in northern hardwood forests. *Natural Areas Journal*, 33(1):21-30.

Loss, S.R., and R.B. Blair. 2011. Reduced density and nest survival of ground-nesting songbirds relative to earthworm invasions in northern hardwood forests. *Conservation Biology* 5: 983-992.

Loss, S.R., G.J. Niemi and R.B. Blair. 2012. Invasions of non-native earthworms related to population declines of ground-nesting songbirds across a regional extent in northern hardwood forests of North America. Landscape Ecology 27(5): 683-696.

GLWW Seminars or Professional Presentations:

1) September 10-11th, 2009 – *Impacts of Invasive Earthworms on Forest Ecology in the Great Lakes Region and Potential Management or Policy Responses*. Invited Keynote Speaker at the Ohio Biodiversity Alliance Soil Science Symposium, Cleveland, OH;
2) October 10th, 2009 – *The Current State of Research on the Impacts of Invasive Earthworms in Northern Temperate Forests*. Invited Seminar Speaker, The Cary Institute of Ecosystems Studies, Millbrook, NY
3) December 4th, 2009 – *Impacts of Invasive Earthworms on Northern Temperate Forest Soils and Implications for Forest Ecology in the Great Lakes Region*. Invited Seminar Speaker - Minnesota Association of Professional Soil Scientists MAPSS Winter Technical Event, St. Cloud, Minnesota.

4) February 23rd, 2010 - INVASIVE EARTHWORM RAPID ASSESSMENT TOOL: Assessing the status of invasive European Earthworms in hardwood forest types for the Western Great Lakes region using visual indicators. Cloquet Forestry Review & Technology Transfer Conference, Cloquet Forestry Center, Cloquet, MN.
5) January 22, 2010 - *Developing Action Recommendations: Responding to the Threat of Invasive Earthworms in Western Great Lakes Forests*. Stewardship & Midwest Invasive Plant Network, East Lansing, MI.
6) March 1-3, 2010 - *Developing Action Recommendations: Responding to the Threat of Invasive Earthworms in Western Great Lakes Forests*. Joint Meeting of the Minnesota Chapters of the American Fisheries Society (AFS), Society for Conservation Biology (SCB), and The Wildlife Society (TWS), Nisswa, MN.
7) March 2010. Jess Johnson and Caleb Bilda. *Vermicomposting – best practices for preventing introduction of invasive earthworms*. Stowe Elementary Environmental Learning Resource Fair.
8) March 16-17th, 2010 - *Developing Action Recommendations: Responding to the Threat of Invasive Earthworms in Western Great Lakes Forests*. Western Great Lakes Research Conference, St. Paul MN.
9) June 8, 2010 - *Developing Action Recommendations: Responding to the Threat of Invasive Earthworms in Western Great Lakes Forests*. The National Tribal Science Forum, Grand Traverse, MI.
10) October 17th, 2011 - St. John's University Lecture series.
11) January 12th, 2012 - *An Invasive Earthworm Rapid Assessment tool for Natural Resource Managers in the Great Lakes Region*. 9th Annual Forest, Wildlife and Natural Resources Research Review, Cloquet MN.
12) April 12th, 2012- *Northland Community College,* Ashland Wisconsin. Guest speaker.
13) April 29th, 2012- Gretchen Anderson. *Vermicomposting – best practices for preventing introduction of invasive earthworms*. Stowe Elementary Environmental Learning Resource Fair.
14) May 1st, 2012 – University for Seniors. University of Minnesota Duluth.
15) June 25th, 2012 - University for Seniors. University of Minnesota Duluth.
16) October 2012. *Itasca Community College,* Grand Rapids, Minnesota. Guest Speaker: Natural Resources: Invasive Species.
17) October 2012- *Project Earth*, St. John's University, Collegeville, Minnesota. Guest Teacher: Project Earth is a day long program aimed at 7th and 8th graders and focusing on nature – science exploration
18) September 2012. Harbor City International School. Delivered presentation open to the entire harbor city school on earthworm impacts and citizen science involvement.
19) August 2012. *Minnesota State Fair*, Minnesota Department of Natural Resources, St. Paul, Minnesota. Invited to be an exhibitor representing Great Lakes Worm Watch. Tabled staffed from August 23 – September 3.
20) September 6-8th, 2012. *Invasive Earthworm Rapid Assessment Tool.* 9th Annual Ohio Conservation Symposium.

GLWW Trainings or Workshops:

A. *Invasive Earthworm Rapid Assessment Tool (IERAT) trainings*

a. Land use mangers, research and NGO's
   i. May 24th, 2011 - Trained researchers based in Duluth from The Nature Conservancy.
   ii. May 25th, 2011 - Trained undergraduate field technicians from the Natural Resources Research Institute.
   iii. June 7th, 2011- Trained researchers, land managers and field technicians from the Chippewa National Forest.
   iv. July 20th, 2011 -Trained researchers, land managers and field technicians from the Superior National Forest.
   v. August, 2011 – Trained ecologist, botanist and field technicians from the National Park Service, Ashland, Wisconsin
   vi. October 6th, 2011. Trained foresters, land managers and technicians from the Fond du Lac Reservation Resource Management division.
   vii. October 19th, 2011. Trained Minnesota Department of Natural Resources researchers, land managers and field technicians.
   viii. June 28th, 2012. Trained undergraduate research crew and Natural Resources Research Institute staff.
   ix. July 10th, 2012.Trained researchers, field techs from the University of Minnesota on the use of the IERAT.
   x. August 12th, 2012. Trained researcher and field techs from the University of Minnesota on the use of the IERAT.

b. College
   i. May, 2011- Trained undergraduate field technicians for the WoBL "Earthworm Bait Label" study
   ii. July 30th, 2011 – Trained graduate researcher from the University of Wisconsin Madison.
   iii. May 10th, 2012 – Undergraduate research student, St. Cloud State College.
   iv. May 21st, 2012 – Graduate researcher, University of Stevens Point, Wisconsin.
   v. June 3rd, 2012 – Trained University of Minnesota Twin Cities graduate researcher with undergraduate field interns
   vi. June 5th, 2012 – Trained university of Minnesota Duluth interns and researchers.
   vii. June 26th, 2012 – Trained University of Minnesota Twin Cities graduate researcher and undergraduate field interns
   viii. June 18th, 2012 - Wisconsin Alliance for Minority Participation (WiscAMP), University of Plattville, Wisconsin

B. *Great Lakes Worm Watch trainings*

a. K-12 Teachers
    i. July 19 thru 21st, 2010 – *Citizen Science Research for Teachers*. Teacher professional development. University of Minnesota, St. Paul Campus
    ii. May 9th, 2012 – Forest For Every Classroom. Teacher professional development, MacKenzie Environmental Education Center, Poynette, WI
    iii. August, 2012 - Fond du Lac Tribal and Community River Watch Program Cloquet, Minnesota
b. K-12 Students
    i. June 26th, 2010 – Rusk County Land and Water Conservation high school camp. Tail End Camp, Bruce, Wisconsin.
    ii. October 11th, 2011 - Superior Wisconsin Middle school 6th gaders in preparation for their “Citizen Science Day”.
    iii. April 25th, 2012 - Iron Range Science and Engineering Festival. Field training with 7 graders in Northern Minnesota.
    iv. June 28th, 2012 – White Earth Science and Math Academy. White Earth, Minnesota.
    v. October 15th, 2012 - Cloquet Middle school 6th grade science class. presentation and earthworm survey.
    vi. October 21st, 2012 - Denfeld High School 10th Grade science Lab. Earthworm identification.
    .
c. College
    i. October 8th, 2011 - In-service teacher training for the College of St. Scholastica Graduate Teaching Licensing program
    ii. October 27th, 2011 - In-service teacher training for the College of St. Scholastica high school science teachers field methods class.
    iii. June 5th, 2012 – University of Minnesota Duluth Interns. Train the Trainer workshop.
    iv. July 17th, 2012 - Wisconsin Alliance for Minority Participation (WiscAMP), Professional Development Training. Pigeon River Field Station, WI.
d. Public
    i. June 25 and 26th, 2011 – BioBlitz, Minnesota Department of Natural Resources, Lake Vermilion State Park.
    ii. July 23, 2011. Sugarloaf Cove public presentation.
    iii. September 25th, 2011 - Big Worming Week training at the Hartley Nature Center, Duluth MN.

iv. August 22nd, 2011 - Sherburne National Wildlife Refuge. Delivered field based hands on workshop for teachers, natural resource professionals and general public.
v. August 15th, 2012 – MinnAqua public program. Detroit Lakes, Minnesota.
vi. August 15th, 2012 – Pikerel Lake Association. Rochert, Minnesota.
vii. August 16th, 2012 – Headwater Science Center. Bemidji, Minnesota.
viii. August 16th, 2012 – Bemidji State Park Event. Bemidji, Minnesota
ix. September 30th, 2012 - Minnesota Department of Natural Resources, Moose Mountain Scientific and Natural Areas, Duluth, Minnesota. Delivered field based hands on workshop for teachers, natural resource professional and general public. Trained participants on quantitative and qualitative sampling techniques.

Print/online/radio/TV media coverage:

1) Fall 2009 – *Wisconsin's second annual big worming week!* Wood Prints, vol.26 number 4, Published by Friends of Beaver Creek Reserve.
2) September 3, 2009 – Cloquet Pine Journal, *Earthworms Invasion? Citizen Scientists needed for Research In Jay Cooke.* By June Kallestad
3) September 5, 2009 – Cook County News Herald, *Earthworms Invasion? Citizen Scientists needed for Research Along the North Shore.* By June Kallestad
4) September 18, 2009 – By Paul Volkmann, *Invasive earthworms on the move*. Inside the Outdoors, online at PeeVee News < http://www.greaterlatrobe.net/pvnews.php>
5) September 21, 2009 - *Spread of European earthworms threatening forests of Northeast Ohio, including sugar maples.* By Michael Scott. The Plain Dealer <cleveland.com>
6) September 21, 2009 - *How to tell if earthworms have invaded a forest*. By Robert Higgs. The Plain Dealer Extra <cleveland.com>
7) November 18, 2009 - *The Dirt on Worms*. By Pam Smith. Published online at www.agweb.com
8) December, 2009 - *Cindy Hale unearths the dark side of wiggly earthworms*. Living North Magazine, by June Kallestad.
9) April 29th, 2010 – *Worm Watch.* Wisconsin Public Television. http://wpt2.org/npa/IW823wormwatch.cfm
10) Winter 2010 – Sustainable Farming Association newsletter, The CornerPost. *Unearthing the dark side of earthworms: Asian "jumping" worms are new gardening threat,* by June Kallestad.

11) June 10th, 2010 - Northland's News Center, NBC 6, CBS3. "Students Work to save forests from invasive species". Story about earthworm monitoring work being done at Hartley Nature Center, Duluth, MN. http://www.northlandsnewscenter.com/news/local/48801247.html
12) April 8th, 2011- Almanac North, WDSE –PBS 8. "Asian Jumping Worms". Story about the potential impacts of the Asian earthworm species *Amynthas.* http://www.wdse.org/shows/almanac/watch/almanac-north-apr-29-2011
13) July 5th, 2011 – *Answer Girl: Rain draws out worms and where to find shelter from storms.* Wyoming's Casper Star Tribune, by Carol Seavey. http://trib.com/news/local/casper/answergirl/article_6d0957c3-4b26-582f-882b-5ee92ed02c3c.html
14) August, 2011 – *Earthworms change the "ground rules" of native forests.* The Vermilion Sportsman Quarterly, By June Kallestad.
15) September 11th, 2011- *Worms in the Woods.* In the Hills, by Chris Wedeles. http://www.inthehills.ca/2011/09/back/worms-in-the-woods/
16) September 12th, 2011- Science Nation "Invasion of the Earthworm!" http://www.nsf.gov/news/special_reports/science_nation/wormwatch.jsp
17) September 16th, 2011 – *Earthworm invasion damages trees.* PBS Newshour, by Jenny Marder. http://www.pbs.org/newshour/rundown/2011/09/earthworm-invasion-damages-trees.html
18) September 26th, 2011- Northland's News Center, NBC 6, CBS3. "Worm Week Wiggles In". Story about participating in Great Lakes Worm Watch annual Big Worming Week. http://www.northlandsnewscenter.com/news/video/Worming-Week-Wiggles-In-130542833.html
19) September 26th, 2011- *As the worm turns*. Ironwood info, by Melanie Fullman. http://www.ironwoodinfo.com/news_2011/09/090411_indawoods/090411_indawoods.htm
20) September 26th, 2011- *The underground master of invasive species – earthworms*. Great Lakes Echo, by Brian Bienkowski. http://greatlakesecho.org/2011/09/26/the-underground-master-of-invasive-species-%E2%80%93-earthworms/
21) September 28th, 2011- *Get your hands dirty during Big Worming Week.* Great Lakes Echo, by Brian Bienkowski. http://greatlakesecho.org/2011/09/28/get-your-hands-dirty-during-%E2%80%9Cbig-worming-week%E2%80%9D/
22) October 12th, 2011 – *US pest invasion dates back to earlier settlers*. Associated Press, by Rick Callahan.
23) November 1st, 2011- *Earthworm research from UMD also highlighted on Science Nation.* Duluth News Tribune. https://secure.forumcomm.com/?publisher_ID=36&article_id=213495&CFID=631372190&CFTOKEN=24421296
24) November 9th, 2011 – *Tiny earthworms, big impacts; Invasive earthworms change North American landscapes, for better or worse.* Science News for kids, by Cecile LaBlanc. http://www.sciencenewsforkids.org/2011/11/tiny-earthworms%E2%80%99-big-impact/
25) January 29th, 2012 – *Are Worms Natural? The Global Worming Debate.*Nature @ WSU, by Rod Sayler. http://wsu-nature.org/2012/01/29/are-worms-natural-the-global-worming-debate/

26) February 2nd, 2012 – Radio interview on the Buckeye Sportsman with Dan Armitage in Ohio. http://www.buckeyesportsman.net/index.php?option=com_content&view=category&layout=blog&id=34&Itemid=55
27) Spring, 2012 – NRRI Now, *K-12 Citizen Scientists*. Natural Resources Research Institute, by June Kallestad
28) Spring/Summer, 2012 – *Impacts of earthworms in North America and around the world.* Northbound, by Cheryl Todea.
29) Spring/Summer, 2012 – *Earthworm impacts in northern forest ecosystems*. Northbound, by Joe Panci.
30) March 7th, 2012 – *Great Lakes Worm Watch.* Ecological Society of America: Ecotones, by Liza Lester. http://www.esa.org/esablog/citizen-science/great-lakes-worm-watch/
31) March 18th, 2012 – *Shalaway:The dark destructive world of earthworms.* Pittsburg Post-Gazette, by Scott Shalaway. http://www.post-gazette.com/stories/sports/hunting-fishing/shalaway-the-dark-destructive-world-of-earthworms-517249/?print=1
32) March 22nd, 2012- *Earthworms, while beneficial, can also destroy.* Farm and Dairy, by Scott Shalaway. http://www.farmanddairy.com/news/earthworms-while-beneficial-can-also-destroy/35754.html
33) March 27th, 2012 – *Earthworms ruin nutrients, moisture on forest floor says researchers.* Canada.com. http://24bdnews7.blogspot.com/2012/03/earthworms-ruin-nutrients-moisture-on.html
34) April 23rd, 2012 – Radio interview with WTIP North Shore Community Radio.
35) May 25th, 2012 – Radio interview with the Lake Superior Binational Forum.
36) May, 2012 – *What you should know about the earthworms in your soil.* Better Farming, by Mike Mulhern.
37) July, 2012 – *Non-native Earthworms on our Shores: Great for Fishing not so Great for the Woods.* From Shore to Shore, by Ryan Hueffmeier. http://www.shorelandmanagement.org/downloads/july_aug_2012.pdf
38) August 27th, 2012 – *Earthworm Experiment: Alien Invaders.* Simple Recipes for real Science, by Kitchen Pantry Scientist. http://kitchenpantryscientist.com/?p=3339
39) September 6th, 2012 – *Ask NRRI*. Natural Resources Research Institute. http://www.nrri.umn.edu/default/asknrri/compost.htm
40) September 23rd, 2012 – *Great Lakes Worm Watch.* Radio station KDAL Duluth, MN. http://kdal610.com/news/articles/2012/sep/27/great-lakes-worm-watch-on-sunday/
41) October 1st, 2012 – "Workshop Teaches People About the Impact of Earthworms" WDIO 10 Duluth MN. http://www.wdio.com/article/stories/S2784166.shtml?cat=11802

Other Outreach Activities: (ie. Exhibits, tabling, etc.)

1) January 15-16th, 2010 - GLWW exhibit booth. The MN Organic Farming Conference, St. Cloud, MN.

2) February 20, 2010 – GLWW exhibit booth. The Sustainable Farming Association of Minnesota's 19th Annual Conference, St. Olaf College, Northfield, MN
3) March 9-10th, 2012 – GLWW Exhibit Booth. Minnesota Families Woodlands Conference, Duluth, MN.
4) April 17th, 2012 – GLWW Poster Session. Western Great Lakes Resource Management Conference. Ashland, WI. Loss, S.R., R.M. Hueffmeier, C.M. Hale, G.E. Host, G.Sjerven, L.E.Frelich. In press. *Earthworm invasions in northern hardwood forests: A rapid assessment method*
5) August, 2012 – Itasca County Fair. Grand Rapids, Minnesota.
6) August, 2012 – Minnesota State Fair Department of Natural Resources Building.
7) October, 2012 – GLWW Poster Session, Hueffmeier, R., G. Sjerven, G. Host. *Exotic Earthworm Invasions: Integrated Research and Education to Achieve Natural Resource Protection.* Minnesota GIS/LIS Consortium 22nd Annual Conference. St. Cloud, Minnesota.
8) October 29-31st, 2012 – GLWW exhibit booth and poster presentation. Upper Midwest Invasive Species Conference, Lacrosse, WI.
    a. Kelly P. Bray, Ryan Hueffmeier, Gerry Sjerven, George Host, David Andow. 2012 *Quantifying The Spread of Invasive Earthworms in Minnesota's Northern Forests.*
    b. Claire Palokangas, Laura Christensen, Ryan Hueffmeier, George Host, David Andow. *Relation Between Fishing Pressure And Earthworm Impacts Near Boat Landings.*
    c. Stephanie Driskell, Jennifer Olker, Ryan Hueffmeier, Cindy Hale. 2012. *Identifying and Evaluating Vernal Pool Habitats Spanning a Continuum of Earthworm Invasion Status*.

Other presentations/publications for which GLWW provided supporting materials:

1) October 2010. Matt Bowser, U.S. Fish & Wildlife Service, Kenai National Wildlife Refuge. Exotic earthworms in Alaska: an insidious threat. Alaska Invasive Species Conference, Fairbanks, Alaska.
2) January 2011. Sarah Reichard. The Conscientious Gardener - Cultivating a Garden Ethic, University of California Press http://www.ucpress.edu/book.php?isbn=9780520267404
3) January 2011. Bernadette Williams, Invasive Species BMP Coordinator, Division of Forestry Bureau of Forest Sciences, Wisconsin Department of Natural Resources. Will be using GLWW images in 2 forestry publications, one addressing county forests and another for distribution to bait dealers. *In press*
4) January 2011. Yan Gu. Order of Buildings and Cities: A Paradigm of Open Systems Evolution for Sustainable Design. *In press*
5) March 2nd 2012. Wild Rivers Invasive Species Coalition received a grant for "Earthworm education for Anglers"
6) June 2nd thru Oct 24th 2012. GLWW partners with the MN DNR and the MN Landscape Arboretum to develop a sign about the effects earthworms can

have on previously earthworm-free forests. The sign has been on display all summer at the "Dirt-o-Rama"

7) April thru October, 2012. Friends of the Sherburne National Wildlife Refuge worked with schools around central Minnesota to educate and collect data on invasive earthworms.
8) June 25th, 2012. Oakland County Parks held “Hooked on Fishing”. Waterford, Michigan.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than December 30, 2009. A final work program report and associated products will be submitted between December 30, 2012 and February 28, 2013 as requested by the LCCMR.

IX. RESEARCH PROJECTS:

Attachment A: Budget Detail for 2009 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: *Prevention and Early Detection of Asian Earthworms and Reducing the Spread of European Earthworms.*

Project Manager Name: *Cindy Hale*

Trust Fund Appropriation: $ *150,000*

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2009 Trust Fund Budget - Result 1	Result 1 Budget:	Amount Spent (Dec30,2011)	Balance (Dec30,2011)	2009 Trust Fund Budget-Result 2	Result 2 Budget:	Amount Spent (Dec30,2011)	Balance (Dec30,2011)	2009 Trust Fund Budget-Result 3	Result 3 Budget:	Amount Spent (Dec30,2011)	Balance (Dec30,2011)	2009 Trust Fund Budget - Result 4	Result 4 Budget:	Amount Spent (Dec30,2011)	Balance (Dec30,2011)	TOTAL BUDGET	TOTAL BALANCE
	Risk-Assessment of Vectors of Earthworm Introduction				Testing Effectiveness of Management Recommendations				Regulatory Responses to Early Detection of Asian Earthworms				Identify Priority Areas for Protection				
BUDGET ITEM				**BUDGET ITEM**				**BUDGET ITEM**				**BUDGET ITEM**					
PERSONNEL: total wages and benefits project total $107,510	34,121	34,121	0	**PERSONNEL: total wages and benefits**	15,514	15,514	0	**PERSONNEL: total wages and benefits**	1,470	1,470	0	**PERSONNEL: total wages and benefits**	56,405	56,405	0	**107,510**	**0**
Cindy Hale, project manager- years 1&2 @ 5% annual salary & fringe (project total $26,574)				**Cindy Hale, project manager**- years 2 &3 @ 5% annual salary & fringe				**Cindy Hale, project manager**- years 3 @ 2% annual salary & fringe				**Cindy Hale, project manager**- all 3 years - 5% annual salary & fringe					
George Host - total of year 1 support @ 1% over 2 year period - experimental design and GIS support (project total $3,889)				**George Host** - total of year 1 support @ 1% over 2nd & 3rd years of project - experimental design and GIS support				**George Host**				**George Host** -year 3 @ 1% consulting, GIS support, manuscript preparation					
Gerry Sjerven (project total $6,777)				**Gerry Sjerven**				**Gerry Sjerven**				**Gerry Sjerven** - GIS specialist, 3% for entire grant period (3 years)					
Jane Reed (project total $1,871)				**Jane Reed**				**Jane Reed**				**Jane Reed** - Website development, 2% for 2 years					
NRRI Junior Scientist (to be named) - 2 years @ 25% annual salary & fringe of $28,000/year + 32.7 fringe (project total $47,289)				**NRRI Junior Scientist** (this effort and $ moved to contract - professional services)				**NRRI Junior Scientist**				**NRRI Junior Scientist (to be named) -** 2 years @ 25% annual salary & fringe of $28,000/year + 32.7 fringe					
Undergradaute field/lab staff - 25% for academic year 09-10 and 50% for summer 2010, based on $16,640 annual salary ($8/hr x 2080 hrs), no fringe (project total $21,100)				**Undergradaute field/lab staff** - 25% for academic year 09-10 and 50% for summer 2010, based on $16,640 annual salary ($8/hr x 2080 hrs), no fringe				**Undergradaute field/lab staff**				**Undergradaute field/lab staff** - 25% for academic year 09-10 and 50% for summer 2010, based on $16,640 annual salary ($8/hr x 2080 hrs), no fringe					
Contracts				**Contracts**				**Contracts**				**Contracts**					
Professional/technical (project total $19,135)	0	0	0	**Professional/technical -** Rebecca Knowles, Leech Lake Band of Ojibwe Departmen of Resource Management, 30% time (salary & fringe) for 1 year	19,135	19,135	0	**Professional/technical**	0	0	0	**Professional/technical** *(with whom?, for what?)*	0	0	0	**19,135**	**0**
Office equipment & computers - project specific data storage (project total $ 900)	300	300	0	**Office equipment & computers -** project specific data storage	300	300	0	**Office equipment & computers**	0	0	0	**Office equipment & computers -** project specific data storage	300	300	0	**900**	**0**
Supplies*:* sampling supplies: mustard for earthworm sampling, baggies, vials, preservation fluids, coolers, ice, misc. (project total $1,700)	800	800	0	**Supplies**: sampling supplies: mustard for earthworm sampling, baggies, vials, preservation fluids, coolers, ice, misc.	800	800	0	**Supplies** *(list specific categories):*	0	0	0	**Supplies** *(list specific categories): sampling supplies: mustard for earthworm sampling, baggies, vials, preservation fluids, coolers, ice, misc.*	150	150	0	**1,750**	**0**
Travel expenses in Minnesota: (project total $19,255) **1)** Field staff will be reimbursed at actual costs since they will be traveling extensively, lodging and meals may be provided in some locations by collaborators and they may be camping in remote locations. Estimated need for 38 days travel at per diem rates (lodging $70/day + M&IE $39/day = $109/day) **2)** 55 days of NRRI vehicle use ($10/day) and 6100 miles @$0.55/mile	8,047	8,047	0	**Travel expenses in Minnesota:** **1)** Field staff will be reimbursed at actual costs since they will be traveling extensively, lodging and meals may be provided in some locations by collaborators and they may be camping in remote locations. Estimated need for 38 days travel at per diem rates (lodging $70/day + M&IE $39/day = $109/day) **2)** 55 days of NRRI vehicle use ($10/day) and 6100 miles @$0.55/mile	8,047	8,047	0	**Travel expenses in Minnesota:** **1)** mileafe for meetings: 5 days of NRRI vehicle use ($10/day) and 721 miles @$0.55/mile **2)** lodging & meals for meetings: estimated 5 days travel (lodging $70/day + M&IE $39/day = $109/day)	992	992	0	**Travel expenses in Minnesota:** **1)** Program staff will be reimbursed at actual costs since they will be traveling extensively, lodging and meals will be provided in many locations by workshop hosts, etc.. Estimated need for 10 days travel at per diem rates (lodging $70/day + M&IE $39/day = $109/day) **2)** 10 days of NRRI vehicle use ($10/day) and 6100 miles @$0.55/mile	2,169	2,169	0	**19,255**	**0**
Other (project total $1,450)	0	0	0	**Other:** publication costs associated with General Technical Report	250	250	0	**Other:** publication costs associated with General Technical Report	0	0	0	**Other: 1)** lab fees -NRRI GIS lab user fees (yrs 1&2 $300 each and yr 3 $200) **2)** postage for mailing sampling supplies to collaborators ($50 in each year) **3)** publication costs for peer-reviewed journal article ($250)	1,200	1,200	0	**1,450**	**0**
COLUMN TOTAL	**$43,268**	**$43,268**	**$0**	**COLUMN TOTAL**	**$44,046**	**$44,046**	**$0**	**COLUMN TOTAL**	**$2,462**	**$2,462**	**$0**	**COLUMN TOTAL**	**$60,224**	**$60,224**	**$0**	**$150,000**	**$0**

List of Supplementary Documents related to deliverable for LCCMR Final Report:
"**Prevention and Early Detection of Asian Earthworms and Reducing the Spread of European Earthworms**

Document Name	Result (1-4)	Nature of Deliverable	Date Completed	Date Submitted to LCCMR
*Appendix 1 *Table X *TableY	1	Appendix 1- Draft manuscript titled: "**Internet Sales as Vectors of Non-native Earthworm Introductions in the western Great Lakes Region."** Appendix 1Table X – Results of internet content searches on earthworm species and common names. Appendix 1Table Y – Results of Species Identification of earthworms purchased via the internet	3/7/2013	3/18/2013
*Appendix 2	1	Informational brochure "**ABC's of Composting with Earthworms Safely"**	2/20/2013	
*Appendix 3	2	Draft manuscript/general technical report titled: **"Non-native Earthworms Transported on Treads of ATVs and Logging Equipment in Northern Hardwood Forests of Minnesota, USA."**	2/20/2013	
*Appendix 4	3	Draft Strategic Plan for **"Interagency Earthworm Regulatory Recommendations.**"	2/20/2013	
#Book	4	"**Earthworms of the Great Lakes**", second edition 2012	March 15th , 2013 scheduled deliver date from printer	
*Appendix 5	4	**IERAT map** illustrating earthworm invasions in MN	2/20/2013	
GIS layer 1	4	Digital GIS data of IERAT sample points are provided through an online interactive map <http://bit.ly/YstIcE>. Link provided in final report.	3/14/13	
GIS layer2	4	Digital GIS data of all Minnesota sample locations as well as species documented is provided through an online interactive map <http://bit.ly/YiIIgV>. Link provided in final report.	3/7/2013	
*Appendix 6	4	"Loss, S R, R M Hueffmeier, C M Hale, G E Host, G Sjerven, and L E Frelich. 2013. A visual method for rapidly assessing earthworm invasions in northern hardwood forests. *Natural Areas Journal*, 33(1):21-30.	2/20/2013	
*Appendix 7	4	Larson, E.R., K.F. Kipfmueller, C.M. Hale, L.E. Frelich, and P.B. Reich. (2010) Tree Rings Detect Earthworm Invasions and their Effects in Northern Hardwood Forests. Biological Invasions **12(5):**1053-1067.	2010	
Attachment A Budget Sheet		Final budget report	2/20/13	

* 3 hard copies and electronic version submitted
# 3 hard copies submitted

Appendix 1 – draft manuscript

Internet Sales as Vectors of Non-native Earthworm Introductions in the western Great Lakes Region.

We propose in this report to identify, describe and quantify the potential vectors of in-state spread of established earthworm species and of interstate transport and introduction of non-established earthworm species (i.e. intentional and unintentional transport of compost, mulch, and soils; fishing bait), including field-based measures of earthworm species and relative abundance present in each vector. Specifically we looked at earthworm information dissemination and potential risk of invasive earthworm introduction through internet based vendors. To begin the process we conducted a literature review on e-commerce as a potential vector of the introduction of invasive species in particular non-native earthworms.

This study will inform stakeholders if there are risks associated with internet based earthworm vendors as a vector for the spread of non-native earthworms. Earthworms are an e-commerce commodity used in such ways as fishing bait, lawn care, bio-remediation, composting and vermicomposting. Many of the earthworm species being sold are non-native to North America and are having negative impacts on regions formally devoid of earthworms (Hale 2008, Proulx 2003, Hendrix & Bohlen 2002, Cameron et al. 2007, Edwards & Arancon, 2006).

Since European settlement, invasive earthworms have transformed the forest floors across large expanses of northern hardwoods. Research demonstrates that invasive earthworm are eliminating and relocating the forest floor, altering the nutrient cycling, increasing nutrient runoff, decreasing plant community diversity, and altering the forest ecosystems in previously earthworm free hardwood forests In earthworm-invaded areas the forest floor is characterized by reduced thickness, lower plant biodiversity, and altered biogeochemical cycling (Bohlen et al. 2004a, Frelich et al. 2006, Hale et al. 2006, Eisenhauer et al. 2007, Holdsworth et al. 2007a, Addison 2009). Soil characteristics and processes are also greatly affected with disappearance of the O horizon, increased soil compaction and leaching losses, reduced soil moisture and nutrient availability, and changes in soil mineral weathering and carbon stabilization (Bohlen et al. 2004b, Hale et al. 2005a, Saurez et al. 2006, Larson et al. 2010, Lyttle et al. 2011). These changes have been shown to alter other aspects of the forest community, including facilitating the spread of other invasive species and altering other vertebrate and invertebrate communities that depend on the forest floor (Migge et al. 2006, Maerz et al. 2009, Loss and Blair 2011). The general view of earthworms is that they are great for the soil and healthy for the local ecosystems. This is true in agricultural setting but is not in native hardwood forests that have been earthworm free since the last glaciations.

Through e-commerce there is the potential for new earthworm species such as the Asian *Amynthas* spp. to be introduced into new environments. Websites selling *Amynthas* spp. commonly known as “Jumping Worms” promote them as “the greatest thing you can do for your yard” and “because they are so active they make great fishing bait”. There is also the potential for the introduction of new genetic material being introduced to already established populations such a *Lumbricus rubellus* which is sold for fishing bait and composting.

One of the main vectors of non-native earthworm introduction that has been studied demonstrates the direct relationship between anglers using live earthworms as bait and the introduction of non-native earthworms (Kilian et al. 2012, Keller et al., 2007, Proulx 2003, Seidl & Klepeis, 2011, Cameron et al., 2007, Hale 2008, Haska et al. 2012). In a study that used the Invasive Earthworm Rapid Assessment tool (Loss et al. 2013) to assess the impact of heavily fished Northern Minnesota lakes versus lightly fished lakes, researchers found that boat launches around heavily fished lakes were more impacted by earthworms (Palokangas et al. 2012). With the growing popularity of vermicomposting and the proliferation of internet based earthworm vendors are we on the verge of new species and genetic material being introduced via this new vector of e-commerce?

I. Review of Literature for e-commerce based Movement of Invasive Species

Currently, there are no known studies looking at the risk of spreading non-native earthworms via the internet. Earthworm products are sold by internet based vendors for many uses: composting, vermicomposting, lawn care, food for pets, gardening and fishing bait. This literature review will first look at the internet as a pathway for the introduction of invasive species. Second, how internet based vendors can facilitate non-native earthworm introductions. Lastly, look at some management practices and regulatory responses that can slow the spread of invasive species.

Methods

This assessment of e-commerce as a vector of spread for earthworms in Minnesota was conducted in three phases. The first phase consisted of this literature review of current knowledge of the spread of non-native species through e-commerce and internet based vendors. The second phase used a content analysis to identifying the overall attitude towards earthworms on public assessable websites and located vendors selling earthworms via the internet. The data gathered in the second phase informed the third phase by identifying the internet based earthworm vendors we contacted. Earthworms were ordered from each vendor, preserved and identified to species, determined the accuracy of vendor identification, analyzed how vendors promote and sell earthworms for fishing bait, pet food, compost, vermicompost, lawn and garden amendments.

The overall goal of the project is to assist in developing highly effective strategies for limiting the spread of established earthworms and preventing introductions of species not yet present in the state, thereby protecting native forests resources from future aesthetic, biologic and economic impacts.

When you do a Google search for invasive species you will get around 11,000,000 results. The results span a wide range of sites from educational to commercial. The question we wanted to understand better was “Do earthworms being sold through e-commerce pose a potential vector introductions into environments formally devoid of earthworms?” E-commerce as a potential introduction source for earthworms is an important vector to consider. The potential for introduction of a species repeatedly and on a large scale into a new area is one of the most important factors that lead to invasiveness (Randall and Marinelli 1996). Currently, the economic damages associated with alien invasive species effects and their control amount to approximately $120 billion/year (Pimental 2005). According to the U.S. Fish and Wildlife Service’s some species are (or have been in the past) deliberately brought into the United States for specific reasons (such as biocontrol or for use as pets) and are either released into the wild on purpose or escape where they then unexpectedly become an invasive species problem. These are called intentional introductions and are mainly plant and vertebrate species (Pimental 2005). One of the newest pathways for intentional introductions is mail order shopping through the Internet. Many species arrive here accidentally, without our knowledge. These are called unintentional introductions. Pathways for unintentional introductions include species arriving in foreign ballast water, hidden in wood packing material, hidden in other vegetation via the nursery trade, hidden aboard ships, hidden on other species, and many other pathways. According to Martin and Coetzee (2011) who looked at the internet as a vector of invasive species spread in South Africa state “the internet is a pathway of potential concern, but it is difficult to quantify its contribution to the trade of invasive species in South Africa”.

Appendix 1 – draft manuscript

One of these intentional and unintentional pathways is e-commerce. A search for research on invasive species being transported via the internet found that: invasive terrestrial and aquatic plants are being transported through the internet worldwide (Kay & Hoyle 2001, Maki & Galatowitsch 2004, Walters et al. 2006, Martin & Coetzee 2011, Drew et al. 2010) and that e-commerce risks the biosecurity associated with the importation via online trade of unwanted flora and fauna into the country, as well as their movement within internal borders (Derraik & Phillips 2010).

The proliferation of hobbyist, domestic commercial, and foreign commercial websites discussing the beauty and qualities of invasive aquatic weeds thus is a very serious concern for federal and state regulatory officials as well as resource managers throughout the United States (Kay & Hoyle 2001). In Minnesota the state law prohibits purchasers and sellers from possessing, importing, purchasing, transporting, or introducing prohibited species (Minnesota Reviser of Statutes, 2000). Nevertheless, compliance with these laws is low as plants illegal to possess in Minnesota (state or federal prohibited) were sent 92% of the time they were requested. While compliance is good within the state of Minnesota, mail-order purchases may be more problematic (Maki & Galatowitsch).

Walters et al. (2006) found that from their e-commerce purchases, retailers frequently do not identify their product scientifically, by including the genus and species. And according to a featured article in the Christian Science Monitor "The Internet sales of plants and other organisms are quite large and there's little if any regulation," says Ted Grosholz, an ecology professor at the University of California at Davis. Marshall Meyers, executive vice president of the Pet Industry Joint Advisory Council, says "Internet sales can be a real problem…you can buy giant salvinia over the Internet out of Europe. [But] this is an area that's confusing to the public.

Not all nonnative species of fish and plants are invasive. Our industry relies on nonnative species. One thing we are trying to do is educate the public not to release these into the environment." (Clayton 2004).

Discussion

At regional to local scales, modern commerce in horticulture, forestry, and vermiculture introduces earthworms widely in urban and managed ecosystems, whereas back-country fishing and off-road recreation (pack animals and motorized vehicles) may be significant vectors of transport into remote areas (Hendrix et al. 2008). In a study looking at the movement of earthworms from bait shops to the forest floor Keller et al. (2007) looked at invasion risk posed by non-native earthworms as being broken down into three categories: They will become invasive; The release/escape of additional individuals of an already invasive species may introduce genetic diversity that can enhance invasiveness (Cox 2004) or establish new populations; The introduction of hitchhiking contaminant species that come in/with the species of primary interest

After conducting this literature review we found no research looking into e-commerce as a potential vector for the introduction of non-native earthworms There is a need for greater awareness of the economic and environmental impacts of invasive species leading many industries and countries to consider how voluntary industry practices, regulatory risk assessments and quarantine measures could be modified to reduce the risks of further harm. Any efforts to reduce invasion risks from trades in live organisms will require retailers and government agencies to know the scientific names of the species sold this suggests that the most effective way to ensure that all species in trade are accurately identified may be through certification programs requiring accurate identifications, and regulations and penalties to encourage accurate identifications by wholesalers (Keller et al. 2007).

Furthermore, better communication between regulators and vendors and less confusion about current state and federal laws may decrease the rate of sale of prohibited aquatic plants. However, because state borders are not policed like national borders, state regulations prohibiting groups of species may not be as effective as federal regulations (Maki and Galatowitsch 2004). And according to Keller and Lodge (2007) there are three options for policy approaches that could be adopted: first, allow any and all new species; second, allow no new species; or third, prescreen species for the likelihood of becoming invasive and allow in trade those that pose low risk.

Conclusion

Looking at the results of research into the introduction and spread of non-native species being sold through internet based vendors, the alarm is raised for looking into the potential risks of non-native earthworm introductions through e-commerce. To start we need to understand: the view of earthworm on the internet; what category the internet based earthworm vendors fit, what products are they promoting and selling and are they accurately identifying the earthworm species they are selling.

Appendix 1 – draft manuscript

II. Content Analysis of Earthworm presence on the Internet

Introduction

The purpose of this content analysis on earthworm information found on the internet, was to understand the current views of earthworms, what types of websites (educational, commercial) contribute to earthworm information, are earthworms scientific names being used, what type of earthworm products are sold and the who are the vendors selling earthworms.

This assessment of e-commerce as a vector of spread for non-native earthworms in Minnesota was conducted in three phases. The first phase consisted of a literature review of current knowledge on the spread of invasive species through internet based vendors. The second phase used a content analysis approach to identifying the overall outlook of earthworms on the internet. The data gathered in the second phase informed the third phase by identifying the websites to contact and order earthworms from to identify species they are selling, how accurate they are at species identification, what they sell them for (bait, compost, etc..). The overall goal of the project is to assist in developing highly effective strategies for limiting the spread of established non-native earthworms and preventing introductions of earthworm species not yet present in the state, thereby protecting native forests resources from future aesthetic, biologic and economic impacts. During the content analysis we focused on the *Amynthas* spp., which is an Asian earthworm species that is yet to be widely established in Minnesota or the Western Great Lakes region. The internet searches will be used to determine the number and nature of sites that provide information and/or products related to earthworms online. These results will help us effectively identify the particular vectors we will target for the quantitative aspect of the risk assessment.

Appendix 1 – draft manuscript

Methods

A content analysis was conducted of websites in which key earthworm search words were the primary research method (See appendix 1).

Sample

Using the Google search engine 25 keywords (appendix 1) were entered and the first 10 websites were recorded for a total sample of 250 websites. Keyword searches were done within a 24 hour period and the 10 websites for each keyword were saved in a Microsoft word document. The survey instrument (appendix 1) was developed to take a broad look on how earthworm information is presented on the internet, it was not pilot tested prior to the content analysis.

Intercoder reliability

To conduct the content analysis three coders were trained in the content analysis protocol. The intercoder reliability was established between the three coders by having all coders code 2 of the same key words or 8% (n=20) of the total websites. Percent agreement, Fleiss' Kappa, Cohen's Kappa and Krippendorff's alpha were all used to assess intercoder reliability for the questions 2 and 3. An online utility that computes intercoder reliability coefficients for nominal data coded by three or more coders called ReCal3 http://dfreelon.org/utils/recalfront/recal3/ was used to assess intercoder reliability (Table 1). Percent agreement (62.22%) tends to be a liberal index and Fleiss' Kappa (0.45), Cohen's Kappa (0.46) and Krippendorff's alpha (0.45) are conservative indexs. Overall there was moderate agreement between coders.

Table 1. Intercoder Reliability for 3 Coders each Coding the same Three Keywords; Invasive Amynthas Earthworm, Asian Earthworm Information, Amynthus Information. Looking at Question 2) Institution? and 3) View of Earthworms? from the survey instrument.

	Percent Agreement	Fleiss' Kappa[a]	Cohen's Kappa[b]	Krippendorfs alpha
Websites(n=60)	62.22%	0.45	0.46	0.45

[a] 0.41 – 0.60 = Moderate Agreement; [b] 0.41 – 0.60 = Moderate Agreement.

Appendix 1 – draft manuscript

Results

Question 1) What was the domain: .org, .com, .edu, .net, others. (Table 2)

Eighty six percent of the total websites (N=250) had the domain of ".com", ".org" and ".edu". Fifty three percent (n=133) of the total websites in the content analysis (N=250) were ".com" domains or primarily used for commercial business (Table 2). Which really is no surprise as the ".com" domain is the worlds' most popular. Nineteen percent (n=47) were ".org" primarily used for non-profits and 14% (n=36) were ".edu" or primarily used by educational organizations. Of the websites selling earthworm products (n=55) or 22% of the total websites, had 82% that were .com's. Websites selling earthworms only (n=24) or 10% of the total websites, had 76% that were .com's with 12% being .org's.

Table 2. Comparison of all websites Domains (N=250) with websites that sell earthworm products (n=55) and those that sell earthworms only (n=24)

	.org	.com	.edu	.net	Other[a]
All websites (N=250)	19%	53%	14%	3%	11%
Websites selling earthworms and earthworm products (n=55)	9%	82%	2%	3%	4%
Websites selling Earthworms only (n=24)	12%	76%	4%	4%	4%

[a]other = .gov, .us, .ca, .info, .uk, .bu

Question 2) Institution: Educational, Commercial, Personal or other. (Table 3)

Based on coders reading of the first page of the website, they were asked to code the site as "Educational", "Commercial", "Personal" or "other" (Table 3). Sixty one percent (n=152) of the total websites were classified by coders as "Educational" meaning they had wording such as "research says", 17% (n=42) of the total websites were classified as "Commercial" meaning they had wording implying they were selling a product or service such as "sale" and 9% (n=24) of the total websites were classified as "Personal" meaning they had wording such as "In my experience”. Of the remaining 13% (n=32) coders were unable to classify website into any of the above categories.

Table 3. Comparison of coder defined intentions for all websites (N=250) with websites that sell earthworm products (n=55) and those that sell earthworms only (n=24)

	Educational	Commercial	Personal	Other[a]	Multiple Codes[b]
All websites (N=250)	61%	17%	9%	10%	3%
Websites selling earthworms and earthworm products (n=55)	13%	58%	9%	13%	7%
Websites selling Earthworms only (n=24)	25%	50%	9%	8%	8%

[a]other = Coder unable to classify website into the three categories; [b]Multiple Codes = Coder classified websites into multiple categories.

Question 3) View of Earthworms: “Positive”, “Negative”, “Neutral” or “other”. (Table 4)

Coders were asked to judge whether the content of the website was “Positive”, “Negative”, “Neutral” or “other”. When looking at the total websites, 50% (n=126) were classified as neutral attitude towards earthworms, 27% (n=67) saw earthworms as being positive towards the environment, 13% (n=32) saw earthworms as having a negative impact on the environment. Websites that sold earthworm products (n=55) had 51% classified as neutral with 47% being classified as positive and 0% classified as negative. Websites that sold earthworms only had 21% classified as neutral with 79% being classified as positive and again 0% being classified as negative.

Table 4. Comparison of coder defined view of earthworms for all websites (N=250) with websites that sell earthworm products (n=55) and those that sell earthworms only (n=24).

	Positive	Negative	Neutral	Other[a]
All websites (N=250)	27%	13%	50%	10%
Websites selling earthworms and earthworm products (n=55)	47%	0%	51%	2%
Websites selling Earthworms only (n=24)	79%	0%	21%	0%

[a]other = Coder unable to classify website into one of the 3 categories.

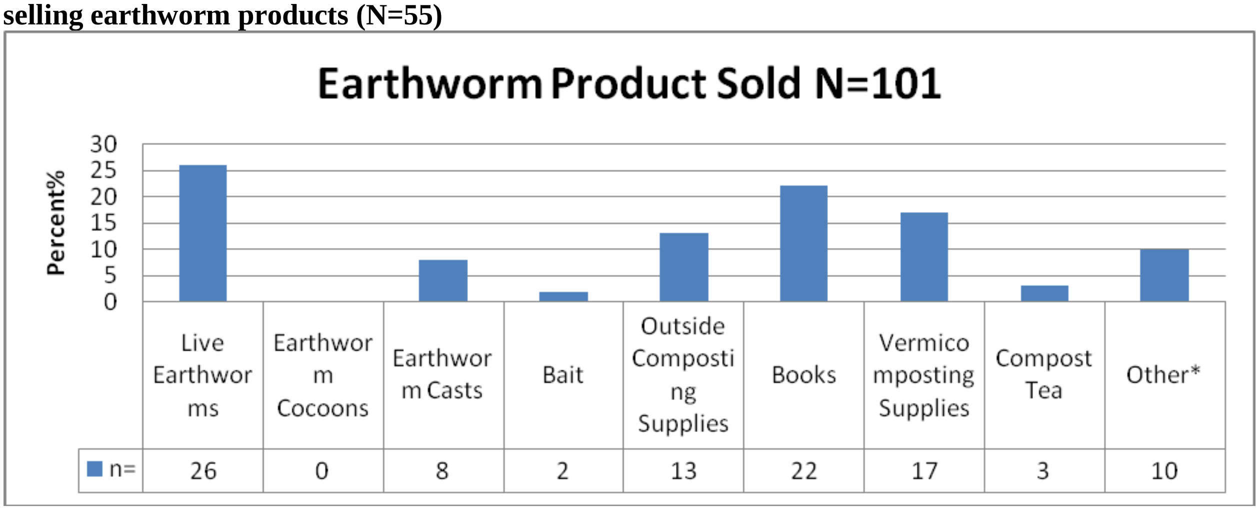
Question 4) Earthworm names; (Appendix 2)

Of the N=735 species, genus and common names used in the N=250 websites 40% (n=294) were common earthworm names, 31% (n=229) listed the genus and 29% (n=215) listed the species name. Websites selling earthworms only had 119 total names with 16% labeled as species, 18% labeled as genus, 66% using common names as identifiers.

Question 5) Earthworm Products (Figure 1)

Twenty two percent (n=55) of the total N=250 websites sold an earthworm product, 24% (n=26) sold live earthworms, 6% (n=6) sold earthworm casting, 2% (n=2) sold earthworms as bait, 13% (n=14) sold outdoor composting supplies, 21% (n=23) sold books, 21% (n=23) sold vermicomposting supplies, 3% (n=3) sold compost tea and 10% sold other products such as pictures,recipes and videos).

Figure 1. Distrubution of total earthworm products sold (N=101) on the total number of websites selling earthworm products (N=55)



*other = pictures, gummy worm recipes, earthworm bins, videos

<u>Discussion</u>

This content analysis is a preliminary investigation into the overall perspective of earthworms on the internet and phase 2 of the overall project. There was moderate intercoder reliability between the three coders but the information gathered is still useful. Discussion of the current views on earthworms and the problems that come with non-native invasive earthworms in previously earthworm free ecosystems is of value. It wasn't a surprise to see that of the websites selling earthworm products and those selling earthworms only were primarily .com's 82% and 76% respectively, compared to all websites surveyed with 53% being .com's. And of those sites selling earthworm products and earthworms only, at least 50% were considered commercial by the coders. It is interesting to note that 25% of the websites that sell earthworms only were classified as educational. This does not support the findings of Kay & Hoyle (2001) who classified websites into Regulatory, Educational, Commercial, Hobbyist, or Foreign. Out of n=1037 total internet hits, the first 100 sites that came up using the Yahoo search engine found that 61% (640) of the hits were websites categorized as "educational" and 14% (146) were regulatory websites.

Of the websites classified as selling some sort of earthworm product coders felt that 0% of the website viewed earthworms in a negative way. When it came to all websites 27% were viewed positive, 13% negative and 50% neutral. It is interesting to note that only 13% of the total websites were viewed as negative, but 61% of the total websites were classified as educational. This can be of great concern for areas that are earthworm free but have advocates for live bait for fishing, composting or gardening with earthworms.

Conclusion

What this preliminary assessment establishes is a lack of educational programs focusing on the potential negative effects of earthworms and that a common theme is that people believe earthworms are only helpful for the soil and ecosystem. This issue has been found in research looking at the spread of invasive species: limited amount of information on proper disposal of unwanted organisms indicates a need for better outreach (Walter et al. 2006); Involvement and education of consumers may provide better oversight outcomes by addressing the moral hazard problem while acknowledging the key characteristics of the industry (Drew et al. 2010); “One thing we are trying to do is educate the public not to release these into the environment." (Clayton 2004). Haska et al. (2012) found that nearly half of those surveyed did not understand the possible ecologic and economic impacts of invasive species and the environmental damage they can cause; lack of knowledge regarding identification as well as regulation of submerged species, which may then result in the unintentional trade of potentially invasive species (Martin & Coetzee 2011).

Appendix 1 – draft manuscript

III. Quantitative Assessment of Earthworms Purchased through Internet Based Vendors

This assessment of the internet as a vector of spread for earthworms in Minnesota was conducted in three phases. The first phase consisted of a literature review of current knowledge of the spread of invasive species through internet based vendors. The second phase used a content analysis approach to identifying the overall outlook of earthworms on the internet. The data gathered in the second phase informed the third phase by identifying the websites to contact and order earthworms from to identify species they are selling, how accurate they are at species identification, what they sell them as. The overall goal of the project is to assist in developing highly effective strategies for limiting the spread of established earthworms and preventing introductions of species not yet present in the state, thereby protecting native forests resources from future aesthetic, biologic and economic impacts.

This study will inform stakeholders if there are risks associated with internet based earthworm vendors as a vector for the spread of non-native earthworms. Earthworms are an e-commerce commodity used in such ways as fishing bait, lawn care, bio-remediation, composting and vermicomposting. Many of the earthworm species being sold are non-native to North America and are having negative impacts on regions formally devoid of earthworms (Holdsworth et al. 2007, Frelich et al. 2006, Hale 2008, Proulx 2003, Hendrix & Bohlen 2002, Cameron et al. 2007, Edwards & Arancon, 2006). Through e-commerce there is the potential for new earthworm species such as the Asian *Amynthas* spp. to be introduced into new environments. Websites selling *Amynthas* spp. commonly known as "Jumping Worms" promote them as "the greatest thing you can do for your yard" and "because they are so active they make great fishing bait". There is also the potential for the introduction of new genetic material being introduced to already established populations such a *Lumbricus rubellus* which is sold for bait and composting.

One of the main vectors of non-native earthworm introduction that has been studied demonstrates the direct relationship between anglers using live earthworms as bait and the introduction of non-native earthworms (Kilian et al. 2012, Keller et al., 2007, Proulx 2003, Seidl & Klepeis, 2011, Cameron et al., 2007, Hale 2008, Haska et al. 2012). In a study that used the Invasive Earthworm Rapid Assessment tool (Loss et al. 2013) to assess the impact of heavily fished Northern Minnesota lakes versus lightly fished lakes, researchers found that boat launches around heavily fished lakes were more impacted by earthworms (Palokangas et al. 2012). With the growing popularity of vermicomposting and the proliferation of internet based earthworm vendors are we on the verge of new species and genetic material being introduced via this new vector of e-commerce?

Currently, there are no known studies looking at the risk of spreading non-native earthworms via the internet. Earthworm products are sold by internet based vendors for many uses: composting, vermicomposting, lawn care, food for pets, gardening and fishing bait. Studies have been conducted on e-commerce as a pathway to the introduction of invasive species such as invasive terrestrial and aquatic plants (Kay & Hoyle 2001, Maki & Galatowitsch 2004, Walters et al. 2006, Martin & Coetzee 2011, Drew et al. 2010) and that e-commerce risks biosecurity associated with the importation via online trade of unwanted flora and fauna into the country, as well as their movement within internal borders (Derraik & Phillips 2010).

These studies have found the proliferation of hobbyist, domestic commercial, and foreign commercial websites discussing the beauty and qualities of invasive aquatic weeds thus is a very serious concern for federal and state regulatory officials as well as resource managers throughout the United States (Kay & Hoyle 2001). In Minnesota the state law prohibits purchasers and sellers from possessing, importing, purchasing, transporting, or introducing prohibited species (Minnesota Reviser of Statutes, 2000). Nevertheless, compliance with these laws is low as plants illegal to possess in Minnesota (state or federal prohibited) were sent 92% of the time they were requested. While compliance is good within the state of Minnesota, mail-order purchases may be more problematic (Maki & Galatowitsch).

Walters et al. (2006) found that from their e-commerce purchases, retailers frequently do not identify their product scientifically, by including the genus and species. And according to a featured article in the Christian Science Monitor "The Internet sales of plants and other organisms are quite a large and there's little if any regulation," says Ted Grosholz, an ecology professor at the University of California at Davis. Marshall Meyers, executive vice president of the Pet Industry Joint Advisory Council, says "Internet sales can be a real problem…you can buy giant salvinia over the Internet out of Europe. [But] this is an area that's confusing to the public. Not all nonnative species of fish and plants are invasive. Our industry relies on nonnative species. One thing we are trying to do is educate the public not to release these into the environment." (Clayton 2004).

<u>Summary of Earthworm Composting Seller study</u>

The purpose of this assessment of internet based earthworm vendors was to assess the risk of introduction of non-native earthworms. Six earthworm species have been identified as potentially the most useful species to break down organic wastes. These are *Eisenia fetida* (and the closely-related *Eisenia andrei*), *Dendrobaena veneta*, and *Lumbricus rubellus* from temperature regions and *Eudrilus eugeniae*, *Perionyx excavatus*, and *Perionyx hawayana* from the tropics. Other species can be used but these species are the commonest (Edwards & Arancon, 2006). This is worrisome as *L. rubellus* is associated with some of the biggest negative effects in northern hardwood forests and *Amynthas* has been documented to have an established population in St. Paul (unpublished data).

Methods

Fourteen sellers of earthworms via the internet were identified from phase 2 the content analysis of earthworms on the internet. Each internet seller was contacted and orders were made online. To keep anonymity orders were made with a personal name not the name of the research organization. Researchers ordered the smallest quantities available. From each earthworm seller we purchased all earthworm species available that were in stock. Once earthworm samples were delivered GLWW staff collected specimens and hand sorted them looking for species that were different (size, color and activity) and then did a hand grab of the rest of the samples and put them into formalin (a cellular fixative) for 48 hours and then sample were moved into 70% isopropyl alcohol for long term storage. GLWW staff identified earthworms down to species where possible. All data was entered into an excel database

Sample

We purchased the smallest amount of earthworms possible from each of the 14 internet vendors. The quantities offered by vendors can be classified in two categories, by the pound and earthworm counts. Researchers ordered a range of samples sizes from ½ lbs to 2lbs and 100 to 1000 count of earthworms. There was a total of N= 14 internet sellers contacted and researchers ordered a total of 33 samples. The amount of different types of earthworms sold by vendors varied: 2 sellers (14%) sold only one type of worm, 6 sellers (43%) sold two types of earthworms, 5 sellers (36%) sold three types of earthworms and 1 seller (7%) sold four earthworms. The mean number of earthworm products offered for sale was 2.4 with the max number was 4 and minimum order available was 1. Of the 33 samples ordered n=4294 earthworms were persevered for identification. Sixty one percent (n=20) of earthworms samples were identified to genus and species by vendors on their websites.

Results

Earthworm Shipping Locations from the 14 Internet Based Vendors

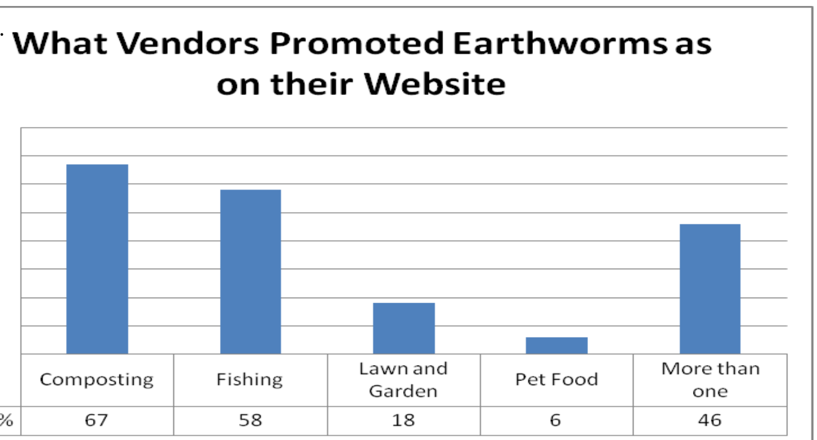
Of the 14 internet sellers, 91% of the samples (n=33) were delivered from out of the state of Minnesota. Earthworm samples were received from nine different states (see Figure. 1) California (n=8), Florida (n=6), Indiana (n=1), Kentucky (n=1), Minnesota (n=3), Pennsylvania (n=7), South Carolina (n=4), Vermont (n=1) , and Wisconsin (n=2).

Fig. 1
Earthworm Shipping Locations for the 14 Internet Based Earthworm Sellers

How are Earthworms being Advertised for Sale via the Internet

Of the 33 earthworm samples purchased 58% were sold as fishing bait as one of the uses, 67% sold as composting worms as one of the uses, 15% lawn a garden as one of the uses, 6% pet food as one of the uses (see Figure 2).

Figure 2.



How Accurate are Vendors in their Identification of Earthworm Species

Thirty nine percent of the earthworm vendors did not list species or genus. Of the N=33 orders from internet earthworm sellers, 27% (n=9) were the species they claimed to be, 33% (n=11) had different species from the same genus and 40% (n=13) had species from a different genus. Eighteen percent (n=6) of the total orders contained *Amynthas* spp. with 2 vendors or 6% of total earthworm orders spp. actually advertised selling *Amynthas* spp. Looking into it further, of the n=1742 specimens that where identifiable to species 39% were misidentified by the vendors (see Figure 3).

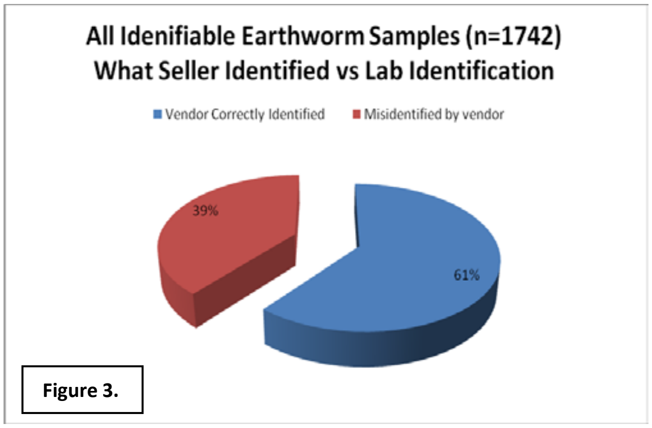


Figure 3.

Discussion

Only 27% of the orders turned out to contain the species that the sellers claimed to sell. This means that 73% of the orders received had different species than what was ordered and 12% of those orders were contaminated by the species *Amynthas.* Thirty nine percent of the earthworm vendors did not list species or genus which is consistent with other research (Walters et al. 2006) that have shown vendors to have a poor record in using scientific names to correctly identify the species they are selling.

Although the sample size of internet earthworm vendors surveyed was relatively small they came from multiple states and all species ordered were non-native to the Great Lakes Region. We were able to order everything we wanted including *Amynthas* spp., *Lumbricus rubellus* and *L. terrstris*. The *Amynthas* spp. are a relatively new species that are not widely established yet in the Western Great Lakes, and their potential impacts are still unknown. Research does show that

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L.rubellus and *L. terrestris* are widely established and are associated with what researchers have coined the forest decline syndrome (Frelich et al. 2008). This could have negative impacts on the ecosystems in the Great Lakes Region and we suggest the following regulatory actions for internet based earthworm trade.

Researcher did not find any information from the vendors warning customers about the potential risk non-native earthworms can have on native ecosystems . This issue has been found in research looking at the spread of invasive species: limited amount of information on proper disposal of unwanted organisms indicates a need for better outreach (Walter et al. 2006); Involvement and education of consumers may provide better oversight outcomes by addressing the moral hazard problem while acknowledging the key characteristics of the industry (Drew et al. 2010); "One thing we are trying to do is educate the public not to release these into the environment." (Clayton 2004). Haska et al. (2012) found that nearly half of those surveyed did not understand the possible ecologic and economic impacts of invasive species and the environmental damage they can cause; lack of knowledge regarding identification as well as regulation of submerged species, which may then result in the unintentional trade of potentially invasive species (Martin & Coetzee 2011).

There is a need for greater awareness of the economic and environmental impacts of invasive species leading many industries and countries to consider how voluntary industry practices, regulatory risk assessments and quarantine measures could be modified to reduce the risks of further harm. Any efforts to reduce invasion risks from trades in live organisms will require retailers and government agencies to know the scientific names of the species sold this suggests that the most effective way to ensure that all species in trade are accurately identified may be through certification programs requiring accurate identifications, and regulations and penalties to encourage accurate identifications by wholesalers (Keller et al. 2007).

Furthermore, better communication between regulators and vendors and less confusion about current state and federal laws may decrease the rate of sale of prohibited aquatic plants. However, because state borders are not policed like national borders, state regulations prohibiting groups of species may not be as effective as federal regulations (Maki and Galatowitsch 2004). And according to Keller and Lodge (2007) there are three options for policy approaches that could be adopted: first, allow any and all new species; second, allow no new species; or third, prescreen species for the likelihood of becoming invasive and allow in trade those that pose low risk.

Conclusion

Looking at vectors of earthworm introductions this study demonstrates that internet based earthworm vendors are a viable source for the introduction of earthworms. This highlights the continued need for outreach and education about the effects non-native earthworms can have on the ecosystems they are introduced into. Not only for the general public but for the vendors that are selling earthworms through interstate e-commerce. With this research in hand the next steps are to develop regulations to address the potential threat internet based vendors will contribute to the continued introduction of non-native earthworm species throughout North America.

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APPENDIX 1: Amynthas Internet Search Protocol

STEP 1: Internet search standardizations

- Use the Internet Explorer browers (not Firefox or any other browser)
- Use Google search Engine (not Yahoo or Bing or any other search engine).
- Follow the step outlined in the "Content Analysis Search Protocol" below.
- Search for each of the key words listed below within a single 24 hour period to establish what 10 sites will be eventually be surveyed in relation to each key word (step 2 below).

Key Words:

Earthworms	Invasive earthworms
Exotic earthworms	Non-native earthworms
Vermicompost	Worms for Vermicompost
Composting	Compost earthworm
Compost worm	Amynthas
Alabama jumpers	Super reds
Dirt worms	Jumping worms
Earthworms information	Red wigglers
Big earthworms	Earthworm ecology
Amynthas ecology	Exotic earthworm ecology
Invasive earthworm ecology	Invasive Amynthas earthworm
Earthworm information	Amynthus information
Asian earthworm information	

STEP2: Content Analysis Survey information

Using the Content Analysis Survey Instrument (see below), for each of the 10 sites:

- Coder write their name in upper left box
- Write date in upper right box
- Write key word being searched in appropriate box
- Write the name of the website and it's full URL (for example: <http://www.duluth.umn.edu/ibs/IBS/ContactUs.htm>)
- In the appropriate box, based on your reading of the content of this page identify:
- it as and Educational, Commercial, Personal, other. List any thematic words that led to y our impression of the site (i.e. Educational = "research says"; Commercial = "sale!"; Pers onal = "my experience was…")
- if the overall message was positive, negative, neutral, other. List any thematic words that led to your impression of the site (i.e. positive = "increase soil fertility"; negative= "h arm"; neutral = "impact (can be positive or naegative)")
- indicate the nature of the domain e.g. ".org", ".edu", ".com", ".net", other
- List any earthworm species, genera and/or common names mentioned on the site
- List any products they sell
- Does the webpage have links to other earthworm related pages? If so list the URL of up 5 external links from this site.
- Note the total amount of time spent on each site (so we can document the level of standardization for the search efforts)

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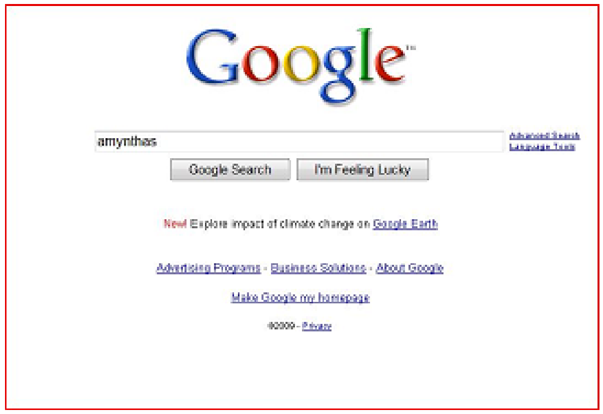
APPENDIX 1: Amynthas Internet Search Protocol

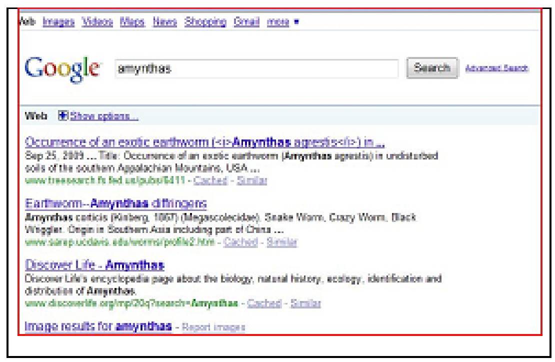
CONTENT ANALYSIS SEARCH PROTOCOL

STEP 1: Internet search standardizations

1) Go to Google home page and type in "Key Word" and hit Google Search.



2) Left click and highlight the first ten search results.

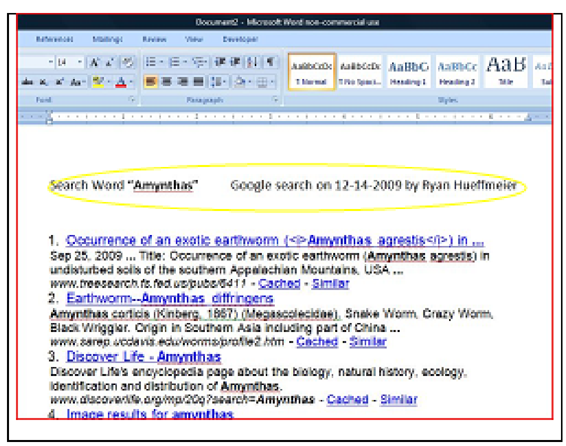


CONTENT ANALYSIS SEARCH PROTOCOL

3) Copy and paste search results into a word document; title the document with the search term, search engine, date of search and researchers name. For example:

"Amynthas-Google-Dec12-09-Ryan.doc"

(do not save in .docx format)

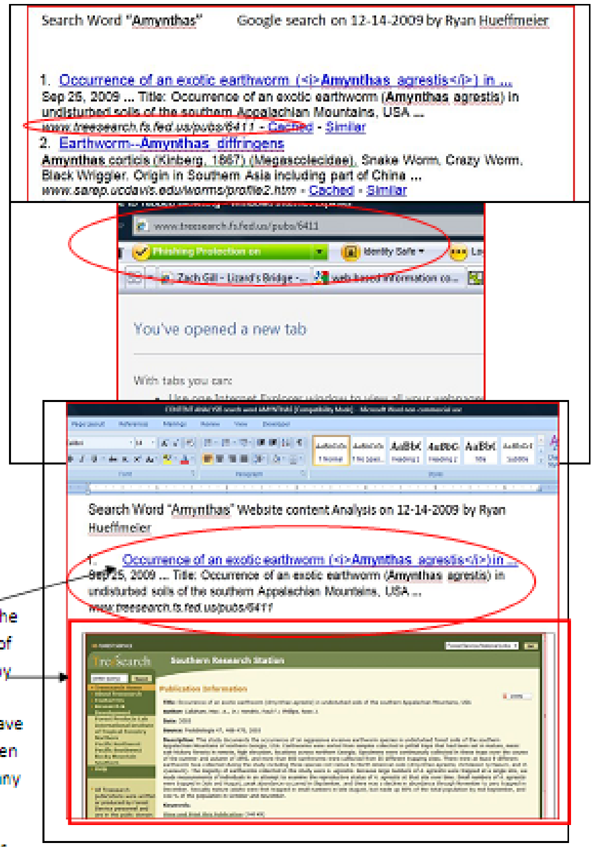


STEP 1 is complete…

CONTENT ANALYSIS SEARCH PROTOCOL

STEP 2: Content Analysis Survey information

1) Copy and paste the URL into Google. So that the first link from the top 10 search results is now on screen.



2) In your Search word document created in Step 1 (example - "Amynthas-Google-Dec12-09-Ryan.doc"), create a heading with the name for this site (ie. "Occurrence of an exotic earthworms…"). Then copy and paste the screenshot(s) of the main page for that site. You may have to scroll down and do multiple screen shots to get the full main page for any given site.

Do the same for all 10 search results.

3) Using the main page only from the link you are examining (do not click on any other pages within the site or links from it), fill out the Content Analysis Survey Instrument.

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APPENDIX 1: Amynthas Internet Search Protocol

Content Analysis Survey Instrument - Amynthus internet searches

Coder:___________________________________ Key Word:

Date:____________________________________ ____

Name of Website: ____ Website http:	1) Domain (Circle one): .org .com .edu .net **Other:**
2) Institution (Circle one): **Educational** **Commercial** **Personal** **Other:** **List Theme Words:**	3) View of Earthworms (Circle one): **Positive** **Negative** **Neutral** **Other:** **List Theme words:**
4) Earthworm names (list all present on site): **Species:** ____ **Genus:** ____ **Common names listed:** **NONE LISTED**	5) Earthworm products (Circle all that apply): **Live Earthworms** **Earthworm cocoons** **Earthworm casts** **Bait** **Outside Composting Supplies** **Books** **Vermicomposting Supplies** **Compost tea** **Other:**

6) External links to additional earthworm sites (List Links):

I.____

II.____

II.____

IV.____

V.____

7) Total amount of time spent on this site:

Other Comments:

APPENDIX 2: Major Taxa Recorded from Conducting Content Analysis

Major Taxa	Species		Common Names
Amynthos spp. (29)	*A. agrestis* (16) *A. gracilis* (8)[†] *A. corticis* (7) *A. hawayanus* (5)[††] *A. hilgendorfi* (3) *A. diffringens* (3) *A. hupeiensis* (1) *A. minimus* (1) *A. morrisi* (1)	*A. rodercensis* (1) *A. pavimentus* (1) *A. biorbis* (1) *A. libratus* (1) *A. hongyehensis* (1) *A. alexandri* (1) *A. aspergillum* (1) *A. mekongianus* (1)	Asian Jumping worms ***Alabama Jumpers***[†] Georgia jumpers Snake worm Crazy worm ***Super reds***[††] Black wriggler ***European nightcrawlers***[†††]
Eisenia spp. (0)	*E. foetida* (27) *E. fetida* (12) *E. hortensis* (5) *E. andrei* (4)		Red wiggler, Red hybred, Red worm, Tiger worms, Brandling worm, Manure worm, California Redworm, ***Spikes Tail***[††††], California golden giant, Florida wiggler, Brown nose, Japanese Tiger, California super red, Garden dirt worms, Compost worms, Red tiger worm, Red tiger, ***European Nightcrawlers***[†††], ***African Nightcrawler***[†††††], ***Alabama jumpers***[†], Belgium nightcrawler, ***super red***[††]
Aporrectodea spp. (1)	*A. trapezoides* (7) *A. tuberculata* (6) *A. longa* (2)[†††]	*A. caliginosa* (1) *A. rosea* (1)	Grey worm, Black headed worm, Canadian worm, Rosey tipped worm
Allolobophora spp. (2)	*A. chloritica* (4) *A. caliginosa* (1)	*A. longa* (1)[†††]	Green worm, field earthworm
Pheretima spp. (2)	*P. hawayana* (2)[†]	*P. hawayanus* (1)[††]	The lazy mans worm
Octolasion spp. (0)	*O. tyrtaeum* (6) *O. cyaneum* (2)		Woodland white worm, Field worm, Blue-grey worm, Grey field worm
Lumbricus spp. (0)	*L. terrestris* (31) *L. rubellus* (24)		Red marsh worm, red wiggler, Dew worm, Canadian nightcrawler, nightcrawler, Common nightcrawler
Megascolex spp. (0)	*M. mekongianus* (1)		Giant Mekong river earthworm
Dendrobaena spp. (0)	*D.octaedra* (12)		Small leaf worm
Driloleirus spp. (0)	*D. americanus* (2)		Giant Palouse earthworm
Perionyx spp. (0)	*P. excavatus* (6)		Blue worm, India blue worm, Malaysian blue worm, Traveling worm, ***spikes tail***[††††]
Bimastos spp. (0)	*B. parvus* (1)		American bark worm
Eudurilus spp. (0)	*E. eugeniae* (5)		***African nightcrawler***[†††††]
Microscolex spp. (0)	*M. dubius* (4)		

[†]Highlights scientific and common names that get attributed to multiple Genus and Species found during content analysis

Table X. Results of protocol-based internet searches assessing the website content of 250 sites that sell earthworms, including the complete list of common names found. The number of sites that provided identifiers of a given genus or species and the number of sites where no genus of species were provided are indicated below in parentheses.

Genus	Species	Common Names
Unidentified (147)	Unidentified (11)	Grey nightcrawler, Leaf worm, Angle worm, Native earthworm, exotic earthworm, T-worms, Common field worm, Giant Gippsland, Hammerhead, Jumping worms
Amynthas spp. (29)	*A. agrestis* (16) *A. gracilis* (8)[*] *A. corticis* (7) *A. hawayanus* (5)[*] *A. hilgendorfi* (3) *A. diffringens* (3) *A. hupeiensis* (1) *A. minimus* (1) *A. morrisi* (1) *A. rodercensis* (1) *A. pavimentus* (1) *A. biorbis* (1) *A. libratus* (1) *A. hongyehensis* (1) *A. alexandri* (1) *A. aspergillum* (1) *A. mekongianus* (1)	Alabama Jumpers[†] Asian Jumping worms[†] Black wriggler Crazy worm European nightcrawlers[†] Georgia jumpers Snake worm Super reds[†]
Eisenia spp. (0)	*E. foetida* (27) *E. fetida* (12) *E. hortensis* (5) *E. andrei* (4)	Alabama jumpers[†], African Nightcrawler[†], Belgium nightcrawler, Brandling worm, Brown nose, California golden giant, California Redworm, California super red, Compost worms[†], Florida wiggler, Garden dirt worms, Japanese Tiger, Manure worm[†], Red tiger worm, Red tiger, European Nightcrawlers[†], Red hybrid, Red wiggler[†], Red worm, Spikes tail[†], Super reds[†], Tiger worms
Aporrectodea spp. (1)	*A. trapezoides* (7) *A. tuberculata* (6) *A. longa* (2)[*] *A. caliginosa* (1) *A. rosea* (1)	Black headed worm, Canadian worm, Grey worm, Rosey tipped worm
Allolobophora spp. (2)	*A. chloritica* (4) *A. caliginosa* (1) *A. longa* (1)[*]	Green worm, Field earthworm
Pheretima spp. (2)	*P. hawayana* (2)[*] *P. hawayanus* (1)[*]	The lazy mans worm
Octolasion spp. (0)	*O. tyrtaeum* (6) *O. cyaneum* (2)	Blue-grey worm, Field worm, Grey field worm, Woodland white worm
Lumbricus spp. (0)	*L. terrestris* (31) *L. rubellus* (24)	Canadian nightcrawler[†], Nightcrawler[†], Common nightcrawler, Dew worm, Red marsh worm, Red wiggler[†]
Megascolex spp. (0)	*M. mekongianus* (1)	Giant Mekong river earthworm
Dendrobaena spp. (0)	*D.octaedra* (12)	Small leaf worm
Driloleirus spp. (0)	*D. americanus* (2)	Giant Palouse earthworm[†]
Perionyx spp. (0)	*P. excavatus* (6)	Blue worm, India blue worm, Malaysian blue worm, Spikes tail[†], Traveling worm
Bimastos spp. (0)	*B. parvus* (1)	American bark worm
Eudurilus spp. (0)	*E. eugeniae* (5)	African nightcrawler[†]
Microscolex spp. (0)	*M. dubius* (4)	

[†]Common names provided from vendors that were variously attributed to multiple genus and species or not attributed to any genus and species.

[*]Species names that were attributed to multiple common names.

Table Y. Results of the identification of 38 batches of earthworms purchased from 14 different vendors via the internet. Samples were purchased from vendors that came up more than once as sources for live earthworms during the 250 site internet content analysis (Appendix 1 and Table X). For each vendor, all different types of earthworms they sold were purchased and identified by Great Lakes Worm Watch staff. In each row, the specific vendor is identified by the number and the specific earthworm product that we sampled from that vendor is identified by a letter.

Vendor and product ID	Common names listed by Vendors	Earthworm Uses Marketed by Vendors	Scientific Name Provided By Vendors	Species Identified by GLWW Staff
1a.	Redworms, Red Wigglers	composting, pet food	*Eisenia fetida*	*Eisenia fetida*
1b.	European Nightcrawler	fishing, pet food	No scientific name provided	*Eisenia fetida* *Eisenia hortensis*
2	Red Hybrid Earthworms	composting	No scientific name provided	*Eisenia fetida*
3	Red Wigglers, Red worms	composting	*Eisenia foetida**	*Eisenia fetida* *Perionyx excavatus*
4a.	Red worms, Red Wigglers	fishing, composting	*Eisenia fetida*	*Eisenia fetida*
4b.	European Nightcrawler	fishing	*Eisenia hortensis*	*Eisenia fetida* *Eisenia hortensis*
5a.	Red Wigglers	composting	*Eisenia foetida**	*Eisenia fetida*
5b.	Euro Nightcrawler, Super Red worms	fishing	No scientific name provided	*Eisenia fetida*
6a.	Africans, Red wigglers, Eisenias	garden	No scientific name provided	*Amynthas* spp. *Eisenia fetida* *Eudrilus eugeniae* *Perionyx excavatus*
6b.	African Nightcrawler	fishing, composting	*Eudrilus eugeniae*	*Eisenia fetida* *Eisenia veneta* *Perionyx excavatus*
6c.	Hybrid Red Wigglers	fishing	*Amynthas (Pheretima) hawayanus*	*Amynthas* spp.
6d.	Eiseni	composting	*Eisenia foetida**	*Amynthas* spp. *Eisenia fetida* *Eisenia veneta* *Eudrilus eugeniae* *Perionyx excavatus*

Vendor and product ID	Common names listed by Vendors	Earthworm Uses Marketed by Vendors	Scientific Name Used By Vendors	Species Identified by GLWW Staff
7a.	Red Worms, Red Wigglers, Brandling Worms, Manure Worms, Trout Worms, Tiger Worms	composting	*Eisenia fetida*	*Eisenia fetida* *Perionyx excavatus*
7b.	European Nightcrawler, Belgian Nightcrawler, Euro's, ENC's	composting, fishing	*Eisenia hortensis*	*Eisenia hortensis* *Eisenia fetida*
8a.	Brandling worms, Tiger Worms, Red Wigglers	composting	*Eisenia fetida*	*Eisenia fetida* *Eisenia hortensis* *Perionyx excavatus*
8b.	European Nightcrawler, Belgian Worms	composting, fishing	*Eisenia hortensis*	*Eisenia fetida* *Eisenia veneta* *Eisenia hortensis*
9a.	Red worms, Red Wigglers	composting	No scientific name provided	*Eisenia fetida* *Perionyx excavatus*
9b.	Red worms, Bait worms	fishing	No scientific name provided	*Eisenia fetida* *Eisenia hortensis* *Perionyx excavatus*
10a.	Canadian Nightcrawler	fishing	No scientific name provided	*Lumbricus terrestris*
10b.	Red worms, Red Wigglers, Sunfish worms, Fishing worms, Tiger worms, Hybrid Reds, Manure worms	composting	No scientific name provided	*Eisenia fetida*
10c.	European Nightcrawler, Jumbo Redworms, Panfish worms, Trout worm, Leaf worm, Belgian Nightcrawler, Euro's, Pan fish worms	fishing, composting	*Eisenia hortensis*	*Eisenia fetida* *Eisenia hortensis*
11a.	Alabama Jumpers	Fishing, yard	No scientific name provided	*Amynthas* spp.

Appendix 1 –Table y

Vendor and product ID	Common names listed by Vendors	Earthworm Uses Marketed by Vendors	Scientific Name Used By Vendors	Species Identified by GLWW Staff
11b.	European Nightcrawler	composting, fishing	*Eisenia hortensis*	*Eisenia hortensis*
11c.	Redworms	composting	*Eisenia fetida*	*Eisenia fetida*
12a.	Red Wigglers, Redworms, Manure Worms	composting	*Eisenia fetida*	*Eisenia fetida*
12b.	European Nightcrawler	fishing	*Eisenia hortensis*	*Eisenia fetida* *Eisenia veneta* *Eisenia hortensis*
12c.	Dendras	fishing	*Dendrobaena veneta* (*Dendrobaena hortensis*)	*Eisenia fetida*
13a.	Manure worm, Red Wiggler, Tiger worm	lawn, garden	*Eisenia fetida*	*Eisenia fetida* *Eisenia hortensis*
13b.	European Nightcrawler	lawn, garden	*Dendrobaena veneta*	*Eisenia fetida* *Eisenia hortensis*
13c.	Common Pasture Worm	lawn, garden	*Aporrectodea caliginosa*	*Eisenia fetida* *Eisenia hortensis*
13d.	Red Tiger Worm	lawn, garden	*Eisenia andrei*	*Eisenia fetida* *Eisenia hortensis*
13e.	Dew-worm, Angle worm	lawn, garden	*Lumbricus terrestris*	*Eisenia fetida* *Eisenia hortensis*
13f.	Red Marsh Worm	lawn, garden	*Lumbricus rubellus*	*Eisenia fetida* *Eisenia hortensis*
13g.	Red Wrigglers	composting, lawn, fishing	No scientific name provided	*Eisenia fetida* *Eisenia veneta* *Eisenia hortensis* *Perionyx excavatus* *Amynthas* spp.
13h.	Super Red Worms	composting, lawn, fishing	No scientific name provided	*Eisenia fetida* *Eisenia hortensis*
14a.	Red Wigglers	composting	No scientific name provided	*Amynthas* spp. *Eisenia fetida*

Vendor and product ID	**Common names listed by Vendors**	**Earthworm Uses Marketed by Vendors**	**Scientific Name Used By Vendors**	**Species Identified by GLWW Staff**
14b.	European nightcrawler, European red worms, Belgium red worms	compost, fishing	*Eisenia hortensis*	*Eisenia veneta* *Eisenia hortensis*
14c.	Red Wigglers, European Nightcrawler	fishing, composting	No scientific name provided	*Eisenia fetida* *Eisenia veneta* *Eisenia hortensis* *Perionyx excavatus*

* *Eisenia foetida* is a synonym for *Eisenia fetida*

JUMPING WORM ALERT!

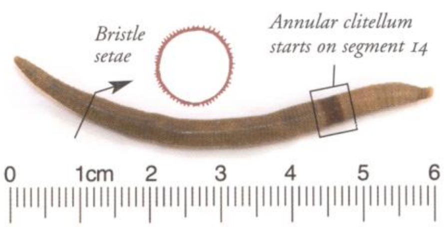
All earthworms in the Great Lakes Region are non-native species, brought over from Europe during early colonization of the United States. But there is a new invasive species causing alarm for the native environment.

Asian earthworms (genus: *Amynthas*) are becoming a threat in Minnesota. They are also known as Jumping Worms because they are very active and hyper -- very non-traditional earthworm behavior.

The *Amynthas* species has an extraordinarily high metabolism and they can live in very high densities. If they become established, their impact on our native ecosystems could be catastrophic.

Amynthas is a common contaminant when you buy "Red Wigglers" for vermi-composting which can lead to the introduction of this species to the wild. To identify *Amynthas*, or to report an infestation, see the Great Lakes Worm Watch website.

www.GreatLakesWormWatch.org



Amynthas: Asian "Jumping Worm"

What can you do to reduce the spread of non-native earthworms?

- **Do not** dump in woods or water. Earthworms don't drown!
- **Do** toss unwanted bait in the trash.
- **Do** tell others about the problems caused by invasive earthworms.
- **Do not** transport leaves, mulch, compost, or soil from one location to another unless certain there are no earthworms or cocoons present.
- **Do** freeze the vermicompost for at least one week before putting it in your garden or other outside environment. This kills the earthworms and egg cocoons.

Support and funding provided by:

University of Minnesota Duluth
Natural Resources Research Institute
Environment and Natural Resources Trust Fund

For questions about the Great Lakes Worm Watch or research contact:

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ABC's of Composting with Earthworms Safely

An introduction to vermicomposting and understanding earthworm use in Minnesota and the Great Lakes Region

Great Lakes Worm Watch

www.GreatLakesWormWatch.org

VERMICOMPOSTING 101

Vermicomposting is similar to traditional microbial/bacterial composting, except that earthworms are added. Together they convert organic waste to nutrient rich compost.

Red Wigglers (*Eisenia foetida)* are the most common worms used in vermicomposting, Red Wigglers are great compost earthworms for northern climates because they do not survive cold winters and are not invasive in the Great Lakes region.

But, several other species are also called Red Wigglers or Red Worms such *Lumbricus rubellus* (sold for bait as Leaf Worm or Beaver tails) and increasingly, the Asian species in the genus *Amynthas*, also called Jumping Worms. These species survive cold winters and can be very detrimental to native forests. They can unintentionally contaminant uncontained vermicompost piles.

HOW DOES VERMICOMPOSTING WORK?

Vermicomposting is done in plastic containers or wooden boxes where earthworms decompose organic material. The earthworm excrement, or cast material, is what creates nutrient-rich finished compost.

EARTHWORM SAFETY:

What you need to know about vermicomposting risks

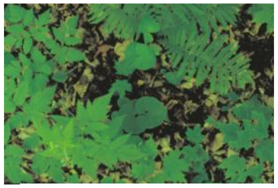
All earthworms in the Great Lakes Region are non-native. Most of the earthworms you know and love are European in origin.

Earthworms are beneficial in artificial environments -- agriculture and gardens -- they can help water move through soil and incorporate organic material to make nutrients more available to plants. *But,* earthworms are *not* good in natural hardwood forests.

Once they invade a native forest, earthworms mix the duff layer into the mineral soil, changing the structure, chemistry and biology of living organisms in the soil.

Duff is the top layer of thick, spongy, decomposing material found on forest floors. It is very important for seedling growth and understory vegetation.

Different species of earthworms have different effects on native forest ecosystems. European earthworms have negative impacts, but the newly arriving Asian species, *Amynthas,* has a particularly strong negative force on native forests and plant communities.



Forest floor: Before

Forest floor: After

CAN I VERMICOMPOST SAFELY?

To prevent accidental introduction of new earthworm species, consider these three things when creating a vermicompost pile.

Where does the material come from?
a. How confident are you that it doesn't contain earthworms or their cocoons?
b. Is it looked at or monitored as it arrives?

How disciplined is on-site management?
a. Is the compost contained or is a barrier in place to prevent earthworms from entering/exiting the material?
b. Do you know what species of earthworms you have in your compost and in the area surrounding your compost site?

How does the compost leave the site?
a. Is the compost frozen, or otherwise treated, to remove or kill all earthworms and their cocoons before being introduced into an outside environment?

Prevent earthworms, and their egg cocoons, from being introduced to a natural environment!

Non-native Earthworms Transported on Treads of ATVs and Logging Equipment in Northern Hardwood Forests of Minnesota, USA

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Abstract: Forests of glaciated regions of North America evolved over thousands of years in the absence of earthworms. Multiple species of European and Asian earthworms now exist in northern forests across the Great Lakes region. Abundant and ecologically diverse communities of non-native earthworms are altering the health and functioning of northern hardwood forests in Minnesota. Humans are a primary source of introduction and spread of these invasive species. This study quantified the relative risk of earthworm spread resulting from soil transport via of all-terrain vehicles (ATVs) and logging equipment in sugar maple (*Acer saccharum*) dominated hardwood forests of the Chippewa National Forest, Minnesota, USA. Soil collected from tires and underbodies of ATVs and logging vehicles were found to contain significant numbers of live earthworms and viable earthworm cocoons. It was concluded that ATV travel and logging activity may be an important vector of continued introductions and spread of invasive earthworms in our region. The greatest threat comes from the transport of earthworm invaded soil picked up on treads and vehicle bodies which move further into the forest from invasion fronts or that are subsequently transported to other sites. We recommend a combination of operator education, equipment hygiene, and land-use and management policies to limit the introduction and spread of non-native earthworms through soil transport by off-road vehicles and logging equipment.

Introduction

Forests of glaciated regions of North America evolved over thousands of years in the absence of earthworms. As human activity expanded into these areas from other continents and parts of North America that had not been subjected to the geologic effects of glaciers, non-native earthworms were introduced (Frelich et al. 2006). Research has demonstrated that multiple species of European and Asian earthworms representing several ecological groups now exist in northern forests across the Great Lakes region (Holdsworth et al. 2007b). The abundance and diversity of these non-native earthworms varies by forest type, soil type, and human activity. Abundant and ecologically diverse communities of earthworms are altering the health, composition, and functioning of northern hardwood forests in Minnesota (Hale et al. 2006; Holdsworth et al. 2007a). Humans continue to introduce non-native earthworms through improper disposal of fishing bait and inadequate containment of compost (Hale 2008). In this study we test hypotheses that earthworms are spread on tire treads of all-terrain vehicles (ATVs) and logging equipment. We identified eight study sites in northern hardwood forests that are invaded by earthworms. We conducted experimental three-kilometer runs of a cleaned ATV on three ATV/snowmobile trails in north-central Minnesota during summer and fall of 2011. After each run, we collected the entire volume of soil from the tires and quantified the earthworms and earthworm cocoons per sample. In cooperation with active logging crews on the US Forest Service Chippewa National Forest (CNF), we collected approximately two gallons of soil from tires of logging vehicles after day-long, normal operations. We quantified earthworms and earthworm cocoons per sample. This observational study was conducted at five sites during summer and fall of 2011. Our overall objective is to quantify the relative risk of ATVs and logging equipment as vectors of earthworm spread so that recommendations aimed at reducing the impact of non-native earthworms may be developed (Callaham et al. *in press*).

Methods

All-terrain vehicles

We identified three study sites that were dominated by *Acer saccharum* Marsh. (sugar maple), invaded by earthworms, and included a well-used ATV trail at least 1.6 km long with fairly consistent trail conditions (Figure 1, Table 1). The extent of earthworm invasion was determined using the Invasive Earthworm Rapid Assessment Tool (IERAT; Hueffmeier 2012; Loss et al. *in press*). Selected sites were on the Leech Lake Reservation, within the CNF, in north-central Minnesota (47°38'N, 94°03'W).

We conducted the experiment using a single ATV that was washed prior to each run so that tire treads and surfaces were free of soil and debris (Figure 2). We trailered the clean, dry vehicle to the study sites and acquired samples by driving the ATV approximately 8-16 km h^{-1} for 1.6 km down the trail and back (n = 16 $site^{-1}$: one sample $visit^{-1}$ x 16 visits $site^{-1}$; approx. one visit $week^{-1}$ from 29 July to 31 October 2011). We documented weather conditions beginning two weeks prior to the first sampling and classified each sample as collected under dry, moist, or wet conditions (for this study, dry = no rain for seven or more previous days; wet = 2.5 cm or more of rain in previous 48 hours; moist = intermediate conditions).

We collected and processed the entire volume of soil on the ATV tires. We determined field weight, homogenized and inspected each sample for earthworms and earthworm cocoons, and transferred sub-samples to determine soil water content (WC; 60°C oven for 48 hours or until weight no longer decreased; WC = field wt – dry wt/ field wt). Using dissecting tools, we collected all earthworms and earthworm cocoons from the bulk samples. Collections were deposited into isopropyl alcohol then stored in formaldehyde. We conducted exploratory analyses of the data and non-parametric tests (Excel 2010; QI Macros for Excel, KnowWare International, Inc., Denver, CO 80224, USA).

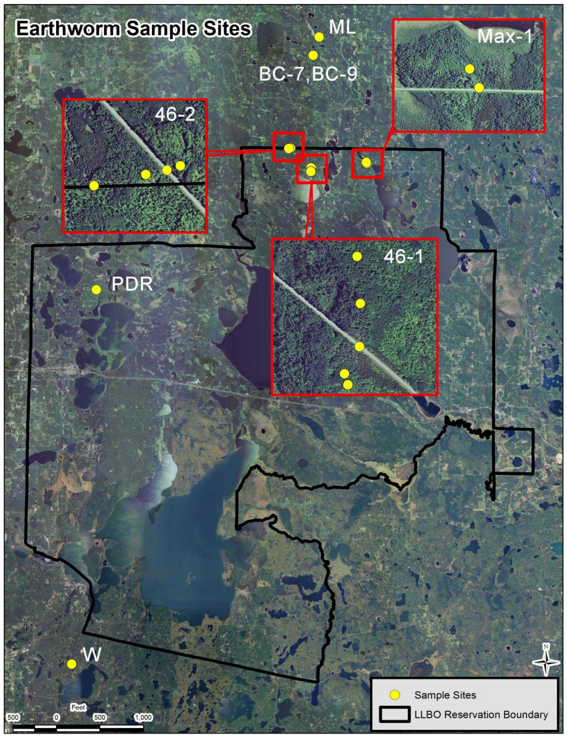

Figure 1. Study sites dominated by *Acer saccharum*, invaded by earthworms, and including a well-used ATV trail or an active logging site in north-central Minnesota, USA.

Table 1. Earthworm vector study sites in north-central Minnesota, USA.

Site	GPS Coordinates		Proximity	IERAT	Vegetation by dominance	Comments
Popple River (46-1)	15T 0413361	5277409	Squaw Lake, MN	2	P. trem, P. grand, Q. rubra, A. sacc, A. rubr, Q. macro, P. glauca, A. balsam, B. papy, C. pensyl	ATV, snowmobile trail
	15T 0413365	5277585		4		
	15T 0413352	5277778		5		
	15T 0413300	5277298		4		
	15T 0413314	5277253		3		
LLR Boundary (46-2)	15T 0410385	5280613	Squaw Lake, MN	4	A. sacc, P. trem, T. amer, C. pensyl	ATV, snowmobile trail
	15T 0410432	5280628		4		
	15T 0410311	5280598		4		
	15T 0410130	5280558		4		
S Rice Lake (Max-1)	15T 0421340	5278414	Max, MN	4	A. sacc, O. virg, B. papy, A. balsam, P. trem, P. grand, C. pensyl, E. scirp	ATV, snowmobile trail
	15T 0421273	5278549		4		
Welch Lake – Eel Lake Impoundments (BC-7 and BC-9)	15T 0413637	5293977	Alvwood, MN	2-3	A. sacc, B. papy, O. virg, T. amer	Select cut (FR 2444 - FR 3355)
Moose Lake/ S Big Calf Lake (ML)	15T 0414508	5296638	Alvwood, MN	2-3	A. sacc, B. papy, P. trem, P. strob, A. balsam, Q. rubra	Select cut
Power Dam Rd (PDR)	15T 0382473	5260201	Bemidji, MN	4	A. sacc, T. amer, P. trem, P. grand, O. virg, B. papy	Select cut
Ten Mile Lake (W)	15T 0378932	5206230	Walker, MN	4	A. sacc, T. amer, O. virg, P. trem, P. resin, P. bank	Clearcut

Figure 2. All-terrain vehicle used to experimentally test the hypothesis that earthworms may be transported via ATV treads.

Logging vehicles

With the assistance of CNF staff, we identified five active logging sites that were dominated by sugar maple, invaded by earthworms, and being logged during the summer or fall of 2011 (Figure 1, Table 1). The timber contracts required that all logging vehicles be cleaned of soil and debris prior to entering the sites. We collected soil samples from the vehicle tires in late evenings following normal harvest operations (n = 3 - 22 site^{-1} depending on size of harvest; 49 samples total were collected from 28 July to 1 November 2011). We classified each sample as collected under dry, moist, or wet conditions as described above.

We collected and processed approximately two gallons of soil from logging vehicle tires per sample. We determined field weight, homogenized and inspected each sample for earthworms and earthworm cocoons, and transferred sub-samples to determine soil water content as described above. We collected and handled all earthworms and earthworm cocoons from the bulk samples as described above. We conducted exploratory analyses of the data and non-parametric tests.

Results

Soil collected from tires of the ATV and logging vehicles contained earthworms and viable earthworm cocoons. All samples were collected under moist conditions except for two dry and three wet days of the ATV study and three wet days of the logging vehicle study. Water content of the collected soil was normally distributed (Mean ± 1SD = 0.37 ± 0.05 for ATV trails; 0.26 ± 0.11 for logging sites). Soil water content did not strongly correlate with earthworm and cocoon counts (Spearman rank correlation coefficient = 0.475 for ATV samples; 0.447 for logging vehicle samples). Counts of earthworms and cocoons per lb of dry soil were right skewed and included zeros so non-parametric tests were conducted (Figure 3). Median counts of earthworms and cocoons were significantly greater than zero in soil collected from ATV and logging vehicle tires (Wilcoxon 1-sample test, α-level 0.05: P-values = 0.002, 0.005, and 0.009 for ATV samples; 0.000 for logging vehicle samples). Median counts of earthworms and cocoons were significantly greater in ATV samples than in logging vehicle samples (Figure 3; Mann-Whitney test, α-level 0.05: P-value = 0.000). Cocoons were determined to be viable as they produced earthworm hatchlings following incubation of the soil samples for one month at room temp (22°C).

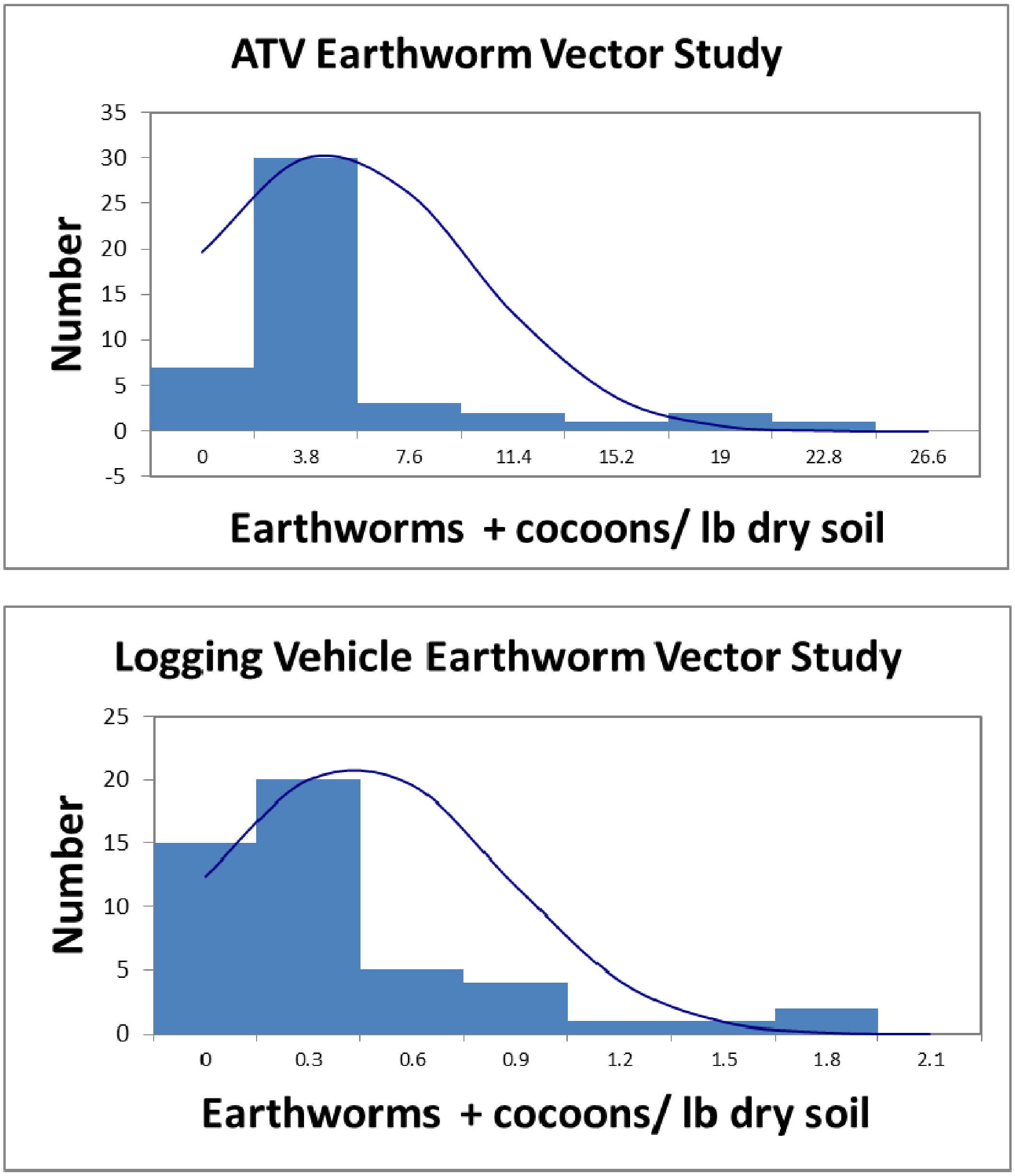

Figure 3. Counts of earthworms plus earthworm cocoons per lb of dry soil collected from tire treads in a study of ATVs and logging vehicles as vectors of earthworm spread in northern hardwood forests of Minnesota, USA.

Discussion

Here we demonstrate experimentally and observationally that soil carried on tire treads of ATVs and logging vehicles can serve as a vector of introduction and spread of earthworms in northern hardwood forests. Under generally moist conditions, soil water content did not correlate with number of earthworms and earthworm cocoons carried on the treads. Moreover, soil samples that were collected from the vehicle tires and initially cleaned of all earthworms but not cocoons later included numerous earthworms. This finding suggests that earthworm cocoons picked up in soil on vehicle treads may be viable under some conditions.

This study suggests then that ATV travel and logging activity in forests invaded by earthworms may contribute to earthworm spread during seasons in which soil is picked up on tire treads. To reduce these routes of spread, we recommend:
(1) that land managers assess the extent of earthworm impact at their sites using the standardized procedures of IERAT prior to timber sales;
(2) that logging activity be restricted in sites ranked at IERAT Level 2 or higher to seasons of frozen soil;
(3) that working groups of regional stakeholders be established to develop plans for using IERAT to generate maps of ATV trails through northern hardwood forests in their areas; and
(4) that these maps be generated and used to guide the development of procedures for cleaning ATV tires so that the spread of invasive non-native earthworms may be reduced.

Acknowledgements

Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources. The Trust Fund is a permanent fund constitutionally established by the citizens of Minnesota to assist in the protection, conservation, preservation, and enhancement of the state's air, water, land, fish, wildlife, and other natural resources. The US Forest Service Chippewa National Forest assisted in identifying timber harvest operations and study sites for this project.

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DRAFT: Strategic Plan to address regulation issue related to earthworms – December 5, 2012

Background:
In recent years the data on the impacts of invasive earthworms has continued to increase. We also have much more data on the distributions of different species and vectors associated with introduction. In particular, the rate of non-target species contamination (selling species X but getting species X,Y,Z) in earthworms sold for all purposes (i.e. fishing bait, composting, gardening, etc.) is very high, exceeding 80% in a recent study we completed on sales via the internet. Similar studies on bait sales indicate comparable levels of contamination. Of particular concern is that in recent years we have documented 5 new species of earthworms, including some that can and some that cannot (yet) survive MN winters.

I recently had a conversation with Laura Van-Piper of the DNR on this subject. They are trying to draft policy/regulatory oversight related to the threat of Asian carp being introduced via fishing bait and this lead to a similar discussion related to earthworms. Their approach with minnows thus far is to focus on identifying species that are allowed to be sold, and those that are not, and then providing training and licensing to minimize the threats. The biggest issue for minnows, as for earthworms, in not identifying species that should be allowed, but rather, developing a licensing and training system that sufficiently ensures reasonable compliance. See side-bar 1 below with links A & B below for what is currently done for minnows.

We can probably come up with an appropriate list of allowed earthworm species based on those that are already well established in the state (i.e. *Lumbricus terrestris, L. rubellus*, *Aporrectodea* spp.). The biggest issue will be to create/provide training so bait sellers know what to look for in earthworm fishing bait to ensure/minimize contamination of non-allowed species. A licensing structure similar to the one they currently have for minnows could also be successful for earthworms. Ideally the sellers of earthworms for fishing bait would be required to receive education on appropriate species and rearing conditions to prevent/minimize contamination of non-allowed species.

A few months ago I had conversations with Tina Seeland (MDA) related to a request for a permit to import earthworms. They faced similar problems in that the current legislative framework seems to limit their ability to deny permits to import. But can impose conditions. However, I was pleased to see the comments from Kevin Connors with APHIS that "My understanding is that earthworms reared in APHIS inspected rearing facilities in the UK and the Netherlands are the only countries that we allow to ship into the US." While this doesn't explicitly address the species identification issue it is a very good place to start. See Side bar 2 below with links to legislation.

Since earthworms are a group of organisms that are terrestrial but have a substantial aquatic pathway for introduction the line between the roles of MDA and DNR get blurry. It appears that an opportunity exists to collaboratively develop recommendations for limiting the continued introduction of earthworms involving DNR, MDA and interested NGO's like the TNC that may be interested in supporting legislative action in some form.

An initial contact list is at the end.

<u>SIDE BAR 1:</u> Mandatory training and licensing programs through the DNR that already exists for bait shops related to minnows - We believe we are now at the place that we have enough information on specific earthworm species impacts and primary vectors; and have developed sufficient training materials via Great Lakes Worm Watch that it would be possible to develop and implement a licensing structure for earthworms similar to that already used for minnow bait sales.

A. **Online Aquatic Invasive Species Training for Minnow Dealer Employees**
   http://www.dnr.state.mn.us/fishing/commercial/mdeinvasivetraining.html

   "If you are an employee of a Minnow Dealer, and plan to work in designated infested waters, you are required by law to take and pass a training course related to Aquatic Invasive Species (AIS) prior to working in designated infested waters. This training course is online and will take about 45 minutes to an hour to complete. There are test questions incorporated into the training and you must answer the test questions correctly in order to advance (but you may attempt the question an unlimited number of times). The online training course is free.

   Upon completion of the online training a certificate will be generated with your name on it. You will need to print the certificate and have it in your possession at all times when working in designated infested waters. The certificate itself is 3.5 inches wide by 2 inches tall (the size of a business card). After you print the page out, you should cut the certificate out so it will be a convenient size to carry with you. You may keep it in your wallet, place it in a plastic cover available for luggage tags and attach it to your work gear, buy a lamination sticker from an office supply store, have it laminated at a copying shop, or another process that makes it easy for you to keep it protected and in your possession when working. You may print out as many copies as you'd like. Your training certificate is good until April 9, 2013. You must complete this online training each year in order to obtain a new certificate to continue to work in designated infested waters."

B. Commercial Businesses
   http://www.dnr.state.mn.us/fishing/commercial/index.html
   The Minnesota Department of Natural Resources offers several different types of licenses that allow for the operation of commercial businesses dealing with wild or cultured aquatic life…

   Commercial Aquaculture Licenses:
   Aquatic Farm License
   Private Fish Hatchery License (sales less than $200)
   Private Fish Hatchery License (sales greater than $200)
   Aquarium Facility License

   Commercial Minnow Licenses:
   Minnow Retailer License
   Minnow Dealer License
   Exporting Minnow Dealer License
   Minnow Dealer, Exporting Minnow Dealer, and Minnow Retailer Vehicle Licenses

   Commercial Fishing Licenses:
   Commercial Fishing Licenses

   Other Commercial Licenses:
   Fish Packer License; Fish Vendor Vehicle License; Turtles; Frogs; Crayfish

SIDE BAR 2:

The USDA website provides links to legislative/regulatory structure in Minnesota
http://www.invasivespeciesinfo.gov/laws/mn.shtml

Administrative Code and Statutes

- **Minnesota Statutes** - State Laws
  - Pest Control (Chapter 18)
    - Minnesota Noxious Weed Law (18.78 **et seq.**)
  - Invasive Species Management and Investigation (18G.12)
  - Conservation (Chapter 84)
    - Invasive Species (Chapter 84D)
  - Roads, general provisions (Chapter 160)
    - Destruction of Noxious Weeds (160.23)

- **Minnesota Rules** - State Regulations
  - Department of Agriculture
    - Pest and Disease Control (Chapter 1505)
    - Seeds, Fertilizers, Feeds (Chapter 1510)
      - Noxious Weed Seed Tolerances (1510.0080)
  - Department of Natural Resources
    - Invasive Species (Chapter 6216)
    - Aquatic Plants and Nuisances (Chapter 6280)

Some links to specific Minnesota Statutes:

1) Invasive Species Chapter 84D MN statutes
   https://www.revisor.mn.gov/statutes/?id=84D
2) 18G.12 INVASIVE SPECIES MANAGEMENT AND INVESTIGATION.
   https://www.revisor.mn.gov/statutes/?id=18G.12
3) Chapter 18J. INSPECTION AND ENFORCEMENT
   https://www.revisor.leg.state.mn.us/statutes/?id=18J

Interstate transport of invasive species…The Pest Control Compact

1) 18.62 ENACTMENT; INSURANCE FUND; ADMINISTRATION; FINANCE.
   https://www.revisor.mn.gov/statutes/?id=18.62

<u>SIDE BAR 3:</u> MN Pollution Control: Composting Rules, regulations and proposed changes
http://www.pca.state.mn.us/index.php/waste/waste-permits-and-rules/waste-rulemaking/proposed-changes-to-compost-rules.html

There is currently regulation related to commercial compost sites -
https://www.revisor.mn.gov/rules/?id=7035.2836
While the rules do not currently exclude earthworms specifically, they set hygiene and operational standards that are amenable to doing so (in my opinion). Further, mandatory training is required of operators and staff of such operations (see below). Providing for inclusion of education related to earthworms would seem reasonable (in H. below) and as with the bait sales recommendations above, we feel that we have sufficient information and educational materials to develop and implement such a program as soon as is possible or desirable. Relevant excerpts of the current regulations are provided:

7035.2545 PERSONNEL TRAINING.

Subpart 1. General.

Solid waste management facility personnel must successfully complete a program of classroom instruction or on-the-job training. The program must prepare facility personnel to maintain compliance with parts 7035.2525 to 7035.2915. Personnel must complete all training within six months after November 15, 1988, or within six months after the date of employment. The owner or operator must record all personnel training on the facility operating record and submit the dates of training in the annual report.

Subp. 2. Owner or operator of a land disposal facility.

Certified owners or operators must be present at a land disposal facility as required by parts 7048.0100 to 7048.1300. A certified operator must be present at a land disposal facility during operating hours.

Subp. 3. Minimum program requirements.

The training program must include training of solid waste management facility personnel about procedures relevant to their positions including contingency action plan implementation. The program must train facility personnel to deal effectively with problems at the site including:

A. using, inspecting, repairing, and replacing facility emergency and monitoring equipment;
B. activating communication and alarm systems;
C. activating automatic waste feed cutoff systems;
D. responding to fires;
E. responding to facility failures, including erosion and failure of liners or monitoring devices;
F. responding to ground water or surface water pollution incidents;
G. accepting and managing waste other than mixed municipal solid waste approved for storage or disposal at the facility;
H. rejecting waste not permitted at the facility; and
I. water sampling.

Initial contacts include:

MN Department of Natural Resources:

1) Laura Van Riper, Terrestrial Invasive Species Coordinator
   651-259-5090, <Laura.Vanriper@state.mn.us>
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4) Jay Rendall, DNR Invasive Species Prevention Coordinator
   (651) 259-5131, <jay.rendall @state.mn.us>
5) Andy Holdsworth, <andy.holdsworth@state.mn.us>

Minnesota Department of Agriculture

1) Kathryn Kromroy, Research Scientist
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     651-201-6448, <Teresa.McDill@state.mn.us>

APHIS

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   - Supervisor: Kevin Connors, *USDA, APHIS, PPQ - State Plant Health Director, MN*
     952-814-1071, <Kevin.J.Connors@aphis.usda.gov>

MN Pollution Control agency

1) Ginny Black, Rule content — Subject matter expert on operations 651-757-2233,
   ginny.black@state.mn.us
2) Tim Farnan timothy.farnan@state.mn.us ,also include Lisa Mojsiej Lisa.Mojsiej@state.mn.us

Others Organizations with an interest or that can serve as resources:

1) Steve Chaplin, *The Nature Conservancy, MN*
   (612)331-0788, <schaplin@tnc.org>
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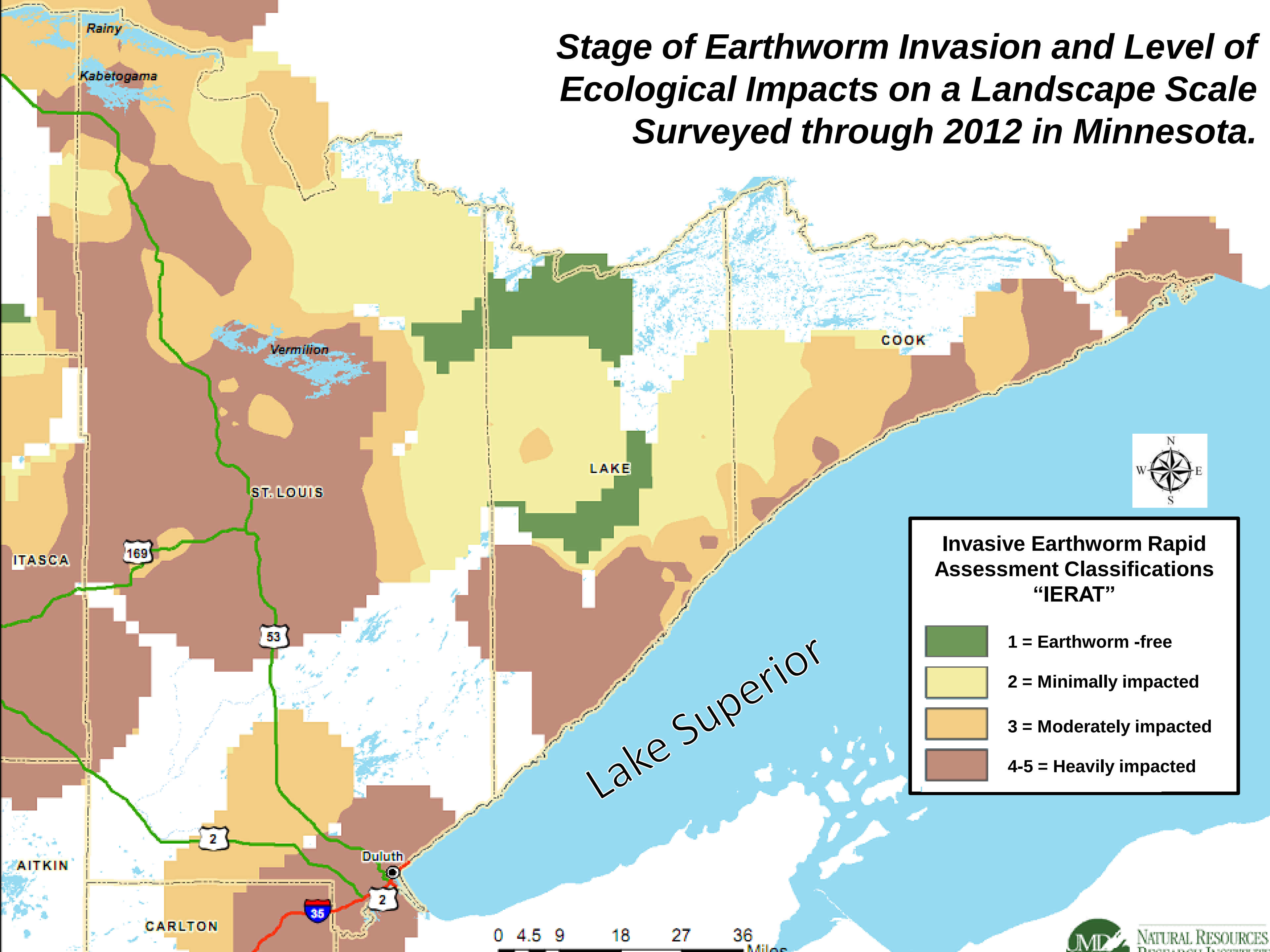
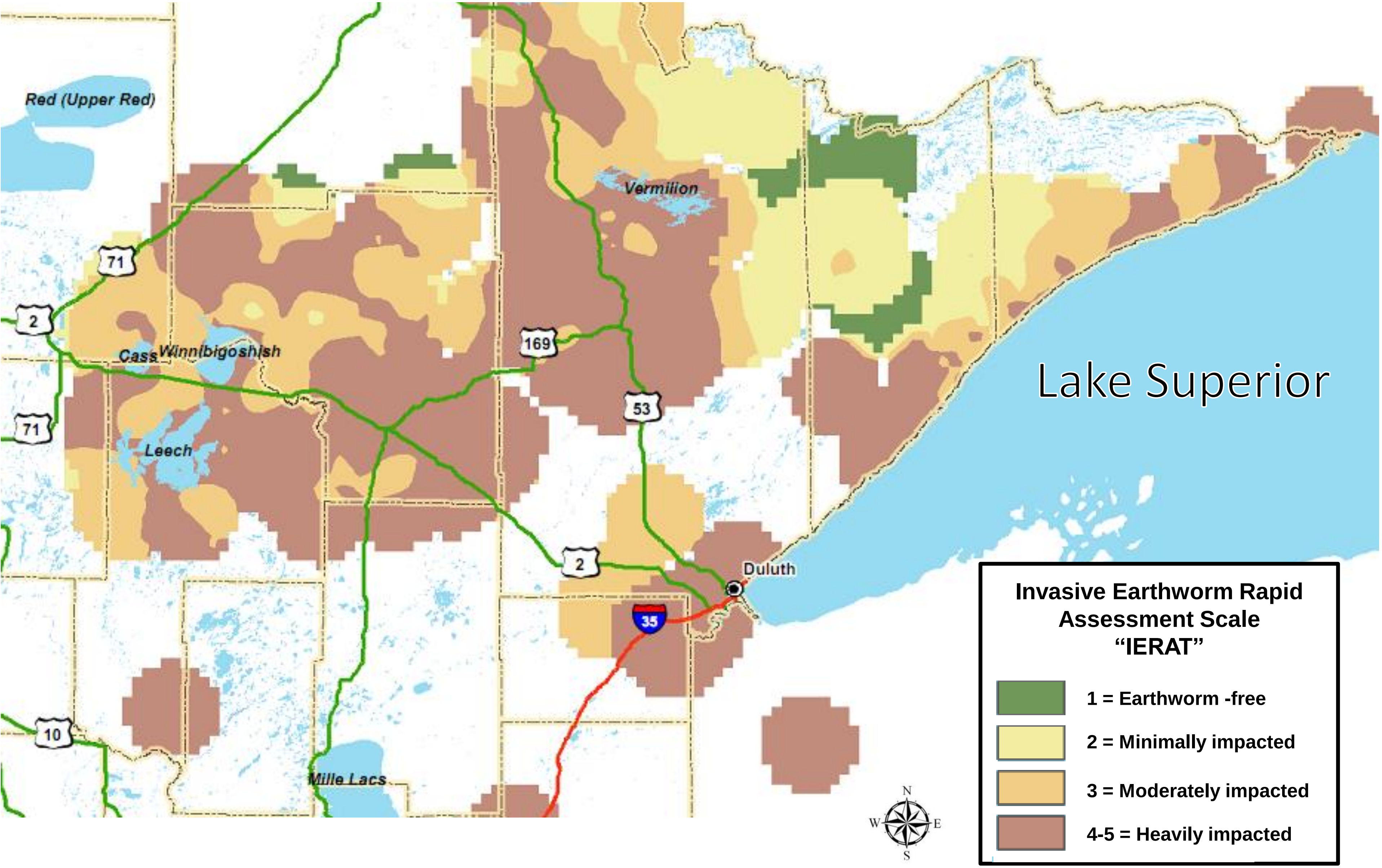
   Department of Forest Resources
   freli001@umn.edu
3) Jonathan Riven, Waste Management Specialist <www.shwec.uwm.edu>
   UW Extension, Solid and Hazardous Waste Education Center
   University of Wisconsin - Stevens Point
   715-346-2793, <jonathan.rivin@uwsp.edu>

Stage of Earthworm Invasion and Level of Ecological Impacts on a Landscape Scale Surveyed through 2012 in Minnesota.



Stage of Earthworm Invasion and Level of Ecological Impacts on a Landscape Scale Surveyed through 2012 in Minnesota.



LCCMR Result 4: Identifying priority areas for protection

A comprehensive and coordinated 3 year effort involving research and educational institutions, governmental agencies, non-governmental organizations and citizen science involving a diverse set of stakeholders identify earthworm-free and minimally invaded areas of the state/region in order to prioritize protection efforts and provide rapid detection and response for new species introductions.

The development of the map came from the development of the development of the IERAT that uses visual assessment of the forest floor characteristics to assign a site to one of five classifications that indicate the stage of earthworm invasion, level of ecological impact and the associated earthworm assemblages most likely present. These classifications and the visual indicators associated with each include:

1 = earthworm free
2 = minimally impacted
3 = moderately impacted
4 = substantially impacted
5 = heavily impacted

- **Classification 1** corresponds to earthworm free areas.
- **Classification 2** corresponds with minimally impacted areas with the common species *Dendrobaena octaedra* found in the forest floor leaf litter. These species are small (XXmm) and feed on the organic material above the mineral soil. You may also find *Lumbricus rubellus* at this point as well. This species is associated with some of the biggest impacts.
- **Classification 3** corresponds with moderately impacted areas. Species found in these area include *Dendrobaena octaedra* and *Lumbricus rubellus* Along with *Aporrectodea* spp. which are soil dwelling species that feed on organic material in the mineral soil.
- **Classification 4 and 5** correspond with heavily impacted areas. Species found are *Dendrobaena octaedra* , *Lumbricus rubellus* and *Aporrectodea* spp. along with the introduction of *Lumbricus terrestris* known as the night crawler. Which is the largest earthworm species in Minnesota at (xxmm). This species is associated with the complete removal of the forest floor.

For the purpose of the map we combined classification 4 and 5 stage four is when you see the indicator species *Lumbricus terrestris* is present and is associated with the removal of the forest floor.

Earthworm Invasions in Northern Hardwood Forests: a Rapid Assessment Method

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Source: Natural Areas Journal, 33(1):21-30. 2013.
Published By: Natural Areas Association
DOI: http://dx.doi.org/10.3375/043.033.0103
URL: http://www.bioone.org/doi/full/10.3375/043.033.0103

RESEARCH ARTICLE

Earthworm Invasions in Northern Hardwood Forests: a Rapid Assessment Method

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Natural Areas Journal 33:21–30

ABSTRACT:
Non-native earthworm invasions in north-temperate North America cause substantial adverse effects to hardwood forest ecosystems. Quantification of invasions is necessary for understanding impacts and identifying remnant earthworm-free areas, but existing sampling techniques are effort-intensive and/or environmentally damaging. We: (1) developed and applied a protocol that allows rapid classification of earthworm invasion into five stages based primarily on visual assessment of the forest floor, (2) sampled earthworms to test whether the protocol's stages can predict invasion by different species, and (3) assessed relationships between individual forest floor characteristics and presence of different earthworm species. Based on differences in biomass among points assigned to different stages, the 5-stage classification protocol accurately identified the onset of invasion by *Lumbricus rubellus* and *Lumbricus terrestris*, the species of greatest management concern in the northern Midwest. Except for middens as a predictor of *L. terrestris* presence, no forest floor variable was useful by itself for assessing invasions. The 5-stage protocol provides an efficient approach for assessing earthworm invasions in hardwood forests of the U.S. northern Midwest, can be implemented with minimal training, and serves as a blueprint for similar protocols in other regions experiencing earthworm invasions.

Index terms: earthworm sampling methods, invasive earthworms, *Lumbricus rubellus*, *Lumbricus terrestris*, northern hardwood forests

INTRODUCTION

Non-native European earthworms are invading previously earthworm-free regions of north-temperate North America, substantially changing hardwood forests (Frelich et al. 2006) and posing a major conservation concern (Sutherland et al. 2010). Invasive earthworms, particularly *Lumbricus* spp., consume organic layers, mix soil horizons (Alban and Barry 1994; Hale et al. 2005b), and alter nutrient dynamics (Burtelow et al. 1998; Costello and Lamberti 2008). Changes to the soil eliminate sensitive plant species (Gundale 2002), reduce cover and diversity of herbaceous plants and tree seedlings, and increase cover of sedges and grasses (Hale et al. 2006; Holdsworth et al. 2007a). These changes can reduce abundance of salamanders (Maerz et al. 2009) and ground-nesting songbirds (Loss and Blair 2011; Loss et al. 2012).

Preventing further spread of earthworms and mitigating effects to soil, plants, and vertebrates requires identification of remnant earthworm-free natural areas and quantification of invasion across broad spatial scales. Several earthworm sampling techniques exist (reviewed by Butt and Grigoropoulou 2010), including removal and hand-sorting of the soil (Raw 1960; Coja et al. 2008), electrical extraction (Weyers et al. 2008), and liquid extraction with permanganate (Svendsen 1955), formalin (Raw 1959; Callaham and Hendrix 1997), or a mustard-water mixture (Lawrence and Bowers 2002; Hale et al. 2005b). These methods are effort-intensive, which precludes efficient sampling at a large number of sites. Some of the methods are also physically destructive or require use of environmentally toxic substances.

Mustard extraction is commonly used in studies of earthworm invasion (e.g., Kourtev et al. 1999; Cameron et al. 2007). This method is environmentally friendly and provides an accurate index of species composition and abundance (Gunn 1992; Lawrence and Bowers 2002; Eisenhauer et al. 2008), especially for the deep-burrowing *L. terrestris* (Chan and Munro 2001). However, the method requires substantial time and effort because large quantities of water must often be transported long distances into remote areas. In one ecological study, field sampling with mustard extraction at 112 points within a 25-km radius required 80 hours of fieldwork (1.4 points/hr, Loss and Blair 2011); and in another study, sampling at 36 points scattered across two national forests required 180 hours of fieldwork (0.2 points/hr, Loss et al. 2012). In addition, earthworms must be identified and measured to estimate biomass upon returning from the field. Development of a protocol that provides a more efficient means for assessing earthworm invasion will benefit conservation, management, and research that requires mapping of invasion at fine resolution or across broad spatial extents.

Earthworm invasions in the U.S. northern Midwest involve multiple species and are thought to progress through five sequential stages, with earthworm-free conditions in stage 1 and the onset of invasion by different taxa in subsequent stages (stage 2 – *Dendrobaena octaedra;* stage 3 – *Lumbricus* juveniles and *Aporrectodea* spp.; stage 4 – *L. rubellus;* stage 5 – *L. terrestris*) (Holdsworth et al. 2007b). Because invasion by additional species of earthworms compounds effects on the forest floor (Frelich et al. 2006), and because earthworm effects are highly visible, it may be possible to use forest floor characteristics (e.g., litter depth, sedge cover, and earthworm castings and middens) to identify the onset of invasion by these different species.

In hardwood forests of the U.S. northern Midwest, we: (1) developed and applied a protocol that allows rapid classification of earthworm invasion into one of five stages based primarily on visual assessment of the forest floor, (2) directly sampled earthworms to test whether the protocol's stages accurately predicted the onset of invasion by different species, and (3) assessed relationships between several forest floor measurements and presence of different earthworm species, including *L. rubellus* and *L. terrestris*, the species with the greatest impact in northern Midwest forests.

METHODS

Study Area and Point Selection

We collected data from two different study areas, one in northeast Minnesota and one in northwest Wisconsin (Figure 1). Minnesota data were collected in nine state parks along Lake Superior's north shore (47°N, 92°W to 48°N, 90°W; hereafter, "Minnesota points"). Wisconsin data were collected at bird nests in earthworm-free and invaded stands in the Chequamegon-Nicolet National Forest (46°N, 91°W; hereafter, "Wisconsin points").

Loss and Blair (2011) reported detailed selection methods for the Wisconsin points. We collected data at 271 ovenbird (*Seiurus aurocapilla*) and hermit thrush (*Catharus guttatus*) nests that were monitored in 2009 (n = 112) and 2010 (n = 159). All nests were in upland-mesic sugar maple (*Acer saccharum*) and sugar maple-basswood (*Tilia americana*) forest sites that were > 60 years old, on sandy loam or loamy sand soils, and had no timber removed in the last 40 years. Earthworm sampling confirmed that sites represented earthworm-free, partially invaded, and completely invaded forest stands (Holdsworth et al. 2007a; Loss and Blair 2011).

The nine state parks containing the Minnesota points were in the North Shore Highlands subsection of Minnesota's Ecological Classification System. We used ArcMap (version 9.3) (ESRI 2008) and a forest type data layer from the Minnesota Native Plant Community Classification (Minnesota Department of Natural Resources 2011) to locate 2000 random points. Field sampling was conducted at a random subset of 163 of these points. The number of points sampled in each park was proportional to the park's size, and the number of points sampled in each forest type was proportional to its cover on the landscape.

The 163 points represented 25 forest types consisting of different combinations of dominant, co-dominant, and sub-canopy tree species. The dominant canopy species were quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and sugar maple. The co-dominant and sub-canopy species were balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), black spruce (*P. mariana*), white cedar (*Thuja occidentalis*), white pine (*Pinus strobus*), red pine (*P. resinosa*), yellow birch (*Betula alleghaniensis*), red maple (*Acer rubrum*), red oak (*Quercus rubra*), basswood, black ash (*Fraxinus nigra*), and big-tooth aspen (*Populus grandidentata*). Much of the analysis for Minnesota focused on points in aspen-birch and sugar maple forests, the most widespread hardwood forest types in the region.

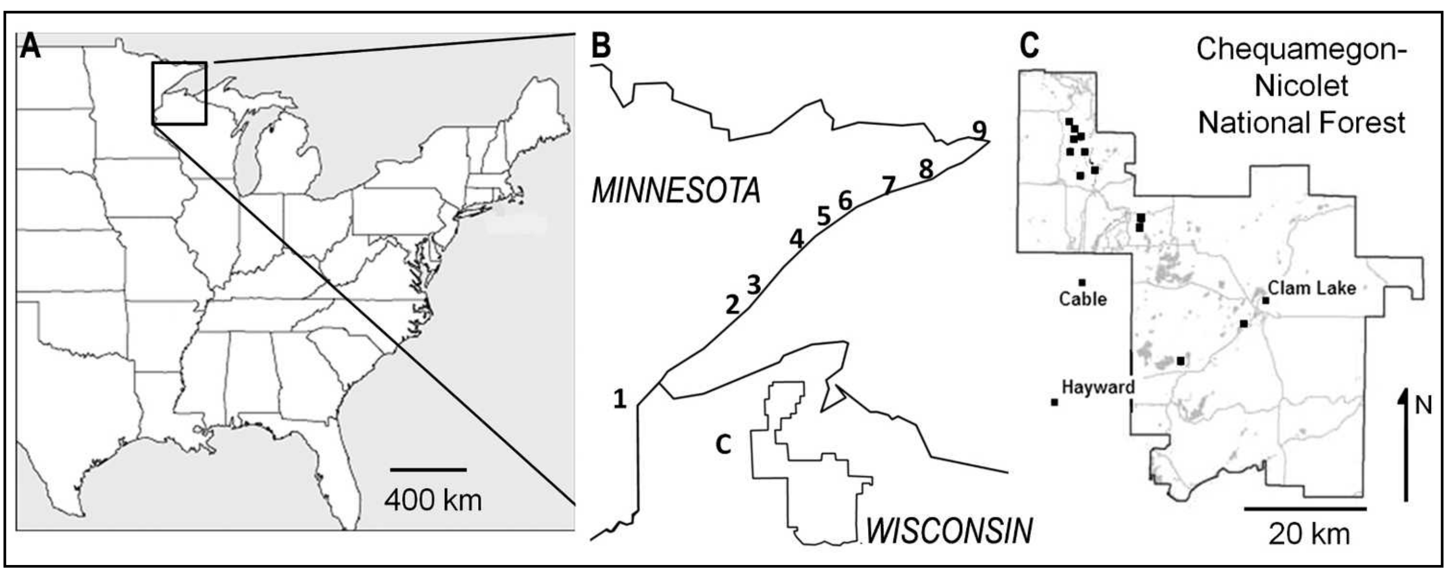

Figure 1. Study area location in the eastern U.S. (A), Minnesota study sites (numbers) along the north shore of Lake Superior (B), and Wisconsin study sites (black squares) in the Chequamegon-Nicolet National Forest (C). Numbers in (B) refer to the following state parks: (1) Jay Cooke; (2) Gooseberry Falls; (3) Split Rock; (4) Tettegouche; (5) Crosby Manitou; (6) Temperance River; (7) Cascade River; (8) Judge C.R. Magney; (9) Grand Portage.

Measurement of Forest Floor Variables

We measured the forest floor at all points, but methods and variables measured differed between Wisconsin and Minnesota points. At Wisconsin points, vegetation and the leaf litter layer were measured between 15–31 July of 2009 or 2010. Within a 2-m x 2-m square centered on each nest, we visually estimated percent cover of the litter layer, maple seedlings < 50 cm tall, all sedges and grasses combined, and total ground vegetation (all grasses, sedges, herbaceous plants, and woody plants < 50 cm tall). Cover estimates were to the nearest 10%. Average litter depth (O_i, O_e, and O_a horizons combined) was measured based on four measurements taken 1 m from the nest at each cardinal direction and by pushing a metal skewer through the litter until meeting resistance from rock or mineral soil. For litter depth and cover estimates, only intact, accumulated leaf litter > 1 year old was measured because presence and depth of the uppermost leaves from the previous autumn is independent of earthworm invasions. We also counted earthworm middens, piles of organic material at burrow entrances created by *L. terrestris* (Figure 2) (Raw 1959; Butt and Grigoropoulou 2010), within 33-cm x 33-cm sub-plots from which earthworms were directly sampled (described in later sub-section). We counted every other midden that fell roughly 50% within the sub-plot.

Figure 2. Earthworm casting material (a) and Lumbricus terrestris middens (b).

At Minnesota points, the forest floor was measured between 1 June–31 August 2009. Because the state parks span > 150 km from south to north and experience different timing of seasonal temperature and moisture patterns, parks were surveyed in a random order to avoid confounding effects of climate. We collected all data within a 5-m radius centered on each point. Fragmentation of the litter layer was classified into one of three categories that reflect increasing earthworm decomposition (1 – Intact, layered forest floor, O_i, O_e, and O_a horizons present; 2 – Litter layer partially fragmented, but with litter from > 1 yr; 3 – No intact litter, only freshly fallen leaves from the previous autumn). Earthworm activity was visually estimated using an earthworm casting index (1 - Castings absent; 2 - Castings present, ≤ 50% of forest floor covered; 3 - Castings abundant, > 50% of forest floor covered) (see Figure 2 for photograph of casting material) and midden index (1 - Middens absent; 2 - Middens present, ≤ 9 middens in 5-m radius; 3 - Middens abundant, ≥ 10 middens in 5-m radius).We extracted soil cores (6 cm diameter; 15 cm depth) from 3 random locations and used them to measure depth of the litter layer (O_i, O_e, and O_a horizons combined) and A-horizon. Soil textural class was determined for the mineral soil component of each core using a manual texture key adapted from Brewer and McCann (1982). Finally, we used a variable radius plot and BAF 10 wedge prism to sample tree species and estimate relative dominance (i.e., proportional representation by each tree species).

The 5-Stage Invasion Classification Protocol

At Minnesota points, we used a dichotomous key (Table 1) that incorporated several of the above forest floor measurements to classify points into one of five earthworm invasion stages. The stages were designed to identify the onset of invasion by different species following Holdsworth et al. (2007b) (stage 1 – potentially earthworm-free; stage 2 – *D. octaedra*; stage 3 – *Lumbricus* juveniles and *Aporrectodea* spp.; stage 4 – *L. rubellus*; stage 5 – *L. terrestris*). The dichotomous key was based on casting and midden indices, degree of litter fragmentation, and on observation of fine root presence in the O-horizon, because fine root abundance decreases following invasion (Fisk et al. 2004; Hale et al. 2005b).

Earthworm Sampling

Earthworms were sampled using the liquid-mustard extraction technique (Lawrence and Bowers 2002; Hale et al. 2005a), which consists of pouring a mustard-water mixture (40 g ground yellow mustard, 4 L water) on the soil surface and collecting all emerging earthworms. At Wisconsin points, sampling was conducted between 15 September–5 October of 2009 or 2010. At Minnesota points, sampling was conducted between 1 September–15 October 2009. This sampling timeframe corresponds to a period of soil moisture conditions favorable for earthworms and in which the population contains a high proportion of adults.

At Wisconsin points, we sampled one-third of the 2009 points (n = 36) using three 33-cm x 33-cm subplots (one at the nest, two random points ≤ 33 m from the nest) and two-thirds of points (n = 76) using one plot at the nest. Because there was no significant difference in biomass of different earthworm species between one-plot and 3-subplot points, we sampled all 2010 points with one plot at the nest (Loss and Blair 2011). At all Minnesota points, earthworms were sampled from three randomly selected 33-cm x 33-cm subplots within 5-m radius plots.

Earthworms were preserved in the field with 70% isopropyl alcohol and transferred to buffered 10% formalin for storage. We counted, identified, and measured length of earthworms using a dissecting micro-

Table 1. Dichotomous key for 5-stage rapid classification of earthworm invasion in hardwood forests of the northern Midwest. Details of measurement methods are in the text.

1. Leaf litter greater than one year present (O_i and O_e layers present).
   - 1a. Yes (go to 2)
   - 1b. No, leaf litter (O_i) from previous autumn only (go to 6)
2. Small, fragmented, relatively un-decomposed leaves greater than one year present.
   - 2a. Yes, O_e present (go to 3)
   - 2b. No, leaf litter (O_i) from previous autumn only (go to 6)
3. Intact, layered forest floor, leaves bleached and stuck together, O_i , O_e , and O_a layers present, fine plant roots in humus (O_a) and leaf fragments (O_e), no earthworms, castings, or middens present.
   - 3a. Yes (Stage 1 – potentially earthworm-free)
   - 3b. No (go to 4)
4. Layered forest floor, but leaves loose, O_i, O_e, and patches of O_a layers present. Some small earthworms and/or earthworm castings present in humus (O_a), fine plant roots present.
   - 4a. Yes (Stage 2)
   - 4b. No (go to 5)
5. Leaf litter (O_i) from previous autumn and small fragmented leaves (O_e) under intact leaves, no humus (O_a), mineral soil (A-horizon) present, earthworm casting present (≤ 50% of forest floor/mineral soil interface covered), fine plant roots absent.
   - 5a. Yes (Stage 3)
   - 5b. No (go to 6)
6. Leaf litter (O_i) from previous autumn, mineral soil (A-horizon) present, earthworm casting *abundant* (> 50% of forest floor/mineral soil interface covered), fine plant roots absent, middens absent or present (≤ 9 middens in 5-m radius).
   - 6a. Yes (Stage 4)
   - 6b. No (go to 7)
7. No forest floor (O_i or O_e), humus (O_a) or fragmented leaves present, mineral soil (A-horizon) present, earthworm casting abundant (> 50% of forest floor/mineral soil interface covered), middens abundant (> 9 middens in 5-m radius).
   - 7a. Yes (Stage 5)

scope. Adult earthworms were identified to species when possible, but most juvenile earthworms were only identifiable to genus. All *Aporrectodea* earthworms were grouped together, because most individuals were juveniles, and adult *A. caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides*, and *A. tuberculata* are morphologically similar (Hale 2007). We used length measurements and regression equations based on allometric relationships (Hale et al. 2004) to estimate earthworm biomass.

Data Analyses

We averaged earthworm biomass (all points) and midden counts (Wisconsin points) across subplots to calculate point-level values and used tree dominance estimates to field-truth forest types at Minnesota points. The forest type at some points did not match the type indicated during point selection; therefore, for statistical analyses conducted separately by forest type, forest types were classified using field-collected dominance estimates (aspen-birch = combined dominance of all aspen and birch species ≥ 0.5; sugar maple = dominance of sugar maple ≥ 0.4). Because different forest floor assessment methods were used for Wisconsin and Minnesota points, all analyses were conducted separately for each state.

For Minnesota points, we compared earthworm biomasses among points classified into the five invasion stages. Because the distribution of biomass values was skewed with zeroes, we were unable to achieve normal distribution of the data. Biomasses were compared with one-way Kruskal-Wallace tests and pairwise comparisons between group medians using Mann-Whitney U-tests. Separate analyses were conducted for *D. octaedra, Aporrectodea* spp., *L. rubellus,* and *L. terrestris*.

Multivariate logistic regression was used to assess relationships between forest floor characteristics and presence of the four earthworm taxa noted above. For Wisconsin points, continuous independent variables were cover of sedge, maple seedlings, total ground vegetation, and leaf litter, as well as litter depth and midden count. A categorical year covariate was also included to account for temperature and moisture variation between 2009 and 2010 that could have affected earthworm sampling results. For Minnesota points, the continuous independent variables were litter depth and A-horizon depth, and the categorical variables were the litter fragmentation, casting, and midden indices. For Minnesota points, regression analyses were conducted separately for aspen-birch (n = 79) and sugar maple forests (n = 42). A preliminary analysis indicated no statistically significant relationships between soil texture and presence of different earthworm species within the above forest types, and soil texture variation was minimal within each type. Therefore, soil texture was likely not a major determinant of earthworm presence within each forest type; and to simplify regression models, we did not include this factor as a covariate.

RESULTS

Of the 271 Wisconsin points, 70 (25.8%) had no earthworms detected. All 163 Minnesota points had at least one earthworm detected; however, samples from three points (1.8%) only contained *D. octaedra*. For the Wisconsin and Minnesota points, 174 (64.2%) and 32 (19.6%) points, respectively, had no *L. rubellus* or *L. terrestris* detected but were invaded by *D. octaedra, Aporrectodea*, and/or other earthworm species.

EFFICIENCY AND ACCURACY OF THE 5-STAGE INVASION CLASSIFICATION PROTOCOL

Characterization of the forest floor using the 5-stage classification protocol required between 5-8 minutes of sampling per point. Minnesota points were assigned to all five stages, including stage 1 (n = 4; 2.5%), stage 2 (n = 11; 6.7%), stage 3 (n = 72; 44.2%), stage 4 (n = 43; 26.4%), and stage 5 (n = 33; 20.2%). Because very few Minnesota points were classified as potentially earthworm-free, we did not include stage 1 in pairwise comparisons of biomass.

D. octaedra biomass was highest at points assigned to invasion stage 2; however, there were no statistically significant biomass differences among stages for this species (H = 5.44, df = 3, p = 0.14) (Figure 3a). *Aporrectodea* biomass was significantly different among stages (H = 8.48, df = 3, p = 0.04), with biomass significantly lower in stage 3 than in stages 4 and 5 but not different between stage 2 and 3 or among stages 2, 4, and 5 (Figure 3b). For *L. rubellus*, we found significant biomass differences among stages (H = 22.74, df = 3, p < 0.01), with biomass in stage 3 significantly greater than all other stages and significant biomass decreases in both stages 4 and 5 (Figure 3c). For *L. terrestris*, there was a significant difference among invasion stages (H = 49.40, df = 3, p < 0.01), with biomass for level 5 greater than all other levels and biomass for level 4 greater than for level 3 (Figure 3d).

Relationships between Forest Floor Variables and Earthworm Presence

We found statistically significant relationships between individual forest floor variables and presence of each earthworm taxa and for both Wisconsin and Minnesota points (see Table 2 for β-coefficients and *p*-values). For Wisconsin points, presence of *D. octaedra* was positively related to sedge cover (odds-ratio=1.40). For *Aporrectodea*, there was an inverse relationship between presence and litter cover at Wisconsin points (odds-ratio=0.40) and a positive relationship between presence and A-horizon depth for Minnesota points in both forest types ($\text{odds-ratio}_{\text{aspen-birch}}$=1.55; $\text{odds-ratio}_{\text{sugar maple}}$=1.57).

Presence of *L. rubellus* at Wisconsin points was positively related to sedge cover (odds-ratio=1.41), total ground cover (odds-ratio=2.10), and *L. terrestris* midden count (odds-ratio=1.49), and inversely related to

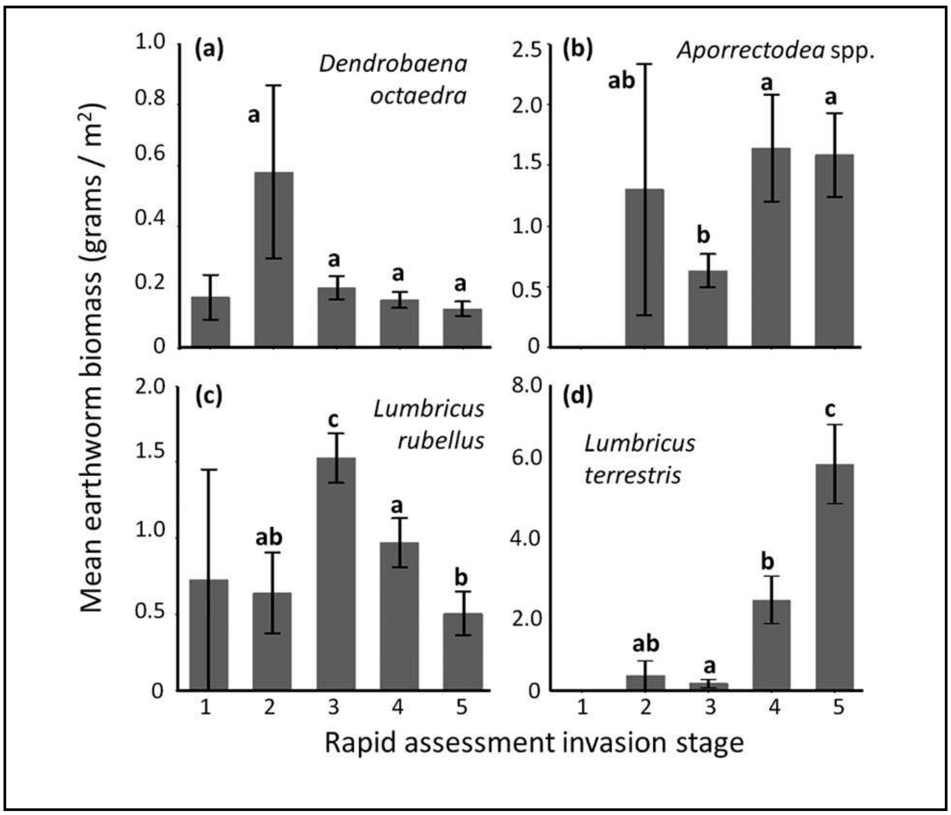

Figure 3. Mean earthworm biomass (± SE) for points classified into 5 earthworm invasion stages along the north shore of Lake Superior, Minnesota: *Dendrobaena octaedra* (a), *Aporrectodea* spp. (b), *Lumbricus rubellus* (c), and *Lumbricus terrestris* (d). Lower-case letters indicate differences among group medians based on Kruskal Wallace and Mann-Whitney U-tests. Units on vertical axis are different for each species; stage 1 was not included in pairwise comparisons due to small sample sizes.

Table 2. Results of multivariate logistic regression models illustrating relationships between forest floor variables and presence of four earthworm taxa at Wisconsin points in sugar maple forest in the Chequamegon-Nicolet National Forest and at Minnesota points in aspen-birch and sugar maple forests along the north shore of Lake Superior.

	Dendrobaena octaedra			*Aporrectodea* spp.			*Lumbricus rubellus*			*Lumbricus terrestris*		
	Coef	Odds-ratio	p	Coef	Odds-ratio	p	Coef	Odds-ratio	p	Coef	Odds-ratio	p
Wisconsin points (n = 271)												
Intercept	-2.00	0.14	0.13	0.44	1.55	0.80	-3.79	0.02	0.04	-1.33	0.26	0.47
Year	0.18	1.20	0.55	0.36	1.43	0.40	0.13	1.14	0.80	0.30	1.35	0.63
Sedge cover	0.33	1.40	<0.01	0.07	1.07	0.62	0.34	1.41	0.04	0.10	1.11	0.60
Maple cover	0.14	1.15	0.22	-0.23	0.80	0.12	-0.34	0.71	0.05	-0.13	0.88	0.51
Total ground cover	0.10	1.11	0.64	0.41	1.51	0.17	0.74	2.10	0.04	0.13	1.14	0.73
Litter cover	0.19	1.21	0.18	-0.91	0.40	<0.01	-0.06	0.94	0.78	-0.39	0.68	0.12
Litter depth	0.17	1.19	0.07	0.09	1.09	0.50	-0.24	0.79	0.22	-0.54	0.58	0.06
Midden count	-0.06	0.94	0.51	0.12	1.13	0.27	0.40	1.49	<0.01	0.42	1.53	<0.01
Minnesota points												
Aspen- birch (n = 79)												
Intercept	-3.12	0.04	0.21	-7.83	<0.01	0.02	-1.96	0.14	0.14	-40.35	<0.01	1.00
Litter depth	2.79	16.33	0.06	1.53	4.62	0.19	0.54	1.71	0.53	-0.44	0.65	0.79
A-horizon depth	0.09	1.10	0.41	0.44	1.55	<0.01	0.11	1.11	0.29	0.05	1.05	0.67
Litter fragmentation	0.13	1.14	0.85	-0.28	0.76	0.71	-0.78	0.46	0.18	0.33	1.39	0.65
Casting index	0.59	1.79	0.48	0.28	1.32	0.81	1.32	3.73	0.10	18.23	8.26E+07	0.99
Midden index	0.50	1.64	0.22	0.60	1.83	0.17	-0.48	0.62	0.18	1.30	3.67	<0.01
Sugar maple (n = 42)												
Intercept	10.36	3.15E+04	0.06	-4.28	0.01	0.16	-2.01	0.13	0.47	-21.13	<0.01	1.00
Litter depth	-0.58	0.56	0.17	0.40	1.49	0.28	0.77	2.17	0.11	-8.82	<0.01	0.32
A-horizon depth	-0.25	0.78	0.28	0.45	1.57	0.01	0.13	1.14	0.42	-0.12	0.89	0.68
Litter fragmentation	-3.25	0.04	0.06	-0.24	0.78	0.83	-0.08	0.92	0.93	-1.21	0.30	0.53
Casting index	-0.20	0.82	0.86	-0.12	0.88	0.90	2.61	13.61	0.06	12.31	2.21E+05	0.99
Midden index	0.91	2.49	0.28	1.06	2.89	0.06	-1.31	0.27	0.09	3.64	38.02	0.03

maple seedling cover (odds-ratio=0.71). At Minnesota points, there were no statistically significant predictors of *L. rubellus* presence. However, there were near-significant ($p \leq 0.10$) positive relationships between *L. rubellus* presence and casting index in both forest types, and odds-ratios for these relationships were relatively high (odds-ratio$_{aspen\text{-}birch}$=3.73; odds-ratio$_{sugar\ maple}$=13.61). Presence of *L. terrestris* was positively related to midden counts at Wisconsin points (odds-ratio=1.53) and to the midden index at Minnesota points, but with a much stronger relationship in sugar maple forests (odds ratio=38.02) than aspen-birch forests (odds ratio=3.67).

DISCUSSION

We found that the 5-stage classification protocol identified the onset of invasion by *L. rubellus* and *L. terrestris*, the earthworm species of greatest management concern in forests of the northern Midwest. Biomass of these species differed significantly among points assigned to different stages, with stage 3 characterized by peak *L. rubellus* invasion, and stages 4 and 5 characterized by the onset and eventual dominance, respectively, of invasion by *L. terrestris*. We also found that the presence of each earthworm taxa was significantly related to at least one forest floor variable, but, except for midden count and midden index as predictors of *L. terrestris* presence, no single variable is likely to be useful for rapid assessment of earthworm presence.

Use of the 5-stage Classification Protocol for Predicting Species Invasions

With some exceptions, the differences in sampled earthworm biomasses suggest that the 5-stage classification system identifies the sequential onset of invasion by different species and is, therefore, a useful tool for quickly quantifying earthworm invasions in hardwood forests of the northern Midwest. Holdsworth et al. (2007b) observed a predictable invasion sequence, with *D. octaedra* invading first, followed by *Aporrectodea* and *Lumbricus* juveniles, then *L. rubellus*, and finally *L. terrestris*. Different species compositions are thought to be a function of time since original invasion (Hale et al. 2005a) and rate and mechanism of dispersal (Proulx 2003; Cameron et al. 2007; Costello et al. 2010). Greater replication is needed to determine the accuracy of stages 1 and 2 of our protocol for identifying potentially earthworm-free and *D. octaedra*-invaded forests, respectively. However, even with a small sample of points assigned to stage 2 (n = 11), this stage had greater *D. octaedra* biomass than any other, suggesting the potential for the protocol to accurately identify invasion by this species. Protocol stage 3 corresponds to the onset of *L. rubellus* invasion, and stages 4 and 5 correspond to the onset and eventual dominance of *L. terrestris* invasion, respectively.

Whereas we observed that onset of *L. rubellus* invasion occurred in stage 3 of our protocol, Holdsworth et al. (2007b) first observed this species in a fourth stage, immediately following invasion by *Aporrectodea* and *Lumbricus* juveniles. However, because they observed *Lumbricus* juveniles in the third invasion stage, and because these individuals likely included *L. rubellus*, invasion by this species probably also occurred in Holdsworth et al.'s (2007b) stage 3. The observed differences in *Aporrectodea* biomass among stages were unexpected. Instead of stage 3 being characterized by the onset of *Aporrectodea* invasion, this stage had the lowest observed biomass among stages where it was present, and there were no biomass differences among the other stages. The 5-stage protocol, therefore, does not appear to diagnose onset of invasion by this group. This negative finding may have resulted from our grouping of all *Aporrectodea* spp. in statistical analyses, an approach that may have obscured unique effects of different species to the forest floor.

Relationships between Forest Floor Variables and Earthworm Presence

Our results suggest that observing the presence and abundance of middens on the forest floor is an efficient way to assess whether forests are invaded by *L. terrestris*, and, therefore, whether they have reached the late stages (4 and 5) of earthworm invasion. With each additional midden counted, *L. terrestris* was 1.5 times more likely to be sampled in sugar maple forest; and with each stepwise increase in the midden index, sampling of *L. terrestris* in aspen-birch and sugar maple forests was 3.7 and 38.0 times more likely, respectively. For all points combined, sensitivity (i.e., accurate assessment of known *L. terrestris* presence by midden counts ≥ 1 *or* index = present or abundant) was 91% and specificity (i.e., correct assessment of known absence by counts of zero middens *or* index = absent) was 77%. Furthermore, the specificity estimate may be conservative because this deep-burrowing species likely escaped detection during mustard sampling at some points where middens were observed.

Although we found significant relationships between individual variables and presence of each earthworm taxa, no forest floor characteristic other than middens is likely to be useful by itself for rapidly assessing invasion by different species. There was a non-significant positive relationship between *L. rubellus* and casting index. However, the utility of this variable for identifying *L. rubellus* presence in the field is uncertain because several earthworm species produce casting material (Edwards and Bohlen 1996), and there is no apparent method for distinguishing among casts of different species. *Lumbricus rubellus* presence was also related to reduced cover of maple seedlings, increased sedge cover, and increased total vegetation cover, in agreement with previous research showing substantial impacts of this species on forest floor plant assemblages (Hale et al. 2006; Holdsworth et al. 2007a). However, other environmental factors also influence understory vegetation cover (e.g., deer herbivory, light availability, and soil productivity) (Powers and Nagel 2008; Reich et al. 2012). Used by themselves, these vegetation cover metrics are unlikely to be useful for predicting *L. rubellus* presence. Further research should address whether incorporation of vegetation measurements into the 5-stage rapid assessment protocol can further improve its identification of *L. rubellus* invasion.

The positive relationship between *L.*

rubellus presence and midden counts at Wisconsin points is unexpected because *L. terrestris* is the only species in the region to create middens. Possible explanations for this correlation are that high-productivity forests favor high abundance of both species or that they are introduced together. The latter explanation is supported by observations that fishing bait is a common vector of introduction for each species and that both species are often present in bait labeled as containing only one or the other species (Keller et al. 2007).

Although presence of *Aporrectodea* was inversely related to litter depth and positively related to A-horizon depth in both forest types, other species co-inhabiting the surface layers of mineral soil – *L. rubellus* in particular – also consume the litter layer and increase A-horizon thickness by incorporating surface organic matter into the soil. Likewise, although *D. octaedra* presence was significantly more likely with increased sedge cover, other earthworm species and environmental factors influence this forest floor variable. Inferring presence of *Aporrectodea* based solely on the presence of a thick A-horizon or thin or absent litter layer and inferring presence of *D. octaedra* based on high sedge cover may, therefore, be inappropriate. Further investigation of relationships with A-horizon depth may allow attribution of varying A-horizon depths to particular earthworm species.

As discussed above, several environmental factors other than earthworms can lead to altered plant communities; and, furthermore, timber management activities can compress the litter layer and cause soil erosion (Yanai et al. 2000). These factors could result in false positive assessments of earthworm invasion. However, *L. rubellus* and *L. terrestris* have substantial effects on multiple aspects of the forest floor. The 5-stage protocol, which includes measurement of several variables, is less likely to result in false positive assessments than a protocol based on one or two forest floor measurements. Classification of points as earthworm-free when they are heavily invaded (i.e., false negatives) is also unlikely given earthworms' substantial effects and that other activities are unlikely to result in forest floors with un-altered soil, extensive plant cover, and a thick, intact litter layer. A limitation of the 5-stage protocol is that accurate assessment of invasion may be difficult when very few individuals of a species are present (e.g., at the invasion's leading edge). This limitation is evidenced by our observation of small numbers and very low biomass of earthworms at points that we classified as potentially earthworm-free.

Recommendations for Implementing the 5-Stage Classification Protocol

The 5-stage classification protocol will be useful across a large proportion of northern Midwest forests. Our analysis focused on sugar maple and aspen-birch forests, which make up a large percentage of forest land in the region, including 51% in Minnesota (Miles et al. 2004) and 29% in Wisconsin (Vissage et al. 2004). Other regions with invasive earthworms (e.g., the northeastern United States and much of Canada) have many of the same European earthworm species, and our protocol may also prove effective for identifying invasions in these areas. Asian earthworms (*Amynthas* spp.) are also invading portions of the eastern U.S., and where they dominate earthworm assemblages, the suite of effects to the forest floor may be different. In these cases, our protocol may be inappropriate; and we encourage development and testing of similar protocols based on assessment of forest floor characteristics.

The 5-stage assessment protocol requires no previous experience with invasive earthworms, and relatively little training. Following a short training session, the method can be easily adopted for use by land managers, biological technicians, researchers, and citizen science monitoring programs. Currently, we regularly conduct two-hour training sessions that prepare surveyors to conduct assessments quickly and independently (see: http://www.nrri.umn.edu/worms/research/IERAT.html*)*; and, in the future, online completion of training will be possible. A preliminary survey indicated that 81% of technicians who had completed training finished each earthworm survey in less than six minutes, and moreover, 90% of surveyors found the training easy to follow and critical for effectively assessing earthworm invasion (R. Hueffmeier and C. Hale, unpubl. data).

Intensive earthworm sampling methods will remain necessary for achieving high-precision estimates of species' composition and biomass. However, these methods are time-consuming and may result in inaccurate population quantification during unusually dry conditions when earthworms are less active (Edwards 1991). Classification based on forest floor characteristics is less sensitive to moisture variation than intensive sampling methods; and, therefore, our protocol can be conducted throughout the summer. The protocol also improves upon other techniques by providing an assessment of the ecological impact of earthworm invasion, rather than simply providing a list of earthworm species present. Results from the rapid assessment protocol can, therefore, be used to indicate locations where rigorous quantitative sampling and monitoring should be conducted or where land protection may be warranted. Furthermore, because earthworm-free and lightly invaded areas generally contain minimally altered plant assemblages, the rapid assessment protocol may be useful for targeting botanical surveys of rare and sensitive plant species. Depending on management objectives, the protocol allows a large number of points to be sampled in a small area to provide a high-resolution picture of invasion (e.g., in forest stands or state parks and natural areas), or numerous points can be sampled across a large scale to coarsely map invasion patterns (e.g., across watersheds, national forests, and national parks).

Budgets for management and conservation activities are limited. At the same time, it is becoming increasingly important to clarify earthworm impacts and to identify remaining earthworm-free areas in which to target conservation and management activities. The 5-stage earthworm invasion assessment protocol that we introduce here provides an efficient and effective method for achieving these objectives in hardwood forests of the northern Midwest and a blueprint for the development of protocols in other regions experiencing earthworm invasions.

ACKNOWLEDGMENTS

Research was funded by the American Museum of Natural History, Bell Museum of Natural History, Dayton-Wilkie Foundation, The Explorers Club, Great Lakes Worm Watch, Minnesota Department of Natural Resources, Minnesota Ornithologists' Union, Natural Resources Research Institute, U.S. Forest Service – Chequamegon-Nicolet National Forest, and Wisconsin Society for Ornithology. S.R.L. was supported by a University of Minnesota Graduate School Fellowship and an NSF IGERT grant: Risk Analysis for Introduced Species and Genotypes (NSF DGE-0653827). R.M.H. was supported in part under the Coastal Zone Management Act, by NOAA's Office of Ocean and Coastal Resource Management, in cooperation with Minnesota's Lake Superior Coastal Program, and also by the Legislative-Citizen Commission on Minnesota Resources. We thank C. Hakseth, L. Lambert, J. Mulligan, M. Sharrow, S.S. Loss, L. Olson, E. Wartman, A. Alness, N. Vander Heiden, M. Hueffmeier, E. Feichtinger, Z. Toland, C. Wright, K. Jeager, J. Johnson and the Minnesota Conservation Corps for field assistance. We also thank R.B. Blair, D.E. Andersen, P. Bolstad, H. Hansen, A. Holdsworth, and B. Sietz for guidance, L. Parker and M. Brzeskiewicz for logistical support, and the Cable Natural History Museum and Minnesota North Shore State Parks for housing and office.

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Biol Invasions
DOI 10.1007/s10530-009-9523-3

ORIGINAL PAPER

Tree rings detect earthworm invasions and their effects in northern Hardwood forests

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Received: 15 November 2008 / Accepted: 1 July 2009

Abstract Invasions of European earthworms into the forests of northern North America are causing dramatic changes in forest floor structure, vegetation communities, biogeochemical cycling, and site hydrology. However, long-term studies on the effects of invasive earthworms are limited because little data exist on the timing and rate of earthworm invasion at specific sites. We successfully used tree rings to identify the timing of earthworm invasions and the effects of earthworm activity on the *Acer saccharum* overstory of two recently invaded sites in northern Minnesota, thereby establishing a method to date earthworm invasions at other sites. In addition to identifying a tree-ring signature related to earthworm invasion, we found trees growing in invaded conditions were more sensitive to drought than trees growing in earthworm-free conditions. Increased drought sensitivity by *A. saccharum* has important implications for possible range shifts under climate change scenarios that include increasing drought frequency and severity.

Keywords *Acer saccharum* · Climate-tree growth relationship · Dendroecology · Earthworm invasion · European earthworms · Northern Minnesota

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Introduction

The ecological effects of invasive species on native ecosystems are of paramount concern among conservation biologists and ecologists and pose one of the greatest challenges faced by land managers today. European earthworms have only recently been recognized as an exotic threat for the deciduous forests of northern North America, yet the changes wrought by these ecosystem engineers have likely been taking place for decades and are fundamentally altering the structure and function of these systems.

Published online: 14 July 2009

Prior to a decade ago, relatively little attention was given to the role of exotic earthworms in forest ecosystems, with most earthworm-related research focused on their roles in nutrient cycling and the benefits of having viable earthworm populations in agricultural settings (Lee 1985). The focus of earthworm research began to change following observations of dramatic declines in forest floor thickness in deciduous forests of northern Minnesota that were eventually linked to the invasion of these forests by European earthworms (Alban and Berry 1994). Soon after this, exotic earthworm invasions into forested communities were documented in Alberta, Canada (Scheu and Parkinson 1994b) and New York State, USA (Burtelow et al. 1998), and eventually throughout the Great Lakes Region (Frelich et al. 2006; Tiunov et al. 2006). In most cases, the invasion of earthworms into previously earthworm-free forest environments resulted in a clear line moving through the forest, in front of which the forest floor appeared relatively unchanged, but behind which the litter layer was eliminated and the abundance and diversity of understory vegetation was diminished (Gundale 2002; Bohlen et al. 2004a; Bohlen et al. 2004c; Hale et al. 2005b, 2006; Frelich et al. 2006). The longer-term effects of these changes in ecosystem function and on the overstory vegetation community, however, are largely unknown (Bohlen et al. 2004b). A broader temporal perspective is required to observe the effects of earthworms on the vegetation communities of northern forests and to explore the implications that these changes hold for land management.

The potential for studying the long-term ecological effects of European earthworms on northern deciduous forests is currently limited by a lack of data describing the timing of invasions at specific sites, the rates at which invasion fronts advance across the landscape, and the environmental factors that affect these processes. Understanding these aspects of earthworm invasions will be critical to develop long-term strategies and management plans for North American forest ecosystems in the presence of European earthworms (Bohlen et al. 2004c). A method is needed to identify the location and timing of past invasion fronts across the landscape (Frelich et al. 2006; Holdsworth et al. 2007). Our research examined the effects of known earthworm invasions on the overstory vegetation of two deciduous forest stands in northern Minnesota to identify and describe an earthworm-related tree-ring signature that could be used to determine the date of earthworm invasions at other sites. Simultaneously, this work documented the impacts of earthworm colonization on the growth of mature trees in invaded stands.

Methods

Study area

The study area lies along the north shore of Leech Lake in north-central Minnesota (Fig. 1a). Our sites were located in sugar maple (*Acer saccharum*, Marshall)-basswood (*Tilia americana*, Linnaeus) forests with clear, active earthworm invasion fronts (Hale et al. 2005a, b, 2006). Additional canopy tree species present included yellow birch (*Betula alleghaniensis*, Britton) and paper birch (*Betula papyrifera*, Marshall). Ironwood (*Ostrya virginiana*, K. Koch), American elm (*Ulmus americana*, Linnaeus), and red oak (*Quercus rubra*, Linnaeus) were also present in the subcanopy and sapling layers. The area was selectively logged during the late 1800s and early 1900s. The soil is a deep, well-drained and light-colored Eutroboralf (Warba series) associated with the Guthrie Till Plain (USDA 1997). Where earthworms have not yet invaded, the forest floor is intact and on average 10 cm thick with O_i, O_e, and O_a layers. The O horizon has been eliminated where earthworms are present (Hale et al. 2005a). Mean annual precipitation is 65.2 cm, mean annual temperature is 4.3° C and mean monthly temperature ranges from 19.9°C in July to −14.5°C in January at the nearby Leech Lake Dam climate station (Easterling et al. 1996).

Five species of European earthworms were present in the study area: *Dendrobaena octaedra*, *Aporrectodea* spp., *Lumbricus rubellus*, and *Lumbricus terrestris* were found at both sites, while *Octolasion tyrtaeum* was present only at Section 19 (Fig. 1a, b; Hale et al. 2005a). Each species of earthworm has a specific set of foraging and burrowing behaviors that result in differential effects on the structure of the forest floor and litter layer. The overall similarity between the earthworm assemblages at these sites suggests that the effects of invasion should be similar in both forests. For a comprehensive description of the earthworm assemblages at these sites see Hale et al. (2005a).

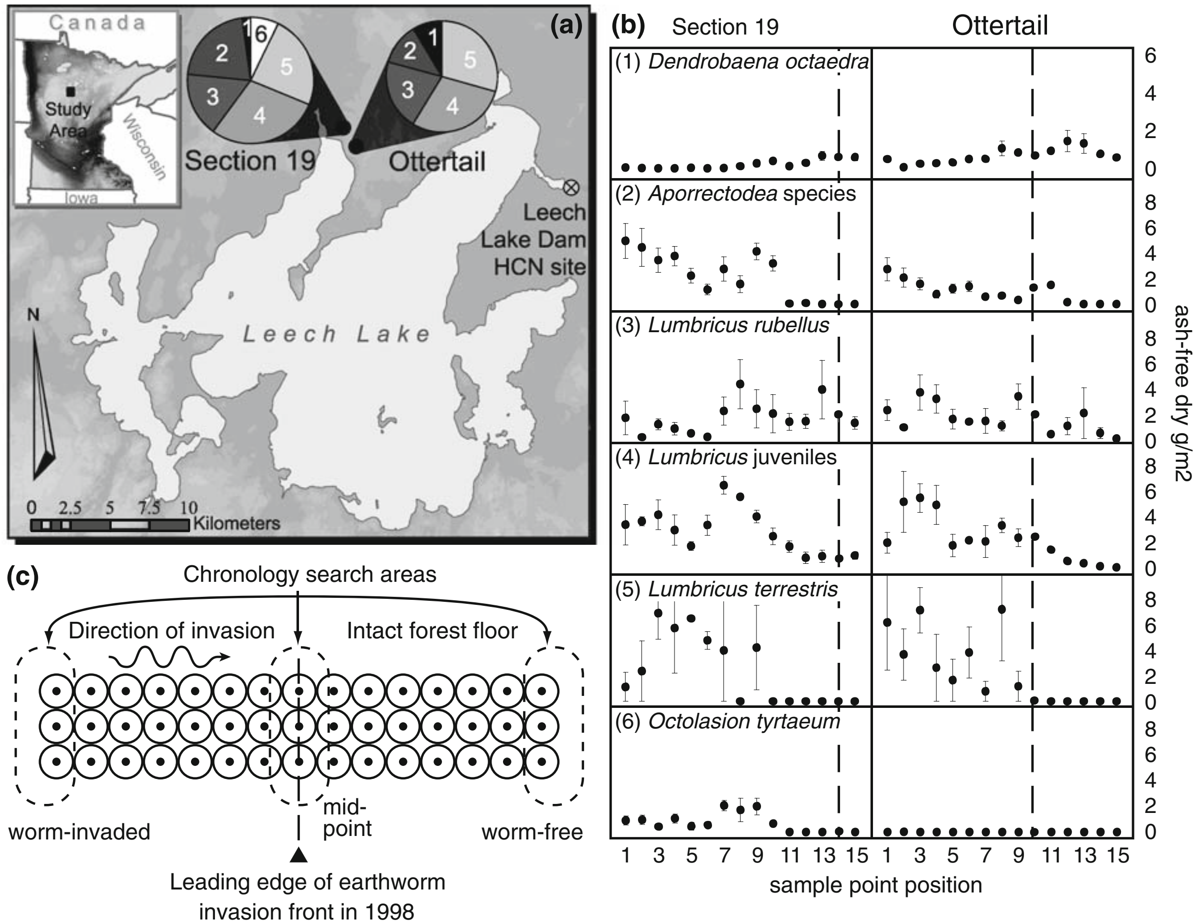

Fig. 1 The **a** study site locations and relative earthworm biomass, **b** earthworm biomass (ash-free dry g/m^2, Hale et al. 2004) relative to the earthworm invasion front, and **c** sample tree search areas relative to the visible leading edge of earthworm invasion at each site in the Chippewa National Forest in north central Minnesota, USA. *Pie charts* show the relative mean earthworm biomass for the species group present at each site in year 2000 and are sized in proportional to mean total earthworm biomass. *Numbers* in the Section 19 pie chart correspond to the numbers listed by each species in *panel* **b**. The *vertical dashed lines* in **b** indicate the position of the leading edge (point where the forest floor had been completely eliminated) for each site in year 2000. The **c** search areas for trees growing in earthworm-invaded conditions, mid-point conditions, and earthworm-free conditions were delineated with respect to the permanent plots (indicated by the *grid* of *circles*) set up at each site by Hale et al. (2005a) in 1998

Field and laboratory methods

A permanent transect of 45 plots was placed perpendicularly to the earthworm invasion front at both sites in 1998 by Hale et al. (2005a). For this study, we collected increment core samples from maple trees at three points along this transect: ahead of the location of the earthworm invasion front in 1998 (earthworm-free conditions); along the 1998 invasion front (mid-point conditions); and behind the location of the invasion front in 1998 (earthworm-invaded conditions; Fig. 1c). Our search areas included the three plots at each point along the permanent transect and extended ca. 20 m beyond the outer plots for total search areas ranging from 700–1,000 m^2 at each point along the transect (Fig. 1c). This effectively provided us with tree-ring data from two control areas (the earthworm-free and mid-point conditions) and one test area (the earthworm-invaded conditions) at each site. In each collection area we identified living canopy maple trees to sample that showed no evidence of recent canopy disturbances. This was done to avoid the potential “noise” that could be introduced to the tree-ring sequences by growth releases or suppressions related to canopy disturbances (Lorimer and Frelich 1989) and that could mask the effects of earthworms on patterns of tree growth. Single increment cores were collected from maple trees growing in the earthworm-free and earthworm-invaded sites at Ottertail for a pilot study in 2004, and two increment cores were collected from each tree at the mid-point of Ottertail and at all three areas of Section 19 in 2006.

The cores were dried, glued into core mounts, and prepared for analysis using a razor blade to obtain a preliminary surface and progressively finer grit sand paper to finish the surface of each core to a high polish (Stokes and Smiley 1996). The rings of each core were counted and visually crossdated using a list of marker rings (i.e., conspicuously narrow rings) common to multiple trees at the site (Yamaguchi 1990). Cores that could not be satisfactorily dated were excluded from subsequent analyses. The rings of all dated cores were measured to the nearest 0.001 mm using a Velmex slide micrometer interfaced with measure J2X software (Voortech Consulting 2005). We assessed our crossdating accuracy using the computer program COFECHA v6.06P (Holmes 1983; Grissino-Mayer 2001), and where necessary, made corrections to the measurement series using the program EDRM v6.0P (Holmes 1999).

We developed standardized ring-width chronologies for each of the three sampling locations at both sites for a total of six chronologies. Our standardization process began by visually inspecting each individual tree ring-width series to determine the forest conditions under which the tree likely established (e.g., in full light under a gap or in shaded understory conditions) and the timing of its canopy ascension (Lorimer et al. 1988). To minimize the effects of growth suppressions and releases due to gap dynamics, we truncated all of our series to the period after which all trees included had attained a canopy position. Truncating the ring-width series also effectively removed growth trends related to juvenile growth, thereby eliminating the necessity of using negative exponential curves or linear regressions as commonly used in the standardization process (Fritts 1976). We considered standardizing our ring-width data using a model developed for closed-canopy ring-width analyses (e.g., cubic splines, Cook and Peters 1981), but decided that the effects of earthworm invasion on tree ring-width patterns could be similar to the expressions of interior deciduous forest dynamics and removed from the final chronologies using this approach. We therefore chose the more conservative approach of standardizing each ring-width series by dividing the measurement of each year by the mean of the series. The ring-width index (RWI) of all series at each site were averaged by year to create standardized RWI chronologies for each site. We used the computer program ARSTAN to conduct our standardizations (Cook 1985).

Identification of an earthworm invasion tree-ring signature

The primary goal of this study was to identify patterns in ring width related to the known invasion of our sites by European earthworms, but tree growth in mature deciduous forests is commonly affected by other factors, particularly gap dynamics (e.g., Runkle 1981; Foster 1988; Lorimer and Frelich 1989). We therefore assessed the growth patterns exhibited by each tree (based on the mean of the two ring-width series for each tree) for release events that would likely be associated with canopy disturbances. We defined a release event as the first year of a 15-year window in the ring-width series that showed a 200% increase in mean ring width relative to the previous 15 years of mean ring width (Lorimer and Frelich 1989; Frelich 2002). We then summed the number of release events by decade for each of the six sites to determine if any stand-level disturbances had occurred that could obscure an earthworm-related tree-ring signature.

To isolate the earthworm-related ring-width signature, we first plotted and inspected the annual RWI chronologies and the RWI chronologies filtered using a 10-year smoothing spline to highlight decadal-scale trends. We supplemented our visual analyses by conducting two-tailed Student's *t*-tests on 15-year sliding windows of the annual RWI chronologies from each site to test our null hypothesis that there would be no difference in growth rates between trees growing in earthworm-free and earthworm-invaded conditions. The result of each *t* test was reported for year 8 of the 15-year window. While this approach ordinarily would call for a one-tailed test, the use of the two-tailed test allows for the identification of significant differences that did not match our expected growth patterns. This was particularly useful since our primary aim was to determine if an earthworm signal could be detected in tree-ring chronologies, regardless of sign. Additionally, given the large number of t-tests that would be required (62 15-year windows for each comparison), it would be expected that by chance alone at least some 15-year periods would be significantly different from one another. To examine the likelihood of chance significant relationships we created random time series equal in length to the chronologies under consideration and with similar means, standard deviations, and autocorrelation structures. While these artificial time series did not reflect an underlying age-structure such as in our tree-ring chronologies, the overall similar characteristics of the artificial and real time series provided a reasonable data set to test for the potential effects of multiplicity on our analysis. T-tests were performed as above with the number of significantly different windows calculated. This was repeated 1,000 times, noting the number of significant windows at each iteration.

Other than the presence of earthworms, the environmental conditions within each site were similar. Therefore, if the changes induced in the environment by earthworm invasion did not affect tree growth then the patterns in the three RWI chronologies from each site should be similar and our null hypothesis would stand. If earthworm activity did affect tree growth we could expect to see different growth patterns in chronologies developed from trees growing in earthworm-invaded conditions than the patterns exhibited by chronologies developed from trees growing at the mid-point and in earthworm-free conditions, thus rejecting our null hypothesis. Additional support for these analyses would be provided if the patterns of difference were similar between the two earthworm-invaded sites. Following this logic we identified the potential dates of earthworm invasion at each site and stratified the RWI chronologies based on these dates. We used Pearson's correlation analyses to compare patterns in tree growth across each site before and after earthworm invasion and Fisher r-to-z transformations to test for differences among the correlation coefficients.

Climate-tree growth analyses

We examined the climate-tree growth relationship recorded in the RWI chronologies at each site to explore potential climatic mechanisms influencing the different ring-width patterns exhibited by trees growing in earthworm-invaded conditions relative to those growing in earthworm-free conditions. We obtained precipitation data and mean, maximum, and minimum monthly temperature data from 1929–2005 for the climate station at Leech Lake dam (Easterling et al. 1996), and Palmer Drought Severity Index (PDSI) data from 1929–2005 for Minnesota State Climate Division 2 (Kalnay et al. 1996). The PDSI is a measure of soil moisture availability, with positive values indicating more moisture available and negative values indicating less moisture available (Alley 1984). We calculated seasonal values for each of these variables as follows: winter (previous year December–current year February), spring (March–May), summer (June–August), and fall (September–November). Because tree growth is affected by the climate of both the current and previous years (Fritts 1976), we used Pearson's correlation analyses to construct a correlation matrix between the six RWI chronologies and all of the variables by individual month and season for the current and the previous year. We identified the variables that showed the strongest relationship to tree-growth over the entire 1930–2005 period and conducted additional correlation analyses between these variables and the RWI chronologies stratified by the timing of earthworm invasion at each site. We tested for differences in the correlation coefficients among the three chronologies from each site for the two time periods using Fisher's r-to-z transformations.

Results

Tree-ring chronology development

We collected 126 core samples from 81 maple trees that showed no evidence of recent canopy disturbances at the six sites. Of these, we successfully crossdated 117 cores from 76 trees. The growth patterns of the 76 dated samples indicated that all of the trees had attained a canopy position by 1930, and the sample depth for each chronology was relatively constant over the period 1930–2005 (Fig. 2). The samples crossdated well over the entire record and the series inter-correlations were relatively high over the period of analysis for RWI chronologies

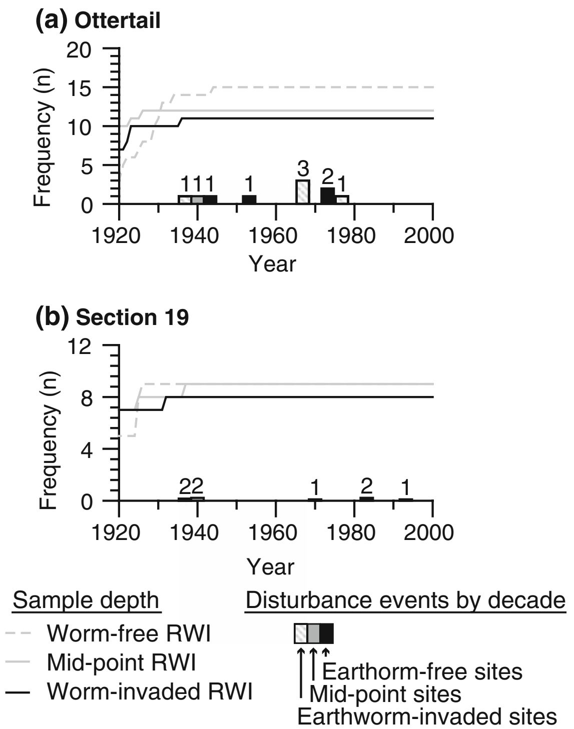

Fig. 2 Sample depth and release event frequency by decade as observed in the ring-width series of the trees sampled for the six chronologies. *Numbers* above the bars indicate the number of trees recording a release event in each decade

Table 1 Descriptions of the tree-ring chronologies developed from sugar maple trees growing in earthworm-free and earthworm-invaded conditions at two sites

Site	Years	No. trees (series)	Series inter-correlation[a]	
			Entire record	1930–2006
Ottertail				
Worm-free site	1884–2006	19 (19)	0.297	0.336
Mid-point site	1843–2006	12 (24)	0.590	0.587
Worm-invaded site	1900–2006	15 (15)	0.336	0.333
Section 19				
Worm-free site	1818–2006	12 (24)	0.474	0.512
Mid-point site	1828–2006	9 (17)	0.446	0.392
Worm-invaded site	1873–2006	9 (18)	0.430	0.499

[a] Series inter-correlation is calculated as the average Pearson's correlation of each ring-width series with a master tree-ring chronology derived from all other individual series at a site. All correlations are significant at $P \leq 0.05$

developed from trees growing in closed-canopy conditions (Table 1). We identified 18 release events across all six RWI chronologies from 1930–2005, with no more than three trees at a site recording releases in any one decade (Fig. 2).

Identification of an earthworm invasion tree-ring signature

The annual RWI chronology plots showed similarly timed periods of low growth among all six chronologies, such as the late 1930s, the mid 1950s, and the early 1980s, but of greater interest to this study were the differences identified between the chronologies developed from earthworm-free areas and earthworm-invaded areas at both sites (Fig. 3). At both Ottertail and Section 19, the chronologies from the earthworm-invaded areas diverged from the earthworm-free chronologies (Point 1 in Fig. 3a, b) during a period of narrower RWI for ca. 20 years at Ottertail and ca. 30 years at Section 19. After that period the earthworm-invaded area RWI chronologies increased relative to the earthworm-free RWI chronologies (Point 2 in Fig. 3a, b). These patterns were more evident in the smoothed RWI chronologies (lower panels in Fig. 3a, b). The *t* tests identified several periods where the annual RWI chronologies from the earthworm-invaded areas at both Ottertail and Section 19 were significantly different than the earthworm-free RWI chronologies (bold lines in top panels of Fig. 3). Both sites showed short periods of contrasting differences early in the period considered (ca. 1944 at Ottertail and 1937 at Section 19), followed by periods of different timing and length but of the same general pattern of significantly narrower RWI initially followed by a shift to significantly wider RWI. Only one 6-year period was identified where either one of the mid-point RWI chronologies was significantly different from the earthworm-free RWI chronology, and of the 1000 randomized time series and associated *t* tests, none showed significant differences for any of the 62 windows tested (or over approximately 62,000 trials). Our observations during previous research of the general rate at which earthworm invasion fronts advanced across these sites (ca. 10 m/year) indicated

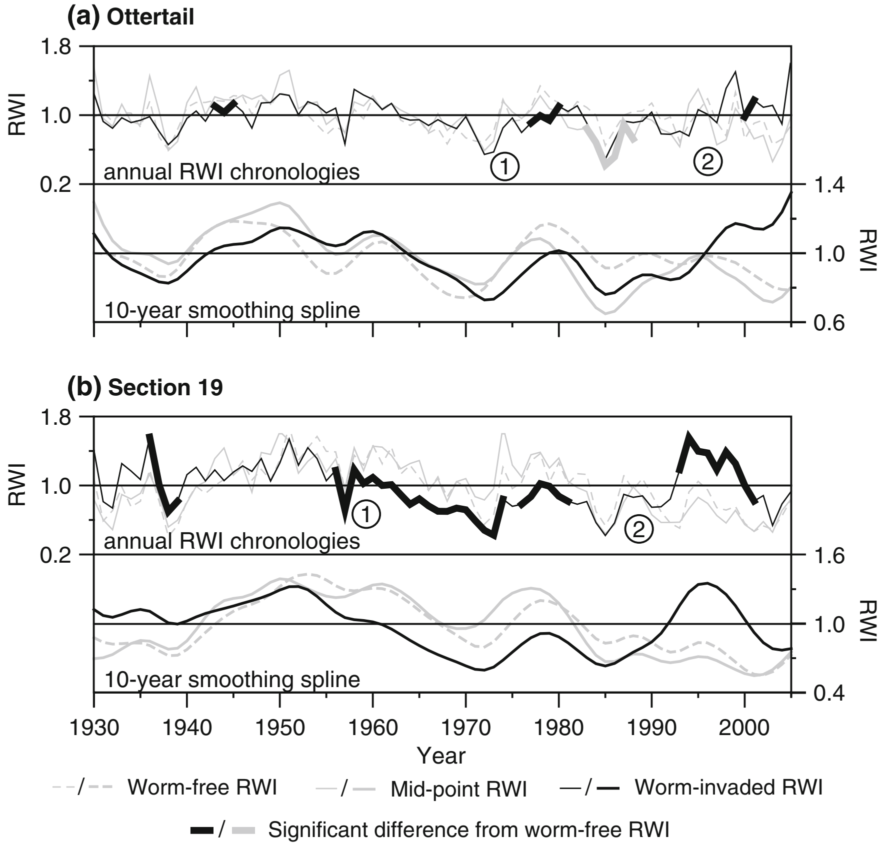

Fig. 3 Annual and smoothed ring-width index (*RWI*) chronologies developed from trees growing in earthworm-free and earthworm-invaded conditions. Differences between the RWI chronologies were tested for using Student's *t* tests on sliding 15-year windows with significance set at $P \leq 0.05$. The *circled numbers* in the *lower panels* indicate (*1*) the timing of earthworm-invasion at that site indicated by the initial departure of the earthworm-invaded RWI chronologies from the reference chronologies and (*2*) a period of recovery by the trees growing at the invaded sites

that the earliest dates most likely precluded the arrival of earthworms to either site. We therefore estimated that the earthworm invasion at Ottertail began sometime during the 1970s and at Section 19 sometime during the late 1950s to early 1960s.

Using the dates obtained from our visual analyses and *t* tests to stratify the RWI chronologies resulted in striking patterns of correlation between the chronologies over different time periods that appeared to pivot most strongly on the years 1970 at Ottertail and 1960 at Section 19. At both Ottertail and Section 19 correlations between all chronologies were highly significant prior to earthworm invasion, yet after that time the correlations between the earthworm-free and earthworm-invaded RWI chronologies deteriorated while the correlations between the earthworm-free and mid-point RWI chronologies, which were earthworm-free until ca. 1998, remained strong (Fig. 4). Correlations between the earthworm-free and earthworm-invaded RWI chronologies following invasion were significantly different ($Z > 2$, $P < 0.05$) from the correlations between all of the RWI chronologies during the earlier earthworm-free period and the earthworm-free and mid-point RWI chronologies following earthworm invasion (Fig. 4). Iteratively examining these patterns using different potential invasion dates consistently showed that the strongest and most consistent differences in tree-ring growth pivoted on the early 1970s at Ottertail and the 1960s at Section 19, thus solidifying these as the approximate dates of invasion.

Climate-tree growth analyses

In general the RWI chronologies developed at Section 19 were more sensitive to climate than those from Ottertail, yet similar patterns emerged in the overall climate-tree growth relationships among all of the chronologies and how earthworm invasion changed these relationships. While not significant in some cases, the climate variables that showed the strongest and most consistent relationship across the six RWI chronologies were mean monthly maximum temperature and mean summer PDSI for the current

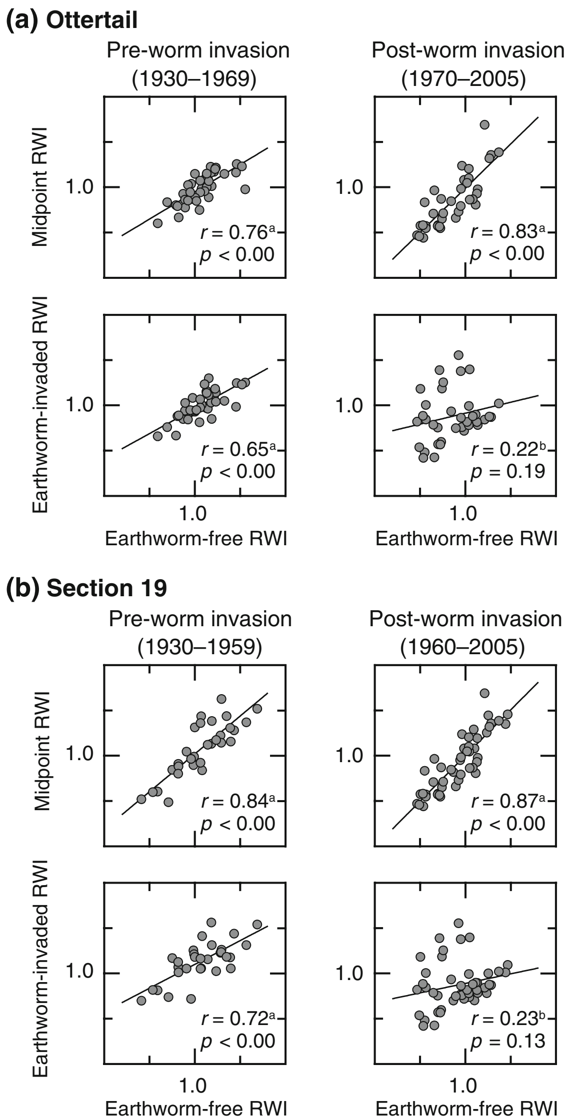

Fig. 4 Correlations between RWI chronologies developed from trees growing in earthworm-free, mid-point, and earthworm-invaded conditions before and after the onset of earthworm invasion. Different *superscript letters* indicate significantly different correlation coefficients

year and total annual precipitation and mean monthly PDSI for the previous year. During the earthworm-free period, all six RWI chronologies were negatively correlated with the current year's mean monthly maximum temperature, and positively correlated with the current year's mean summer PDSI, the previous year's total precipitation, and previous years mean monthly PDSI (Fig. 5). After earthworm invasion, the RWI chronologies from the earthworm-free and mid-point areas showed negative or neutral correlations with all of these climate variables, while in contrast, the earthworm-invaded sites showed positive correlations with all of these climate variables, including significantly different correlations ($Z > 2$, $P < 0.05$) with the current year's mean summer PDSI and the previous year's total precipitation at both Ottertail and Section 19 (Fig. 5a, b). The shifting correlations between the chronologies developed from trees growing in the earthworm-free and mid-point areas and climate likely reflected the generally different climate conditions during these periods, with cooler, drier conditions during the 1930s to 1950s and warmer, wetter conditions prevailing during the 1960s up to the 2000s (Fig. 5c). Plotting the correlation coefficients between the RWI chronologies and the temperature and precipitation variables illustrates the contrasting directional changes in climate-tree growth relationships between the earthworm-free and earthworm-invaded sites (Fig. 6).

Discussion

Synthesizing the evidence of an earthworm-related tree-ring signature

The use of tree rings to study the dynamics and ecological effects of biotic disturbances in forested ecosystems has a well-developed history. Researchers have analyzed patterns in tree-ring width to identify past outbreaks of defoliating insects (e.g., Blais 1958; Morin et al. 1993; Swetnam and Lynch 1993; Asshof et al. 1999; Speer et al. 2001) and have used stand age structure or non-host species growth releases to identify outbreaks of pests that commonly lead to the mortality of mature trees (e.g., Baker and Veblen 1990; Veblen et al. 1991; Perkins and Swetnam 1996; Bergeron 2000; Fraver and White 2005). Most of these studies have focused on pests that directly affect the foliage or cambium of their host trees, and only a few have investigated the effects of pest species on below-ground biomass and function of trees (e.g., Koenig and Liebhold 2003). Our research builds on this rich literature by developing a novel application of tree-ring width information to reconstruct the timing of past invasions by European earthworms into previously earthworm-free forests and to examine the effects that these ecosystem

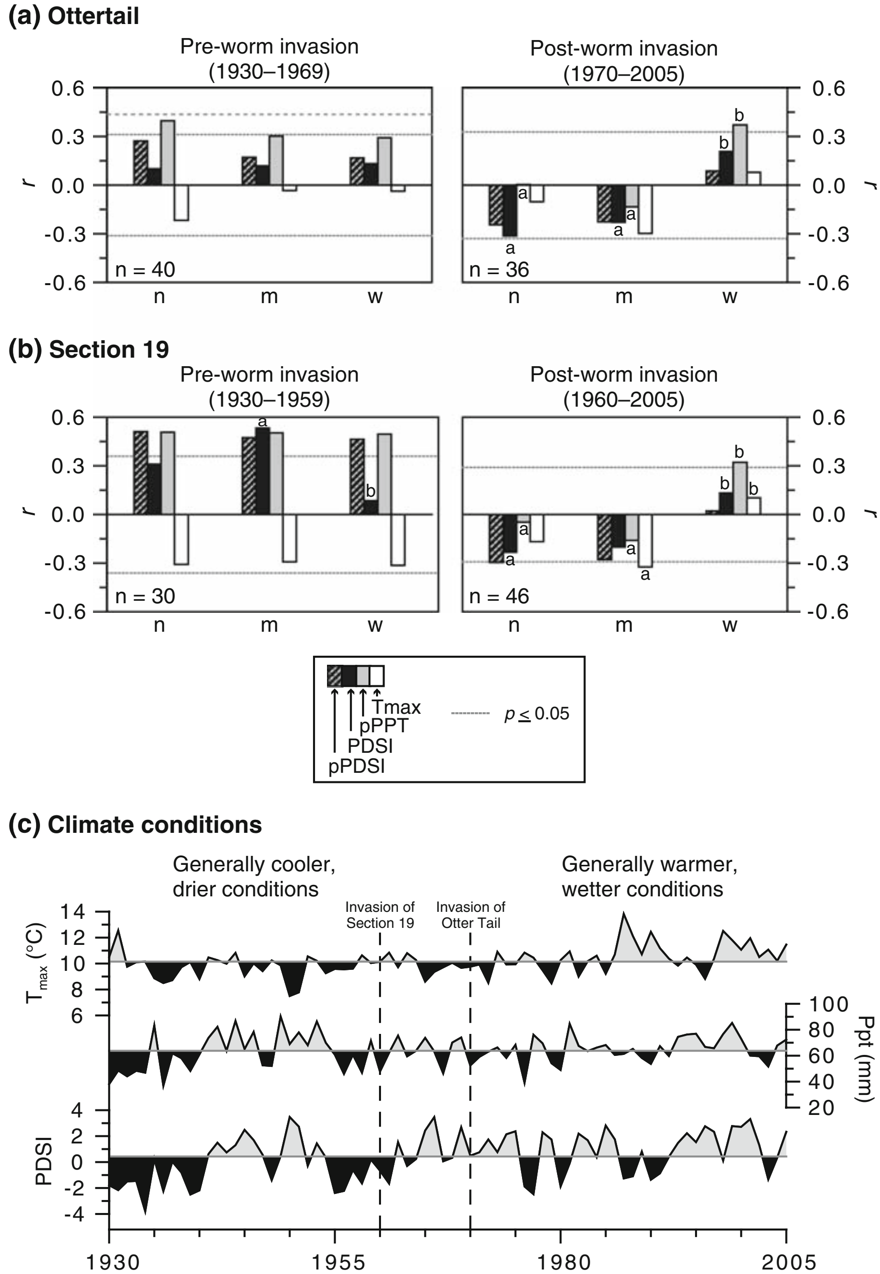

Fig. 5 Pearson correlations between climate and the standardized ring-width chronologies from **a** Ottertail and **b** Section 19, and **c** general climate conditions for our study area since 1930. Different *letters* indicate significantly different correlation coefficients. The climate variables are graphed relative to their 1930–2005 averages

engineers have on the relationship between tree growth and climate.

The relatively high series inter-correlations of the RWI chronologies, the significant correlations among the RWI chronologies at each site, and the similar relationships between the RWI chronologies and climate variables from the earthworm-free and earthworm-invaded areas over the early periods at Ottertail and Section 19 indicate that the trees growing at these sites before earthworm invasion were responding to the same stand-scale, and perhaps regional, environmental conditions (Fritts 1976; Schweingruber 1996). Additionally, the lack of widespread release events at any of the sites suggests that no stand-wide disturbance events occurred over our period of analysis that would influence our results. Therefore, our comparisons between the RWI chronologies developed from trees growing in earthworm-free conditions to those

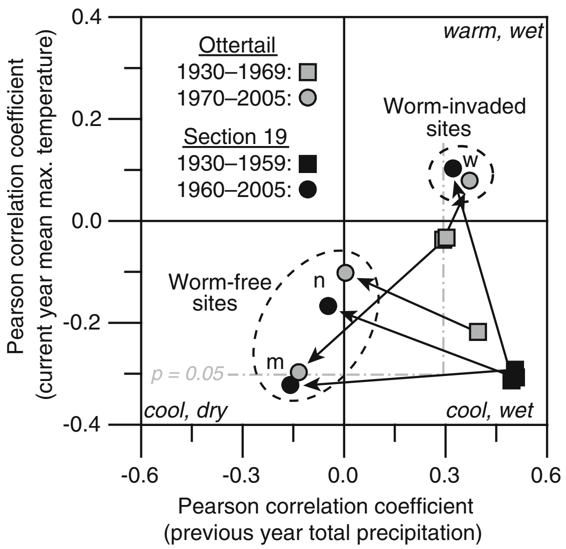

Fig. 6 Conditions promoting wider rings in trees growing in the earthworm-free sites (*n*), mid-point sites (*m*), and earthworm-invaded sites (*w*) before and after earthworm invasion. The delineation of $P = 0.05$ is based on the interval with the fewest years in the analyses (1930–1959, $n = 30$)

developed from trees growing in earthworm-invaded conditions is effectively using a host/non-host approach common in dendroecology studies (cf. Fritts and Swetnam 1989; Kipfmueller and Swetnam 2001).

The tree-ring signature of earthworm invasion, as identified in this study, includes three components: (1) a period of 20–30 years of reduced radial growth rates in trees growing in newly invaded areas relative to trees growing in the same stand but in earthworm-free conditions; (2) a subsequent period of increased growth by the trees in the earthworm-invaded areas relative to trees in the same stand growing in earthworm-free conditions; (3) the deterioration of the common ring-width signal between trees growing in earthworm-free and earthworm-invaded areas. It is important to note that this signature was identified through a series of complementary analyses. Taken alone, each analysis would likely be unable to identify the arrival of earthworms into these stands, yet when used in conjunction with one another they provide a clear illustration of the earthworm-related tree-ring signature and the date when earthworms first entered these sites. It should also be noted that while the results of some of our analyses are contingent on the dates of invasion estimated from the initial visual examination of the tree-ring chronologies, iterative testing of other dates consistently showed our selected dates of invasion as the point that distinguished the strongest most consistent differences between the earthworm-free and earthworm-invaded sites. Additionally, the persistent patterns of deviation identified by the *t* test approach using the real time series, in contrast with the lack of significant differences found in the randomized trial, improves our confidence that these differences are indeed real. The sampling design attempted to control for climate (sites are similar), disturbance, and other factors that may have led to differences suggesting that the differences between chronologies are indeed evidence of the effects of invading earthworms.

The earlier invasion at Section 19 is corroborated by its geographic location and the earthworm biomass data. A primary source of earthworm invasions in northern Minnesota is the release of unused fishing bait and the intentional introduction of earthworms for the purpose stocking fishing bait (Frelich et al. 2006). The likely epicenter for the earthworm invasion fronts moving through Section 19 and Ottertail is a fishing resort on Leech Lake that was established in the 1950s. Our site at Section 19 directly borders the resort property, while the Ottertail site is separated from the resort by a paved road and several hundred yards further inland. The earthworm biomass data indicate a more advanced invasion at Section 19, with a more complete species assemblage and greater total earthworm biomass (Fig. 1a, b).

Interestingly, the mid-point chronologies at both Ottertail and Section 19 exhibited lower growth rates relative to the earthworm-free chronologies ca. 1985. While the visible leading edge of earthworm activity was at the midpoint of each site in 1998, the earthworm biomass data indicates that both *D. octaedra* and *L. rubellus* were found in front of this line. These species, and in particular *L. rubellus*, may affect site conditions sufficiently to initiate the early declines in tree growth that we have identified in the earthworm-related tree-ring signature. Additional research is needed to explore the species-specific effects of earthworm invasions.

Potential mechanisms linking earthworm activity to a tree-ring signature

The tree-ring signal that we observed at our sites was not a discrete event typically associated with biotic disturbance agents, such as a period of defoliation (e.g., Duncan and Hodson 1958; Speer et al. 2001), but instead reflected relatively sudden and long-lasting changes wrought in the growing environment of the sampled maple trees following the invasion of European earthworms into these previously earthworm-free sites. The most conspicuous and consistently observed change in forest systems after invasion by earthworms is the elimination of the O horizon (Gundale 2002; Bohlen et al. 2004a, c; Hale et al. 2005b; Frelich et al. 2006). The loss of the O horizon results in cascading ecological changes that are likely the primary cause of the tree-ring signature exhibited by trees growing in earthworm-invaded conditions.

Earthworms eliminate the O horizon by mixing the organic material of which it is composed as well as each annual litter fall material throughout the top layers of the soil profile (Ponge and Delhaye 1995; Gundale 2002). This creates an organic rich, homogenous A horizon (Burtelow et al. 1998) subject to increased rates of erosion at the exposed soil surface and an overall compaction of the soil due to the higher bulk density of earthworm casts than that of undisturbed forest soils (Hale et al. 2005b). These changes, in turn, result in decreased rates of water infiltration (Francis and Fraser 1998).

The O horizon of an intact northern deciduous forest floor contains the greatest amount of fine roots for understory plants, tree seedlings, tree saplings, and adult trees of the soil profile (Fisk et al. 2004). By eliminating this layer, earthworm invasions are linked to reductions in fine root mass (Fisk et al. 2004; Hale et al. 2005b) and high rates of mortality of plants in the understory community (Hale et al. 2006). In addition to the loss of fine roots, the loss of the O horizon disrupts mycorrhizal communities in sugar maple forests (Lawrence et al. 2003). The uptake of several important nutrients, including nitrogen and phosphorus, occur through the fine root systems of trees and their associated mycorrhizal communities (Wood et al. 1984; Nadelhoffer et al. 1985; Marschner and Dell 1994). The physiological shock that trees must experience following earthworm invasion and the loss of their fine root systems is likely the cause of the initial decrease in growth rates we identified in the ring-width chronologies developed from trees growing in earthworm-invaded conditions. Following this shock, as trees reestablish their fine roots systems, an ephemeral pulse of phosphorus and nitrogen released when the O horizon is first mixed into the soil profile following earthworm invasion (Scheu and Parkinson 1994a; Hale et al. 2008) would become available and could explain the increased growth rates exhibited by trees growing in worm-invaded conditions. As illustrated at Section 19, however, these increased growth rates are short-lived. This likely reflects the accelerated leaching of nutrients such as nitrogen and phosphorous following their mineralization and concentration by earthworms (Steinberg et al. 1997; Bohlen et al. 2004b; Suarez et al. 2004; Szlavecz et al. 2006).

The effects of earthworm invasion on climate-tree growth relationships

Climate-tree growth relationships in northern deciduous forests vary over space and time (Graumlich 1993; Tardif et al. 2001). Trees in different canopy positions also respond to the same climate conditions differently (Orwig and Abrams 1997). It was therefore not surprising to find the strength of the relationships between the RWI chronologies and the different climate variables shifted over the period of our analyses. The inverse correlations with temperature and positive correlations with precipitation and PDSI of all six RWI chronologies during the early period indicated that the growth of these trees was largely related to drought conditions, where cool moist conditions encouraged wider rings and warm, dry conditions resulted in narrow rings. The 1930s and 1950s were very dry in this region (Karl and Koscielny 1982), and small variations in moisture and temperature likely had large effects on what may have been chronically moisture-stressed trees. In contrast, the 1960s through the early 2000s were relatively wet (mean PDSI for 1961–2001 = 1.06) and reduced the sensitivity of trees growing in earthworm-free conditions to variations in precipitation. At the same time, tree growth in the earthworm-invaded areas remained significantly and positively correlated with precipitation, which one would expect for trees with poorer water status. These contrasting relationships are perhaps the result of changes in site hydrology and/or tree fine root systems due to earthworm invasion.

The O horizon in unaltered forests acts as a buffer between the soil and the atmosphere by moderating the moisture and temperature regimes of the soil surface (Kimmins 2003). The removal of this buffer leads to higher evaporation rates and less insulation from temperature extremes, and when coupled with the compaction of forest soils by earthworms (Hale et al. 2005b), results in increased runoff, decreased rates of moisture infiltration, and increased frequency and amplitude of drought conditions in the soil (Francis and Fraser 1998). The loss of the O horizon in earthworm-invaded sites therefore decreases the residence time of moisture in the forest following rainfalls by increasing runoff rates and removing the buffer provided by the decomposing organic material. In turn, this likely increases the frequency in occurrence of drought conditions at earthworm-invaded areas so that tree growth may be limited by low moisture availability even during periods where the average climate conditions are relatively wet, as shown in our climate-tree growth analysis of trees growing in earthworm-invaded conditions. In contrast, an intact O horizon during generally wetter climate conditions may in fact prolong saturated soil conditions (sensu Hook 1984), particularly following spring melt off and rains (Payette et al. 1996; Tardif et al. 2001). This could explain the negative correlations between PDSI and tree growth in earthworm-free sites during the later period of our analyses.

The results of our climate-tree growth analyses hold potentially important implications for the effects of earthworm invasion on forests in a changing global environment. Models predict that the future climate of the Midwestern US will likely include more frequent and severe droughts (Tebaldi et al. 2006). The range of many tree species that are currently found in this region are expected to change as a result (Auclair et al. 1996), with suitable habitat for sugar maple being eliminated in Minnesota over the next century (Iverson et al. 2008). Increased sensitivity of sugar maple trees to drought conditions following earthworm invasion may accentuate and accelerate the loss of this species from the state and region if droughts do indeed become more frequent and severe.

Conclusions and implications for future research

The results of our research indicate that dendrochronology can serve as a viable tool to date past European earthworm invasions into previously earthworm-free environments and creates new opportunities to study the long-term ecological effects of earthworm invasions on North American forests. Our research also illustrates the effects that earthworm invasions have on the climate-tree growth relationships in northern hardwood forests, with important implications for forest management under changing climate conditions and drought frequency. Future research should use both tree-ring widths and chemical evidence contained in tree rings (e.g., biogenic element concentrations, isotopic ratios of nitrogen and/or oxygen) to explore if these results are consistent among forests with different stand histories and environmental settings and address in more detail how the environmental conditions created by earthworms affect the climate-tree growth relationships of trees growing in northern deciduous forests.

Acknowledgments We thank Ian Aldrich, Ruth Baker, Chaïna Bapikee, Ryan Hueffmeier, Danny Margoles, and Julia Sawa for their assistance in the field, and Kenny Blumenfeld for his insightful comments on the most recent climate predictions for Minnesota. The comments of two anonymous reviewers and Daniel Simberloff improved this manuscript.

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