THE USE OF FIRE IN THE CONTROL OF INVASIVE, EPIGEIC EARTHWORM SPECIES IN THE SOUTHEASTERN UNITED STATES

by

JAMES HINES BLACKMON IV

(Under the Direction of PAUL F. HENDRIX)

ABSTRACT

Invasive species have become one of the largest problems in the field of ecology in the past few decades. Invasive earthworms have recently gained attention in North America where they have become a growing problem. Invasive earthworm species have been shown to have severe impacts on many ecosystem level properties such as community structure and nutrient cycling. In order to fully understand the invasive success of the exotic, invasive species *Amynthas agrestis* we tested temperatures as an environmental clue for cocoon hatching and determined 10ºC was the optimal temperature for emergence. We then tested whether or not fire, either directly or indirectly, impacts invasive earthworm survival and demonstrated that survival is diminished indirectly post-fire. These results will help in future control of the spread of invasive earthworm species in the southeastern United States.

INDEX WORDS: Invasive species, Earthworm, *Amynthas*, *Lumbricus terrestris*, Wildfire, Prescribed fire, Cocoon, Temperature, Eastern deciduous, Forest

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Perhaps the reason Paul considered me worthy of becoming one of his graduate students was the expert mentoring provided by one Becky Ball. Entering into my senior year of undergraduate education, I decided to better myself (and résumé) by applying for a laboratory position somewhere at UGA. I saw an e-mail from Becky needing help and I applied. I showed up for an "interview" in a suit and tie and was almost laughed at (always dress for success, right). Unbeknownst to me, I was applying for a very grungy and dirty job assisting Becky in her Ph.D. work. After about a year of grinding, sorting and counting samples (not to mention a multitude of other tasks I constantly complained about) I was finally molded into a shape resembling a soil ecologist (at least I thought so). So Becky, for taking all of my complaining and making something out of it, I give you unsurpassed gratitude. I have said it before and I say it again, were it not for you, I would not be where I am today.

My complete gratitude goes out to my family for their support. My wife, bless her, has tolerated me for many years now through thick and thin, good times and bad and supported me throughout, even when she was the primary “bread-winner.” My mom has not only been my best friend throughout my lifetime but has lent her generous financial support and backing throughout my college career and without this, I would be nowhere. Mom, I could not have done any of it without you and I will probably never be able to return all that you have given me but I will try. Thanks also goes out to my grandparent, Francia and Donald Cofer (and like-grandparent Patsy and Marvin Rathbone), for their raising of me and financial help along the way. As a single parent, my mother had it tough and these people helped her and me. My father, James H. Blackmon III, piqued my interest in most scientific things related to the outdoors and taught me many life lessons along the way. To the multitude of other family (step and in-laws included) your contribution doesn’t go unnoticed they are merely too generous and numerous to name here. Thanks to you all.

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Introduction and Literature Review

Effects of Temperature on Hatchling Emergence in Amynthas agrestis Cocoons

Exotic, invasive earthworm species are a growing problem in North American ecosystems, as well as around the globe, because these organisms have been linked to changes in many ecosystem level properties (Steinberg et al. 1997; Bohlen and Parmelee 1999; Bohlen et al. 2004 A; Groffman et al. 2004; Hale et al. 2005, 2006; Hendrix et al. 2008). Invasive earthworms have been shown to affect forest floor dynamics by changing understory plant and faunal communities (Hale et al. 2006). Invasive earthworms have also been linked to decreases in organic horizons of soil (Oa, Oe, and Oi), as well as soil transformations from a mull-type soil profile to a mor-type (Groffman et al. 2004; Hale et al. 2005, 2006). Most studies of exotic earthworms have focused on ecosystem effects and documentation of occurrences of invasions, but much less attention has been paid to basic biology and life history traits (Terhivuo and Saura 2006). However, information of this kind is increasingly available in applied earthworm research fields because of a rapid expansion in the past 20 years in use of earthworms for ecotoxicological studies, soil restoration, and biomonitoring (Lowe and Butt 2005). In this context, reproductive rates and life history traits have been extensively studied for many lumbricid (Family: *Lumbricidae*) species (Lowe and Butt 2005; Terhivuo and Saura 2006; Grigoropoulou et al. 2008), but little is known about the reproductive capacities of many other important invasive species around the globe (Hendrix et al. 2008).

Although the invasion of European earthworms into North America has long been recognized and studied in the United States (Bohlen et al. 2004 B; Hendrix 2006), the newer, secondary invasions of Asian species have been little studied until recently and are not well understood. Asian earthworms in the genus *Amynthas* (Family: *Megascolecidea*) are currently invading areas around the globe, including North America, Central America, and Europe (Fragoso et al. 1999; Hendrix 2006). Of particular interest is the invasion of the southern Appalachian Mountains by the aggressive invader *Amynthas agrestis*. *Amynthas agrestis* is now known in numerous places in this region, including sites in Tennessee (Reynolds 1978; Snyder 2008), Georgia (Callaham et al. 2003 B) and North Carolina (Reynolds 1978; Callaham et al. 2003 B). Many of these locales were undisturbed habitats that were considered less likely to be invaded, and were expected to serve as refugia for native earthworm species (Callaham et al. 2003 B). In order to effectively study and possibly control invasions of this relatively new exotic invader, life history traits must be studied and assessed.

Little is known about the life history of *Amynthas* earthworms. Most original descriptions of the genus place their origin in southeastern Asia (Gates 1972; Reynolds 1978; Zhang et al. 2006). Incomplete information on life history traits for this species has led to uncertainties about basic issues such as reproductive rates, temperature preferences, and whether or not the species overwinters in an adult stage, or only in a cocoon stage. Callaham et al. (2003 B) reported that all *A. agrestis* found in the southern Appalachians were fully clitellate, and therefore reproductively mature (i.e., adult) by September and no adult earthworms were found during winter months. When earthworms were again collected the following spring, only juveniles were collected in pitfall traps. This data suggests that *A. agrestis* becomes reproductively active in late fall, lays cocoons (through obligate parthenogenesis), then dies

(Blakemore 2003; James et al. 2005). In spring, due presumably to some external cue, *A. agrestis* hatch from their cocoons and emerge. However, this behavior has yet to be observed under field conditions. Laboratory incubations of adult *A. agrestis* have yielded cocoons showing that reproductive success is possible in a laboratory setting (Snyder 2008), and this has provided an opportunity to delve further into determining if there are any external cues that may cause hatchlings to emerge from cocoons.

Biological or environmental cues for emergence in earthworms are not well understood and the assumption is that emergence is either temperature dependent or water dependent (Lowe and Butt 2005). Holmstrup et al. (1991) showed that embryonic development of *Allolobophora chlorotica* (a European lumbricid) can be inhibited at 3°C. Lowe and Butt (2005) noted the significance of temperature in embryonic development of many European earthworm species, but also stated that minimum temperature requirements for hatchling emergence are species dependent. Therefore, we hypothesized that external temperature is an important environmental cue for *Amynthas agrestis* emergence. We expected that because *A. agrestis* originated in sub-tropical climates, cocoons would have higher temperature requirements than do European species (as described in the literature), and that emergence would occur at higher temperatures than many, if not all, European species.

The Use of Fire on Exotic, Invasive Earthworm Species

Earthworms are of growing worldwide concern (Steinberg et al. 1997; Hendrix and Bohlen 2002; Bohlen et al. 2004 A; Hendrix 2006; Hendrix et al. 2008) as there are over 100 species distributed beyond their native habitats (Fragoso et al. 1999). Exotic, invasive earthworm species are known to have severe ecosystem impacts in deciduous forests such as removal of forest floor litter, changes in forest floor species assemblages, changes in N and C cycling (Bohlen et al. 2004 A, B; Fisk et al. 2004; Hale et al. 2005, 2006). Although invasions of North America by earthworm species have been well studied (Grigoropoulou et al. 2008; Hendrix et al. 2008), little is known of the effects that varying land management strategies may have on them (Callaham et al. 2003 A).

Fire is an integral part of many ecosystems around the globe (Keeley 1991, 2002; Keeley and Bond 2001). Forest fires disturb the natural balance of forests, and soil, being an integral part of forests, is exposed to many of these disturbances (Bezkorovainaya et al. 2007). Soils, and for that matter, forest ecosystems, are dominated by below-ground activity and are dependent on soil faunal activity for many ecosystem level services including nutrient cycling (Nadel et al. 2007).

Impacts of fire are well studied and documented (Viro 1974; De Ronde 1990; Keeley 1991, 2006; Vinton et al. 1993; DeBano et al. 1998; Choromanska and DeLuca 2001; Keeley and Fotheringham 2001; Rhoades et al. 2002; Parr and Chown 2003; van Langevelde et al. 2003; Keeley et al. 2005; Mabuhay et al. 2006) but little research has been devoted to the impacts of fire on below-ground species. What little research literature is available does not focus on the effects on earthworms (DeBano et al. 1998; Callaham et al. 2003; Dawes-Gromadzki 2007). Even more disparate in the literature are the impacts of fire on earthworms in deciduous forest ecosystems (Viro 1974; DeBano et al. 1998). Fire has long been used a method to control fuel loads and understory growth in forested ecosystems (Viro 1974; Keeley 1991; Keeley and Fotheringham 2001; Parr and Chown 2003; Keeley et al. 2005, 2006). It is also considered a reliable method for soil restoration and for restoration of nutrient recycling (DeBano et al. 1998). Fire could play an important role in abating the distribution of invasive earthworm species by either direct or indirect mortality.

In this study, it was hypothesized that fire in an eastern deciduous forest would significantly impact survival of an invasive earthworm species. Epigeic (litter dwelling) earthworms live and move through the upper organic layers of soil (Jegou et al. 1998; Bossyut et al. 2005); therefore the removal of litter, or food material, could be a possible mechanism for any deleterious effects of fire on earthworms. Fire causes a number of other changes within soils such as increased pH, nitrogen removal through combustion and release of micronutrients (De Ronde 1990; DeBano et al. 1998; Choromanska and DeLuca 2001; Rhoades et al. 2002; Mabuhay et al. 2003; Parr and Chown 2003). All of these could also affect the survival of earthworms indirectly and it is not known what, if any, response earthworms will have to fire in an eastern deciduous forest.

References

Bezkorovainaya, I. N., E. N. Krasnoshchekova, et al. (2007). Transformation of soil invertebrate complex after surface fires of different intensity. Biology Bulletin, Springer Science & Business Media B.V. **34:** 517-522.

Blakemore, R. J. (2003). Japanese earthworms (Annelida: Oligochaeta): a review and checklist of species. Organisms Diversity & Evolution. **3:** 241-244.

Bohlen, P. J., P. M. Groffman, et al. (2004) (A). Ecosystem Consequences of Exotic Earthworm Invasion of North Temperate Forests. Ecosystems. **7:** 1-12.

Bohlen, P. J. and R. W. Parmelee (1999). "Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates." Soil Science Society of America Journal **63**(4): 882.

Bohlen, P. J., D. M. Pelletier, et al. (2004) (B). Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests. Ecosystems. **7:** 13-27.

Bossyut, H., J. Six, et al. (2005). "Interactive effects of functionally different earthworm species on aggregation and incorporation and decomposition of newly added residue carbon." Geoderma **130**(2006): 14-25.

Callaham, M. A., J. M. Blair, et al. (2003) (A). "Macroinvertebrates in North American tallgrass prarie soils: effects of fire, mowing and fertilization on density and biomass." Soil Biology and Biochemistry **35**(2003): 1079-1093.

Callaham, M. A., P. F. Hendrix, et al. (2003) (B). "Occurence of an exotic earthworm (*Amynthas agrestis*) in undisturbed soils of the southern Appalachian Mountains, USA." Pedobiologia **47**: 466-470.

Choromanska, U. and T. H. DeLuca (2001). "Prescribed Fire Alters the Impact of Wildfire on Soil Biochemical Properties in a Ponderosa Pine." Soil Science Society of America Journal **65**(1): 232.

Dawes-Gromadzki, T. Z. (2007). Short-term effects of low intensity fire on soil macroinvertebrate assemblages in different vegetation patch types in an Australian tropical savanna. Austral Ecology, Blackwell Publishing Limited. **32:** 663-668.

De Ronde, C., Ed. (1990). Impact of prescribed fire on soil properties- comparison with wlidfire effects. Fire In Ecosystem Dynamics. The Hague, The Netherlands, SPB Academic Publishing.

DeBano, L. F., D. G. Neary, et al., Eds. (1998). Fire's Effects on Ecosystems. New York, John Wiley and Sons.

Fisk, M. C., T. J. Fahey, et al. (2004). Earthworm Invasion, Fine-root Distributions, and Soil Respiration in North Temperate Forests. Ecosystems. **7:** 55-62.

Fragoso, C., P. Lavelle, et al. (1999). Earthworm communities of tropical agroecosystems: origin, structure and influences of management practices. Earthworm Management in Tropical Agroecosystems. P. Lavelle, L. Brussard and P. F. Hendrix. New York, CABI Publishing**:** 27-55.

Gates, G. E. (1972). "Burmese Earthworms: An Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia." Transactions of the American Philosophical Society **62**(7): 1-326.

Grigoropoulou, N., K. R. Butt, et al. (2008). Effects of adult Lumbricus terrestris on cocoons and hatchlings in Evan's boxes. Pedobiologia. **51:** 343-349.

Groffman, P. M., P. J. Bohlen, et al. (2004). Exotic Earthworm Invasion and Microbial Biomass in Temperate Forest Soils. Ecosystems. **7:** 45-54.

Hale, C. M., L. E. Frelich, et al. (2006). "Changes in harwood forest plant communities in response to European Earthworm invasions." Ecological Society of America **87**(7): 1637-1649.

Hale, C. M., L. E. Frelich, et al. (2005). "Effects of European Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA." Ecosystems **8**: 911-927.

Hendrix, P. F. (2006). "Biological invasions belowground-earthworms as invasive species." BIological Invasions **8**: 1201-1204.

Hendrix, P. F. and P. J. Bohlen (2002). "Exotic Earthworm Invasions in North America: Ecological and Policy Implications." Bioscience **52**(9): 801-811.

Hendrix, P. F., M. A. Callaham Jr., et al. (2008). "Pandora's Box Contained Bait: The Global Problem of Introduced Earthworms." Annual Reviews of Ecological and Evolutionary Systematics **39**: 593-613.

Holmstrup, M., I. K. Ostergaard, et al. (1991). "The relationship between temperature and cocoon incubation time for some lumbricid earthworm species." Pedobiologia **35**(3): 179-184.

James, S., S. Hsi-Te, et al. (2005). Seven new species of Amynthas (Clitellata: Megascolecidae) and new earthworm records from Taiwan. Journal of Natural History, Taylor & Francis Ltd. **39:** 1007-1028.

Jegou, D., Cluzeau, D., Balesdent, J. and Trehen, P. (1998). "Effects of four ecological categories of earthworms on carbon transer in soil." Applied Soil Ecology **9**: 249-255.

Keeley, J. E. (1991). "A Continent on Fire." Natural History **100**(4): 82-84.

Keeley, J. E. (2002). Native American impacts on fire regimes of the California coastal ranges. Journal of Biogeography, Blackwell Publishing Limited. **29:** 303-320.

Keeley, J. E. (2006). Fire Management Impacts on Invasive Plants in the Western United States. Conservation Biology, Blackwell Publishing Limited. **20:** 375-384.

Keeley, J. E. and W. J. Bond (2001). "On Incorporating Fire into Our Thinking about Natural Ecosystems: A Response to Saha and Howe." American Naturalist **158**(6): 664.

Keeley, J. E. and C. J. Fotheringham (2001). Historic Fire Regime in Southern California Shrublands. Conservation Biology, Blackwell Publishing Limited. **15:** 1536-1548.

Keeley, J. E., C. J. Fotheringham, et al. (2005). Factors affecting plant diversity during post-fire recovery and succession of mediterranean-climate shrublands in California, USA. Diversity & Distributions, Blackwell Publishing Limited. **11:** 525-537.

Keeley, J. E., C. J. Fotheringham, et al. (2006). DEMOGRAPHIC PATTERNS OF POSTFIRE REGENERATION IN MEDITERRANEAN-CLIMATE SHRUBLANDS OF CALIFORNIA. Ecological Monographs. **76:** 235-255.

Lowe, C. N. and K. R. Butt (2005). Culture techniques for soil dwelling earthworms: A review. Pedobiologia. **49:** 401-413.

Mabuhay, J., Y. Isagi, et al. (2006). Wildfire effects on microbial biomass and diversity in pine forests at three topographic positions. Ecological Research, Springer Science & Business Media B.V. **21:** 54-63.

Mabuhay, J. A., N. Nakagoshi, et al. (2003). Microbial biomass and abundance after forest fire in pine forests in Japan. Ecological Research, Springer Science & Business Media B.V. **18:** 431-441.

Nadel, R. L., M. C. Scholes, et al. (2007). Slash burning, faunal composition, and nutrient dynamics in a Eucalyptus grandis plantation in South Africa. Canadian Journal of Forest Research, NRC Research Press. **37:** 226-235.

Parr, C. L. and S. L. Chown (2003). Burning issues for conservation: A critique of faunal fire research in Southern Africa. Austral Ecology, Blackwell Publishing Limited. **28:** 384-395.

Reynolds, J. W. (1978). "The earthworms of Tennessee (Oligachaeta). IV. Megascolecidae, with notes on distribution, biology and a key to the species in the state." Megadrilogica **3**(7): 117-129.

Rhoades, C., T. Barnes, et al. (2002). Prescribed Fire and Herbicide Effects on Soil Processes During Barrens Restoration. Restoration Ecology, Blackwell Publishing Limited. **10:** 656-664.

Snyder, B. A. (2008). Unpublished Dissertation Work. Athens University of Georgia.

Steinberg, D. A., R. V. Pouyat, et al. (1997). "Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient." Soil Biology and Biochemistry **29**(3/4): 427-430.

Terhivuo, J. and A. Saura (2006). "Dispersal and clonal diversity of North-European parthenogenetic earthworms." BIological Invasions **2006**(8): 1205-1218.

van Langevelde, F., C. A. D. M. van de Vijver, et al. (2003). EFFECTS OF FIRE AND HERBIVORY ON THE STABILITY OF SAVANNA ECOSYSTEMS. Ecology. **84:** 337.

Vinton, M. A., D. C. Hartnett, et al. (1993). "Interactive Effects of Fire, Bison (Bison bison) Grazing and Plant Community Composition in Tallgrass Prairie." American Midland Naturalist **129**(1): 10-18.

Viro, P. J., Ed. (1974). Effects of Forest Fire on Soil. Fire and Ecosystems. New York, Academic Press.

Zhang, W.-X., J.-X. Li, et al. (2006). New earthworms belonging to the genus of Amynthas Kinberg (Megascolecidae: Oligochaeta) and Drawida Michaelsen (Moniligastridae: Oligochaeta) from Guangdong, China. Journal of Natural History, Taylor & Francis Ltd. **40:** 395-401.

CHAPTER 2

TEMPERATURE AS AN ENVIRONMENTAL CUE FOR *AMYNTHAS AGRESTIS* HATCHLING EMERGENCE[1]

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[1] Blackmon, J.H., B A. Snyder, M A. Callaham Jr., and P F. Hendrix. To be submitted to *Southeastern Naturalist*.

Abstract

This study addressed the effect of temperature on *Amynthas agrestis* (an exotic, invasive, epigeic earthworm species from southeastern Asia) hatching rates and life history strategies. The hypothesis that hatching is temperature dependent was tested by selecting cocoons from three distinct populations of *A. agrestis* and subjecting them to randomly assigned temperatures (i.e., 5, 10, 15, 20, 25, or 30°C) and assessing hatching rates. We also assessed hatching rates of cocoons held at relatively cold temperatures (i.e., 5, 10, or 15°C) for 15, 30 or 60 days to determine if temperature change is an environmental cue for emergence. As expected, temperature was a determining factor in *A. agrestis* hatching, and 10°C was the optimal temperature at which hatching occurred, regardless of time. Hatching rate was also highest in cocoons held at 10°C for 60 days versus 15 or 30 days or any other temperature. Although temperature was believed to be the determining factor in hatchling emergence, 10°C was not expected to be the optimal temperature for emergence of *A. agrestis* due to their subtropical origins. These life history results are another step forward in the understanding of the invasive success of *A. agrestis* and other Asian invaders and could have implications in invasion biology as well as global climate change.

Introduction

Exotic, invasive earthworm species are a growing problem in North American ecosystems, as well as around the globe, because these organisms have been linked to changes in many ecosystem level properties (Steinberg et al. 1997; Bohlen and Parmelee 1999; Bohlen et al. 2004 A; Groffman et al. 2004; Hale et al. 2005, 2006; Hendrix et al. 2008). Invasive earthworms have been shown to affect forest floor dynamics by changing understory plant and faunal communities (Hale et al. 2006). Invasive earthworms have also been linked to decreases in organic horizons of soil (Oa, Oe, and Oi), as well as soil transformations from a mull-type soil profile to a mor-type (Groffman et al. 2004; Hale et al. 2005, 2006). Most studies of exotic earthworms have focused on ecosystem effects and documentation of occurrences of invasions, but much less attention has been paid to basic biology and life history traits (Terhivuo and Saura 2006). However, information of this kind is increasingly available in applied earthworm research fields because of a rapid expansion in the past 20 years in use of earthworms for ecotoxicological studies, soil restoration, and biomonitoring (Lowe and Butt 2005). In this context, reproductive rates and life history traits have been extensively studied for many lumbricid (Family: *Lumbricidae*) species (Lowe and Butt 2005; Terhivuo and Saura 2006; Grigoropoulou et al. 2008), but little is known about the reproductive capacities of many other important invasive species around the globe (Hendrix et al. 2008).

Although the invasion of European earthworms into North America has long been recognized and studied in the United States (Bohlen et al. 2004 A; Hendrix 2006), the newer, secondary invasions of Asian species have been little studied until recently and are not well understood. Asian earthworms in the genus *Amynthas* (Family: *Megascolecidae*) are currently invading areas around the globe, including North America, Central America, and Europe (Fragoso et al. 1999; Hendrix 2006). Of particular interest is the invasion of the southern Appalachian Mountains by the aggressive invader *Amynthas agrestis*. *Amynthas agrestis* are now known in numerous places in this region, including at sites in Tennessee (Reynolds 1978; Snyder 2008), Georgia (Callaham et al. 2003) and North Carolina (Reynolds 1978; Callaham et al. 2003). Many of these locales were previously undisturbed habitats that were considered less likely to be invaded, and were expected to serve as refugia of native earthworm species (Callaham et al. 2003). In order to effectively study and possibly control invasions of this relatively new exotic invader, life history traits must be studied and assessed.

Little is known about the life history of *Amynthas* earthworms. Most original descriptions of the genus place their origin in southeastern Asia (Gates 1972; Reynolds 1978; Zhang et al. 2006). Incomplete information on life history traits for this species has led to uncertainties about basic issues such as reproductive rates, temperature preferences, and whether or not the species overwinters in an adult stage, or only in a cocoon stage. Callaham et al. (2003) reported that all *A. agrestis* found in the southern Appalachians were fully clitellate, and therefore reproductively mature (i.e., adult) by September, and no adult earthworms were found during winter months. When earthworms were again collected the following spring, only juveniles were collected in pitfall traps. This data suggests that *A. agrestis* becomes reproductively active in late fall, lays cocoons (through obligate parthenogenesis), then dies (Blakemore 2003; James et al. 2005). In spring, due presumably to some external cue, *A. agrestis* hatch from their cocoons and emerge. However, this behavior has yet to be observed under field conditions. Laboratory incubations of adult *A. agrestis* have yielded cocoons, showing that reproductive success is possible in a laboratory setting (Snyder 2008), and this has provided an opportunity to delve further into determining if there are any external cues that may cause hatchlings to emerge from cocoons.

Biological or environmental cues for emergence in earthworms are not well understood and the assumption is that emergence is either temperature dependent or water dependent (Lowe and Butt 2005). Holmstrup et al. (1991) showed that embryonic development of *Allolobophora chlorotica* (a European lumbricid) can be inhibited at 3^{o}C. Lowe and Butt (2005) noted the significance of temperature in embryonic development of many European earthworm species, but also stated that minimum temperature requirements for hatchling emergence are species specific. Therefore, we hypothesized that external temperature is an important environmental cue for *Amynthas agrestis* emergence. We expected that because *A. agrestis* originated in sub-tropical climates, cocoons would have higher temperature requirements than do European species (as described in the literature), and that emergence would occur at higher temperatures than many, if not all, European species.

Methods

Adult *Amynthas agrestis* earthworms from three distinct populations (Table 2.1) were collected and stored in the laboratory in plastic culture containers at room temperature (22˚C) for approximately 60 days (from September to November, 2008). Soil in the cultures was maintained at water holding capacity, and cultures were periodically amended leaf material collected from the Great Smoky Mountains National Park site (CH) (Table 2.1). All material added to the cultures was air dried and crushed prior to use as feed in order to reduce the likelihood of introducing new cocoons into the cultures. Cocoons were collected from each culture by wet-sieving the soil and leaf material through a 1 mm sieve and carefully removing the cocoons with forceps. Cocoons were stored in water for seven days at room temperature (22°C) until being used in the incubation trials.

Constant temperature incubation

Collected cocoons were placed into petri dishes and submerged in tap water (20mL) between two pieces of filter paper (Whatman #1 qualitative filter paper; 5.5 cm diameter) to prevent drying (Lowe and Butt 2005, 2007). Ten or 12 cocoons from each population were placed into separate petri dishes and assigned randomly to incubators at six different temperatures (5, 10, 15, 20, 25, and 30˚C). Cocoons were then gradually adjusted to their assigned temperature. All incubators were kept dark to simulate field light conditions.

Cocoons were checked weekly to assess hatching and to add tap water as needed to keep them submerged. As hatchings occurred, immature *Amynthas agrestis* and their hatched cocoons were taken from the petri dish and placed into sieved soil collected from Great Smoky Mountain National Park. Numbers of hatchlings were recorded in order to determine which temperature had the highest success rate of hatchlings.

Limited-cold temperature incubation

In order to test temperature change as an environmental cue for *Amynthas agrestis* hatchling emergence, a separate set of cocoons from the Great Smoky Mountain National Park (CH) (Table 2.1) was used in a second experiment. Ninety cocoons were placed into petri dishes in a 3 x 3 factorial design with three different low temperatures and three different incubation lengths (9 petri dishes with 10 cocoons each). Cocoons were gradually lowered to one of three cold temperatures (5, 10, and 15°C) and allowed to incubate for varying amounts of time (15, 30, or 60 days). All petri dishes were kept at 20˚C for 24 hr and were gradually brought down to the experimental temperature in 5˚C increments over 24 hr. After the experimental incubation period, cocoons were brought back to 20˚C in 5˚C increments over 24 hr. Cocoons then remained at 20˚C, and rates of hatching success were assessed for 180 days after temperature treatments were applied (Table 2.2).

Statistical analysis

Hatching data from incubation trials were expressed as mean percent hatched from each population. Results from hatching trials were analyzed using analysis of variance (ANOVA) in SAS (SAS Institute Inc., Cary, NC, USA). Normal distribution of data was examined using a univariate analysis in SAS and data was heavily skewed. Therefore, a Kruskal-Wallis one-way analysis of variance was performed using SAS to establish significance of main effects variable using $\alpha=0.05$ (reported as p-value). The main effects variable for the constant temperature incubation was temperature, and main effects variables in the limited-cold incubation were temperature and duration of cold treatment.

Results

Constant temperature incubation

Of the six different temperatures examined, 10ºC proved to be the optimal temperature at which hatching occurred ($p=0.02$) (Fig. 2.1). At 10ºC, the Nantahala (NT) population demonstrated the highest hatching rate (100%) followed by the Tremont (TR) population (60%) and then the Great Smoky Mountain Nation Park (CH) population (33%). No hatching was observed at either 5 or 20ºC. Hatching rate declined at temperatures above 10ºC with no hatching observed at 20, 25, and 30ºC; the exception being the TR population (50% hatch rate at 25ºC). A 20% and 10% hatch rate was observed for the TR and CH populations, respectively, at 15ºC. Overall, hatching rate for all populations was significantly higher at 10ºC ($p=0.02$) with an overall success rate of 65%. Hatching rate at 15, 25 and 30ºC were not significantly different from each other (p=0.1) with rates of 8.8, 14.7 and 2.3%, respectively.

When hatching rate was plotted against time, interesting dynamics were observed (Fig. 2.2). Cocoons at 25ºC hatched before all other cocoons and after week three, no further emergence was observed. At week ten, cocoons held at 15ºC hatched, with only a single cocoon hatching thereafter. In week twelve, one hatching occurred from a cocoon held at 30ºC. At week 17, cocoons held at 10ºC began to hatch with five hatchings in week 17 followed by a rapid increase in the number of hatchings (14) on week 18. Hatching ceased after week 21 in the 10ºC incubation, with 22 out of 34 cocoons hatched - significantly greater success than any other temperature (p=0.02).

Limited-Cold temperature incubation

Dynamics observed in the constant temperature incubations were again seen when temperatures were lowered for varying amounts of time (15, 30 and 60 days) then subsequently brought back to room temperature (Fig. 2.3). Hatching rate was higher at 10ºC than at any other temperature (5 or 15ºC) (p=0.01). No cocoons lowered to 15ºC hatched, regardless of duration of incubation at this temperature, and only one cocoon that incubated at 5ºC for 60 days hatched. However, hatching occurred at 10ºC for all incubation lengths. Three cocoons held at 10ºC for both 15 and 30 days emerged and half (5) of the cocoons held at 10ºC for 60 days hatched.

Timing of hatching was also interesting, as the length of time held at a given lowered temperature seemed to affect emergence after cocoons were brought back to room temperature. Cocoons held at their assigned temperatures for 60 days expectedly took longer to emerge than cocoons held for 15 or 30 days (Fig. 2.4). Cocoons held for 15 days at 10ºC hatched sooner than cocoons held for 30 days. Temperature also affected cocoons held at 5ºC as hatching occurred after all cocoons at 10ºC had hatched. Once temperatures were raised again after being lowered, hatching was rapid for cocoons held at 10ºC. Once brought back to room temperature, cocoons held at 10ºC for 15 days took six weeks to hatch and all hatchings ceased after twelve weeks. Cocoons held at 10ºC for 30 days also began hatching six weeks after being brought back to room temperature and completed hatching after 10 weeks. Cocoons lowered to 10ºC for 60 days took only five weeks to begin hatching and completed hatching after 13 weeks whereas cocoons held at 5ºC for 60 days took 13 weeks to begin hatching and only one hatched.

Discussion

Results from the manipulation of *Amynthas agrestis* cocoons using temperature were somewhat expected. In general, temperature was expected to affect the hatching rate of cocoons. However, it was thought that *A. agrestis* cocoons would be adapted to higher temperatures for hatching due to their subtropical origins. It was not expected that 10˚C would be the temperature at which optimal emergence would occur. Given the significance of hatching at this temperature ($p=0.02$), *A. agrestis* cocoons are more adapted for colder temperatures than previously thought.

Since it is extremely difficult to identify *Amynthas* earthworms to species in the field, a possibility exists that parents from the TR population were not all *A. agrestis* and it could have contained individuals of *A. corticis* or other *Amynthas* species. This would presumably explain the larger rate of hatching, though not significant, at 25ºC for the TR population. Different life history strategies have been observed in unpublished laboratory incubations of *A. corticis*. *Amynthas corticis* is commonly found mingled within populations of *A. agrestis* in the southern Appalachian Mountains (Reynolds 1978; Snyder 2008). Only slight size variance under field conditions can sometimes be detected between these two species and this is far from conclusive. The only way to definitively determine species within the genus *Amynthas* is to dissect specimens. Since this would have prevented reproduction, dissection was not performed and any viable specimens after reproduction had taken place had long since died and decayed. Laboratory incubations of both species have shown differences in longevity (Snyder 2008), and field observations suggest that *A. agrestis* seems to experience full mortality at the end of each fall (Callaham et al. 2003). If the two do in fact have different life history strategies, it is not unreasonable to expect different cocoon hatching strategies.

Since significantly different rates of hatching were seen at 10˚C (p=0.02), this is the optimal temperature cue for *Amynthas agrestis*. In a natural setting, cocoons over-winter in the organic horizons of the soil and experience temperatures at this level or lower. Once the soil temperatures reaches 10˚C for a period of time, it will again warm, leading to emergence of hatchlings. This was demonstrated in the limited-cold incubations as cocoons held at 10˚C hatched approximately six weeks after incubation. This occurred in every case; the exception being that cocoons held at 10˚C for 60 days emerged a week earlier (Fig. 2.4). Under field conditions, cocoons would presumably not experience 60 days of temperatures at 10˚C, and after being subjected to this temperature for a longer period of time, embryonic development would be more rapid once temperatures were raised.

Timing of hatching was also important as cocoons held at 10˚C took longer to hatch than cocoons held at any other temperature (Fig. 2.2). Hatching did not occur until after 17 weeks of incubation and ceased by the 21st week. This is a relatively short time period for emergence and is biologically significant when considered in the context of invasion biology. Before death, *Amynthas agrestis* is likely to produce large numbers of cocoons to ensure success (e.g. as in Table 2.1). Once temperatures drop during winter months and a warming is experienced in spring months, cocoons hatch at approximately the same time. This information could be useful to prevent or inhibit the invasional success of *A. agrestis*. If cocoons can be destroyed before hatching, future population numbers can be significantly decreased. Cocoon removal by either mechanical, human or burning methods could be employed in order to decrease population numbers of juvenile *A. agrestis*; further investigation into these methods is warranted.

Limited-cold manipulations again showed 10˚C was the optimal temperature for emergence as only one cocoon from any other incubation temperature (5 and 15˚C) hatched. Cocoons held for 60 days at 10ºC had a higher rate of hatching (50%) than any other combination of incubation temperature and time interval and although not statistically significant does demonstrate that length of colder temperatures is important and could impact future generations and preventative methods. For example, if an unseasonably warm winter occurs, perhaps cocoons of *A. agrestis* will have less success, which could also be important in the face of global climate change as mean annual temperatures are expected to increase. This could lead to future invasions in a poleward direction for *Amynthas agrestis* into habits where they are not presently known to occur (Hendrix et al. 2008).

Conclusions

In order to fully understand invasion biology, life history traits must be fully explored. Little is known about life history traits for many exotic invasive earthworm species. As knowledge of these characteristics increases, so shall the understanding of the invasional success of many Asian species of earthworm, including how they are able to invade areas that have are already inhabited by European invaders and/or native earthworm species. We have demonstrated that external temperature cues affect hatching rates of *Amynthas agrestis* cocoons and that 10ºC is the optimal temperature at which hatching occurs. Knowledge of the life history strategies of Asian invaders will be important in unlocking the mystery of their invasional success if not the key itself. Knowing that temperature plays such a crucial role in cocoon emergence of *A. agrestis* will give insight into how other *Amynthas* species are able to survive away from their places of origin. It will also present a chance to prevent or inhibit future invasions.

References

Blakemore, R. J. (2003). Japanese earthworms (Annelida: Oligochaeta): a review and checklist of species. Organisms Diversity & Evolution. **3:** 241-244.

Bohlen, P. J., P. M. Groffman, et al. (2004) (A). Ecosystem Consequences of Exotic Earthworm Invasion of North Temperate Forests. Ecosystems. **7:** 1-12.

Bohlen, P. J. and R. W. Parmelee (1999). "Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates." Soil Science Society of America Journal **63**(4): 882.

Bohlen, P. J., D. M. Pelletier, et al. (2004) (B). Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests. Ecosystems. **7:** 13-27.

Callaham, M. A., P. F. Hendrix, et al. (2003). "Occurence of an exotic earthworm (*Amynthas agrestis*) in undisturbed soils of the southern Appalachian Mountains, USA." Pedobiologia **47**: 466-470.

Fragoso, C., P. Lavelle, et al. (1999). Earthworm communities of tropical agroecosystems: origin, structure and influences of management practices. Earthworm Management in Tropical Agroecosystems. P. Lavelle, L. Brussard and P. F. Hendrix. New York, CABI Publishing**:** 27-55.

Gates, G. E. (1972). "Burmese Earthworms: An Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia." Transactions of the American Philosophical Society **62**(7): 1-326.

Grigoropoulou, N., K. R. Butt, et al. (2008). Effects of adult Lumbricus terrestris on cocoons and hatchlings in Evan's boxes. Pedobiologia. **51:** 343-349.

Groffman, P. M., P. J. Bohlen, et al. (2004). Exotic Earthworm Invasion and Microbial Biomass in Temperate Forest Soils. Ecosystems. **7:** 45-54.

Hale, C. M., L. E. Frelich, et al. (2006). "Changes in harwood forest plant communities in response to European Earthworm invasions." Ecological Society of America **87**(7): 1637-1649.

Hale, C. M., L. E. Frelich, et al. (2005). "Effects of European Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA." Ecosystems **8**: 911-927.

Hendrix, P. F. (2006). "Biological invasions belowground-earthworms as invasive species." BIological Invasions **8**: 1201-1204.

Hendrix, P. F., M. A. Callaham Jr., et al. (2008). "Pandora's Box Contained Bait: The Global Problem of Introduced Earthworms." Annual Reviews of Ecological and Evolutionary Systematics **39**: 593-613.

Holmstrup, M., I. K. Ostergaard, et al. (1991). "The relationship between temperature and cocoon incubation time for some lumbricid earthworm species." Pedobiologia **35**(3): 179-184.

James, S., S. Hsi-Te, et al. (2005). Seven new species of Amynthas (Clitellata: Megascolecidae) and new earthworm records from Taiwan. Journal of Natural History, Taylor & Francis Ltd. **39:** 1007-1028.

Lowe, C. N. and K. R. Butt (2005). Culture techniques for soil dwelling earthworms: A review. Pedobiologia. **49:** 401-413.

Lowe, C. N. and K. R. Butt (2007). "Earthworm culture, maintenance and species selection in chronic ecotoxicological studies: A critical review." European Journal of Soil Biology **43**(Supplement 1): S281-S288.

Reynolds, J. W. (1978). "The earthworms of Tennessee (Oligachaeta). IV. Megascolecidae, with notes on distribution, biology and a key to the species in the state." Megadrilogica **3**(7): 117-129.

Snyder, B. A. (2008). Unpublished Dissertation Work. Athens University of Georgia.

Steinberg, D. A., R. V. Pouyat, et al. (1997). "Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient." Soil Biology and Biochemistry **29**(3/4): 427-430.

Terhivuo, J. and A. Saura (2006). "Dispersal and clonal diversity of North-European parthenogenetic earthworms." BIological Invasions **2006**(8): 1205-1218.

Zhang, W.-X., J.-X. Li, et al. (2006). New earthworms belonging to the genus of Amynthas Kinberg (Megascolecidae: Oligochaeta) and Drawida Michaelsen (Moniligastridae: Oligochaeta) from Guangdong, China. Journal of Natural History, Taylor & Francis Ltd. **40:** 395-401.

Table 2.1. Source populations for *Amynthas agrestis* adults and number of cocoons produced.

Collection Location	Population Code	Number of Adults Collected	Earthworm Collection Date - Sieving Date	Number of Cocoons Produced
Great Smoky Mountains National Park, along edge of pond near US Hwy. 129/Lake Chilhowee (35º31’57” N, 83º 59’27” W)	**CH**	13	July 2008 - September 2008	210
Roadside of NC 28 in Nantahala National Forest (35º26’31” N, 83º49’21” W)	**NT**	3	July 2008 - September 2008	89
Ditch along road in Great Smoky Mountains Institute at Tremont (35º38’23” N, 83º41’24” W)	**TR**	4	July 2008 - September 2008	76

Table 2.2. Temperature and time treatments for *A. agrestis* cocoons in the limited-cold incubations.

Number of cocoons	Treatment temperature (ºC)	Days spent at treatment temperature
10	5	15
10	5	30
10	5	60
10	10	15
10	10	30
10	10	60
10	15	15
10	15	30
10	15	60

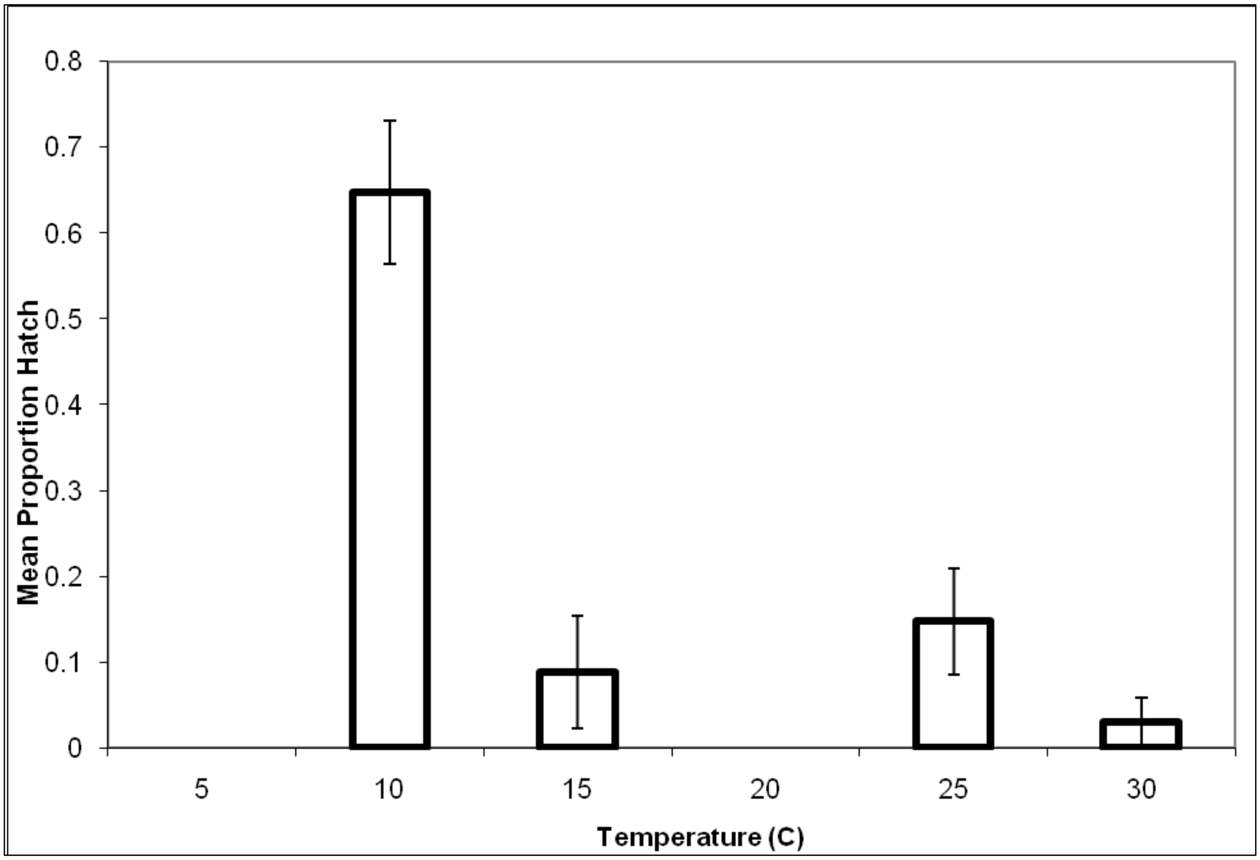

Fig. 2.1. Mean proportion hatched of *A. agrestis* cocoons (± SE) from the constant temperature incubations.

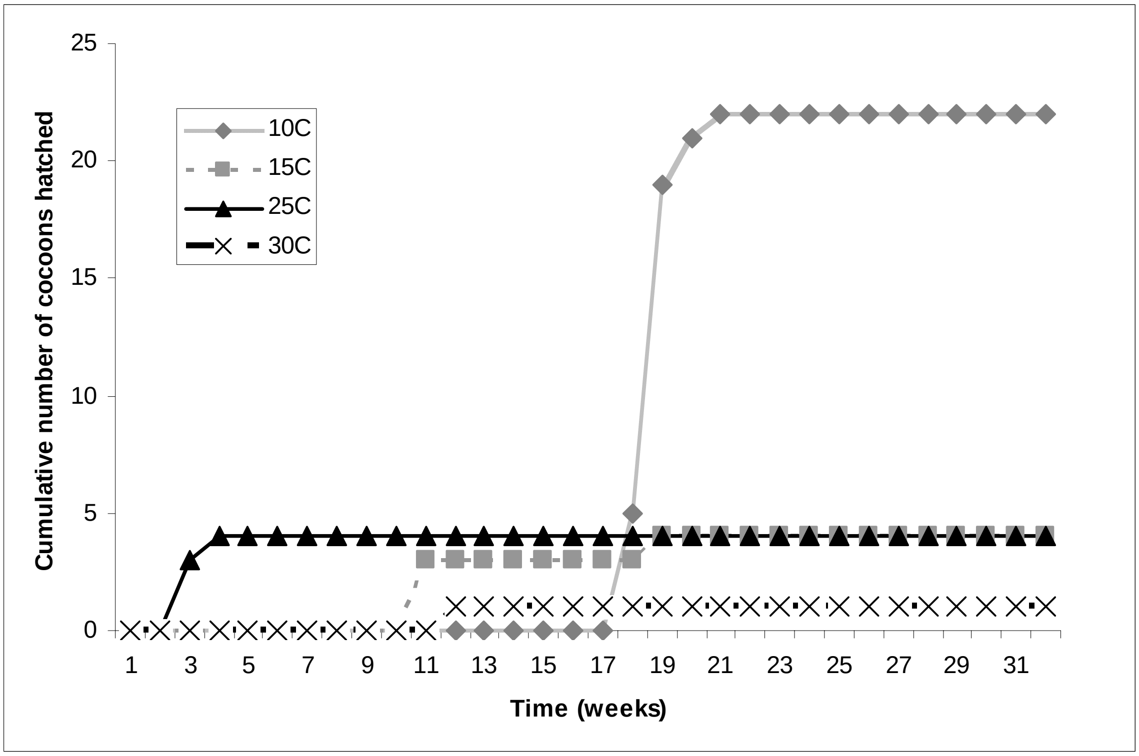

Fig. 2.2. Cumulative number of hatchings of *A. agrestis* cocoons over time during the constant temperature incubations.

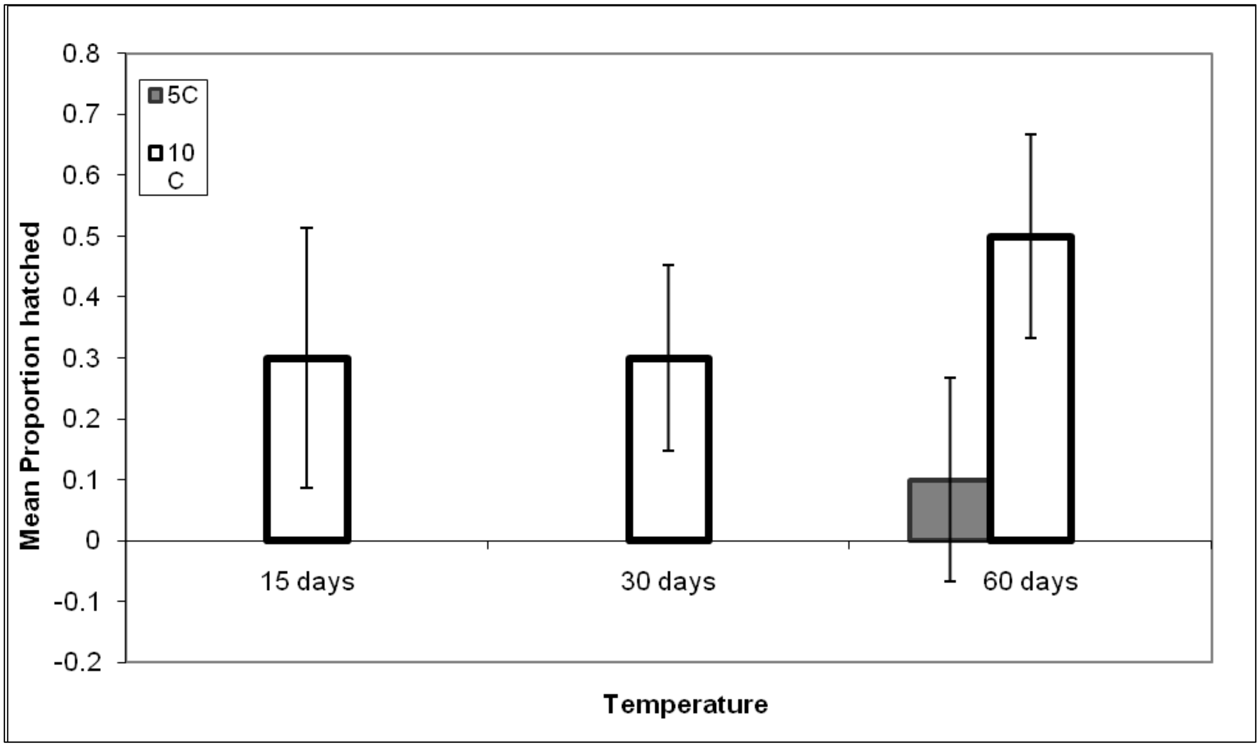

Fig. 2.3. Mean proportion hatched of *A. agrestis* cocoons (± SE) during the limited-cold incubations.

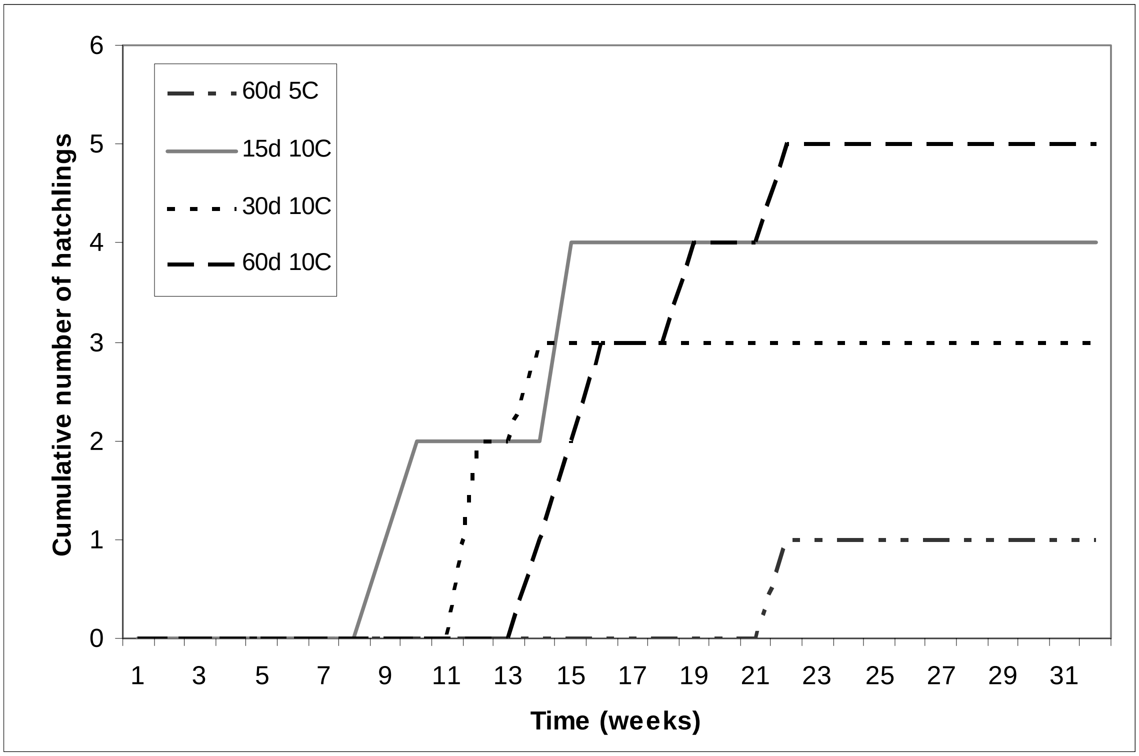

Fig. 2.4. Cumulative number of hatchings of *A. agrestis* cocoons over time during the limited-cold incubations.

CHAPTER 3

DIRECT AND INDIRECT EFFECTS OF FIRE ON SURVIVAL OF INVASIVE, EPIGEIC EARTHWORM SPECIES IN FORESTS OF THE SOUTHEASTERN UNITED STATES[2]

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[2] Blackmon, J.H., M A. Callaham Jr., and P F. Hendrix. To be submitted to *Southeastern Naturalist*.

Abstract

This study examines the direct and indirect effects of prescribed fire and wildfire on survival of invasive, epigeic earthworm species commonly found in forest ecosystems of the Southeastern United States. It is hypothesized that fire would significantly impact survival of invasive, earthworm species either 1) through a lack of food resources following fire; or 2) through direct mortality of earthworms from fire. To determine if direct mortality of earthworms occurs during fire, a prescribed fire was simulated and earthworm mortality was assessed. To test the post-fire effects on earthworms after a wildfire, intact soil cores were collected in Great Smoky Mountains National Park after a wildfire and earthworms were introduced in a laboratory setting. Survival and biomass loss of earthworms were assessed and chemical analyses were performed on the soil. No direct mortality of earthworms due to fire was seen in this study but indirect mortality and significant loss of biomass post-fire was seen. Earthworms in soil that experienced wildfire continuously lost biomass whereas earthworms in soil that had not been burned gained biomass after four weeks, and then lost. After chemical analyses, a mechanism for earthworm mortality could be detected: starvation. These results could be used for future policy decisions on land management strategies as well as a possible way to abate invasive earthworm spread.

Introduction

Exotic, invasive earthworms are of growing worldwide concern (Steinberg et al. 1997; Hendrix and Bohlen 2002; Bohlen et al. 2004; Hendrix 2006; Hendrix et al. 2008) as more than 100 species have become distributed beyond their native habitats (Fragoso et al. 1999). Invasive earthworms are known to have severe ecosystem impacts in some deciduous forest ecosystems, such as removal of forest floor litter, changes in forest floor species assemblages, changes in N and C cycling, as well as detrimental effects to other soil biota (Bohlen et al. 2004 A,B; Fisk et al. 2004; Hale et al. 2005, 2006). Although invasions of North American soils by non-native earthworm species have been well studied (Hendrix and Bohlen 2002; Hendrix et al. 2008), little is known of the effects varying land management strategies may have on them (Callaham et al. 2003, 2006).

Fire, for instance, is one land management strategy that could have impacts on invasive species. Impacts of fire on vegetation and soil are well studied and documented, with most studies demonstrating a response of vegetative communities and changes in nutrient status of soils (Viro 1974; De Ronde 1990; Keeley 1991; DeBano et al. 1998; Choromanska and DeLuca 2001; Keeley and Fotheringham 2001; Rhoades et al. 2002; Parr and Chown 2003; Keeley et al. 2005; Mabuhay et al. 2006). Fire has long been used as a method to control fuel loads and understory growth in forested ecosystems (Viro 1974; Keeley 1991; Keeley and Fotheringham 2001; Parr and Chown 2003; Keeley et al. 2005, 2006). It is also considered a reliable method of soil restoration and to restore of natural nutrient cycling regimes (DeBano et al. 1998). However, little is known about fire effects on below-ground communities (Callaham et al. 2003) and the value of using prescribed fire for controlling invasive earthworm species has yet to be evaluated. Fire could play an important role in abating the distribution of invasive earthworms by either direct or indirect mortality.

Epigeic (litter dwelling) earthworms live and move through the upper organic layers of soil (Jegou et al. 1998; Bossyut et al. 2005). The removal of litter (the primary food resource for epigeic earthworm species) by fire could be a possible mechanism for controlling their spread. However, fire causes a number of other changes within soils such as increased pH, nitrogen removal through combustion and release of micronutrients (De Ronde 1990; DeBano et al. 1998; Choromanska and DeLuca 2001; Rhoades et al. 2002; Mabuhay et al. 2003; Parr and Chown 2003). These could also affect the survival of earthworms indirectly and it is not known what, if any, response earthworms will have to fire in an eastern deciduous forest. This study was conducted using soils from two different locations and two different invasive epigeic earthworm species to test the hypothesis that fire would significantly impact survival of invasive, earthworm species either 1) through a lack of food resources following fire; or 2) through direct mortality of earthworms from fire.

Methods

Simulated Prescribed Fire Experiment

Simulation of a prescribed fire in a deciduous forest floor was achieved by first constructing microcosms from soils collected from a deciduous forest ecosystem at Horseshoe Bend, Athens, GA (33º 55’ 59” N; 83º 21’ 15” W). Organic material (Oa, and Oe horizons) was collected from the site, as well as mineral soil from the A-horizon (to approximately a 5 cm depth). Soil (A-horizon), litter (Oi) and organic material (Oa/Oe) were then placed into aluminum cores that were 10 cm in diameter and 20 cm in length. A-horizon soil made up the bottom 10 cm of soil in the microcosms, covered by approximately five cm of Oa horizon material and the remainder of the core filled with Oe and Oi material. *Lumbricus rubellus,* an epigeic, invasive, European earthworm species commonly found in the Southeastern United States, were collected from the same site as was the soil and then added to the microcosms at approximately the same densities and biomass as field conditions (200-400 g fresh weight m^{-2}soil at time of collection). Microcosms were sealed with fiberglass mesh screens and earthworms were allowed to acclimate for 48 h.

Microcosms were placed into a bed of granite sand bordered by concrete blocks. Just prior to application of fire, sand was moistened in order to facilitate heat removal from the aluminum surface of the cylinders and prevent thermal displacement of heat throughout soil. Sand beds containing microcosms were covered with a layer of forest floor organic material (Oe/Oa) and dry litter (Oi) to simulate a forest floor (~ 1 kg m^{-2}). The sand bed was divided into two sections separated by concrete blocks and fire was applied to one section.

Different moisture treatments were randomly assigned to microcosms in order to test fire residence time and temperature in a two-way design. To simulate shorter residence times with higher temperatures, soil cores were allowed to dry to 30% of field capacity before earthworms were introduced. Longer residence times were simulated using cores that were wet to field capacity prior to earthworm introductions. Microcosms were randomly sampled either immediately after the fire (one h), or four weeks after fire. Each combination of treatments was replicated eight times for a total of 64 microcosms.

To determine maximum surface fire temperatures and residence times of fire, a forward looking infrared camera was used to film the experimental fire in its entirety. Soil heating was assessed by inserting thermocouples into 13 randomly assigned microcosms at two depths (1cm and 5.5cm below soil surface). Four randomly chosen microcosms also had thermocouples inserted 11.5 cm below the soil surface to assess heating at depth. Thermocouples were attached to Campbell Scientific® data loggers to record temperature data. Thirty thermocouples were used.

Data from the simulated prescribed fire study were first arcsine transformed then analyzed using a two-way analysis of variance (ANOVA) using Statistical Analyzing Software (SAS Institute Inc., Cary, NC, USA) to determine any statistical significance. The main effects variables in this analysis were fire, moisture and time, with mortality of earthworms as the response variable. Responses were considered significant when $p < 0.05$.

Post-wildfire Survival Experiment

Site description Soils were collected from along the western edge of Great Smoky Mountains National Park (GSMNP) from a mesic-xeric secondary forest. Soils are a complex of moderately deep Junaluska and deep Brasstown soils, fine-loamy, mixed, subactive, mesic Typic Hapludults (USDA and NRCS 2008).

Experimental Methods In August 2007, this site experienced a wildfire which provided an opportunity to sample soils from burned and unburned patches side by side. Samples were collected the following October two meters away from either side of a fire control line (burned and unburned). Twenty, 10 cm (diameter) by 20 cm (height) aluminum cores were driven 15 cm into the soil, including through any leaf litter present, yielding intact soil samples. Additional samples of burned and unburned mineral soil from two depths (0-5 cm and 5-15 cm) were collected for chemical analyses. Intact cores were transported to the laboratory and allowed to air dry for six months.

Once cores had dried sufficiently, mass of the dry sample was taken. Moisture was added to field capacity and differences in weights were assessed. Cores were subsequently monitored for moisture loss, and water was added as needed to maintain cores at field capacity. Once at field capacity, earthworms were randomly introduced to the cores to test their post-fire survival. Each core had introduced approximately the same mass of *Amynthas agrestis* earthworms (mean fresh weight 1.9 g ± 0.17 g standard deviation; 3-5 individuals). Cores were then destructively sampled at random at two time periods (after four and eight weeks incubation) for measurement of earthworm survival and biomass loss. Additionally, soil from each core was analyzed for total C and N following incubation (micro-Dumas combustion on a Carlo Erba CHN analyzer, Italy). Cations (Mg, K, and Ca) were also extracted from the samples using a double-acid extraction method and analyzed by atomic absorption (Mehlich 1953; Jones Jr. 1990).

Data were analyzed using a two-tailed t-test in Microsoft Excel to determine the significance of fire effects. The main effects variable was presence/absence of fire and response variables included earthworm mortality and biomass change. C, N and cation data were analyzed using a two-way analysis of variance (ANOVA) using Statistical Analysis Software (SAS Institute Inc., Cary, NC, USA). Responses were considered significant when $p < 0.05$.

Results

Simulated Prescribed Fire Experiment

Earthworm mortality was significantly higher ($p=0.0004$) in samples with less moisture (Fig. 3.1). Mortality was not significantly affected by fire treatments and there was no interaction between fire and moisture treatments. Mortality was not significantly different between burned and unburned samples for each moisture treatment.

After four weeks of incubation, earthworm mortality was again significantly higher ($p=0.03$) in samples with less moisture (Fig. 3.2). There was also a significant effect of fire after four weeks as burned samples had significantly higher (p=0.005) earthworm mortality (Fig. 3.2). No interaction between fire and moisture was seen on earthworm mortality after four weeks of incubation post-fire.

Average soil temperature one cm below the soil surface increased by 13.36°C during combustion (26.03°C) (Fig. 3.3). Average soil temperature 5.5 cm below the soil surface increased by 6.27°C during combustion (17.20°C) (Fig. 3.3). As expected, surface soil heated faster and reached a higher maximum temperature during combustion than deeper soil (Fig. 3.3). The highest maximum temperature achieved one cm below the soil surface of any microcosm was 42.1°C; this was considerably higher than the average maximum temperature.

Post-wildfire Survival Experiment

After four weeks incubation, earthworms in cores from the burned site lost biomass whereas earthworms in soil from the unburned site gained biomass (Fig. 3.4). Earthworms in soil collected from the burned site lost an average of 1.89 (± 0.99 standard error) percent biomass and earthworms in soil from the unburned site gained an average of 18.07 (± 4.7 standard error) percent biomass during incubation. Differences in biomass were statistically significant (p=0.005) between treatments. After four weeks incubation, one earthworm in soils from the burned site was lost to mortality and no earthworms in soils from the unburned site were lost. All but one core containing soil from the burned site experienced biomass loss after four weeks and all cores containing soil from the unburned site experienced biomass gain.

After eight weeks incubation, mortality in both treatments increased. Although not statistically significant, observed mortality was 62 percent in microcosms with soil from the burned area, and 25 percent in microcosms with soil from the unburned site (Fig. 3.6). Mean percent biomass loss was significantly (p=0.02) higher in soils from the burned site with loss being 58.87 (± 17.2 standard error) percent in soils from burned cores and 12.67 (± 22.4 standard error) percent in soils from unburned cores (Fig. 3.5). All cores containing soil from the burned site experienced biomass loss and three experienced 100 percent mortality. Two of five cores containing soil from the unburned site had biomass loss with one core having 100 percent mortality after eight weeks.

There were significant differences in Ca concentrations in the 0-5 cm (A-horizon) depth ($p = 0.006$) at sampling time two (after eight weeks) (Fig. 3.7). However, there were no significant differences in the concentrations of Mg or K for any of the samples at any treatment level ($p = 0.3$ and 0.5, respectively) (Figs. 3.8 and 3.9). There was also no significant difference between sampling times for Mg or K ($p = 0.5$ and 0.6 for Mg, respectively, and $p = 0.3$ for K at both time periods) (Figs. 3.8 and 3.9).

No significant differences were seen in percent N, percent C, or C/N ratios of soil at sampling time one (after four weeks) at any depth regardless of fire ($p = 0.2$, 0.5 and 0.3, respectively) (Figs. 3.10, 3.11 and 3.12). Percent N was significantly higher ($p = 0.04$) in soils from the unburned site and significantly lower at the 5-15cm depth ($p < 0.0001$) at time two (after eight weeks) (Fig. 3.10). Percent C was significantly lower ($p < 0.0001$) in soils from the 5-15 cm depth at time two but no differences between burned and unburned soils were detected (Fig. 3.10). C/N ratios were also significantly lower ($p < 0.0001$) in soils from the 5-15cm depth and no differences between burned and unburned soils were detected at time 2 (Fig. 3.12).

Discussion

Simulated Prescribed Fire Experiment

No direct effect of fire on earthworm mortality was immediately seen in the simulated burn portion of this study; mortality was not significantly different between burned and unburned cores. However, there was an effect of moisture on earthworm survival (regardless of fire treatment), which is not surprising. Recent studies have shown that the epigeic earthworm species *Lumbricus rubellus* is much more tolerant of high moisture conditions and has much greater success in high moisture conditions than in lower moisture conditions (Zorn et al. 2008). What was surprising was the fact that little to no direct mortality was seen due to fire. Microcosms at field capacity moisture that experienced fire had a six (± 4 standard error) percent rate of mortality whereas microcosms that did not experience fire had two (± 2 standard error) percent mortality (Fig. 3.1) but they were not significantly different from one another ($p = 0.39$). Samples with low moisture content were also not significantly different from one another ($p = 0.15$) with low moisture microcosms that experienced fire having a 40 (± 8 standard error) percent mortality compared to a 23 (±9 standard error) percent mortality in microcosms that were not burned (Fig 3.1). These trends suggest a higher overall mortality for burned cores but significance was not detected immediately due to large variances in mortality. However, significant results were achieved four weeks after fire. Results from samples incubated four weeks reflect patterns that were seen in the post-wildfire survival experiment: earthworm survival is detrimentally impacted post-fire. This is presumably due to a lack of edible substrate.

The lack of direct mortality during fire may be an artifact of experimental methods and not due to the actual effects of fire (see methods). Microcosms were buried in sand in a test bed that was surrounded by a raised concrete wall. Microcosms were reconstructed from deciduous forest floor soils and litter collected at the same site was placed on top of the microcosms after earthworms were introduced. Once fire was applied, high temperatures were achieved at the surface of the sand. Fire moved over the cylinders but complete combustion of the litter material contained in the microcosms was not achieved. This led to relatively low temperatures one cm below the soil surface. The highest temperature recorded one cm below the soil surface was 41ºC which, apparently, was not high enough to significantly impact earthworm survival during fire.

These rates of mortality can also be attributed to earthworm escape. Earthworms were observed to have escaped from sealed microcosms before fire was applied by crawling through the mesh and out of the microcosm. Differentiation of mortality and escape however, cannot be made and are therefore reported as mortality. Though novel, methods used for simulating a controlled burn of a deciduous forest ecosystem could be improved upon.

Post-wildfire Survival Experiment

Indirect mortality over time was seen in the post-fire survival portion of this study and definitive biomass changes were also observed. This shows that earthworm survival is indeed impacted after wildfire in an eastern deciduous forest. To determine the mechanism of decreased survival, chemical analyses of soil were completed and results of analyses are presented above. It has been shown that during fire, C and N volatilize and the amounts of soil C and N post-fire are much smaller than pre-fire (Choromanska and DeLuca 2001, 2002). Cation concentrations are also much higher post-fire as Ca, Mg, and K are generally not volatilized (Viro 1974; DeBano et al. 1998). Results confirm these expected trends and also show that earthworms had an impact on nutrient and cation availability within the soils (see results).

The significant difference of Ca at time two (after eight weeks) is as expected, as Ca concentrations have been shown to be higher in upper soils (A-horizon) post-fire (Viro 1974; DeBano et al. 1998). Significant differences in N were also as expected, with a higher N content being in soils that had not experienced fire (Choromanska and DeLuca 2001, 2002). An effect of depth was also expected, with N and C concentrations being lower in deep soil. However, the results presented above do not account for the significant differences in earthworm biomass between soils from burned and unburned sites.

The combination of C, N, and cation concentrations, along with biomass change and mortality, allows for a mechanism of earthworm decline to be hypothesized: the mechanism for mortality was starvation or at least unfavorable habitat conditions after a fire. The removal of organic material (food resources) could be a possible mechanism for deleterious effects of fire on earthworms. Fire does causes a number of other changes within soils such as increased pH, nitrogen removal through combustion, and release of micronutrients (De Ronde 1990; DeBano et al. 1998; Choromanska and DeLuca 2001; Rhoades et al. 2002; Mabuhay et al. 2003; Parr and Chown 2003). However, these changes were not significant after four weeks incubation where the largest differences in biomass were observed.

Earthworms in soils from the burned site exhibited a constant decrease in biomass over eight weeks. No surface organic material was present in cores from the burned site at the time of collection due to combustion by wildfire. Earthworms in soils from the unburned site first gained, and then lost biomass, presumably due to consumption and subsequent lack of food resources over the eight weeks. Little to no organic material (Oe, or Oa) remained on soils from the unburned sites after four weeks incubation due to mixing and incorporation into the mineral soil by earthworms. Only earthworm castings and some whole litter (Oi) could be observed.

During the first four weeks, earthworms were active and mixed the upper soil (A-horizon) as expected and these results were seen in the chemical analyses (Figs3.7-3.11) as well as through lack of organic material. The lack of organic material on soils from the unburned site after eight weeks may reflect a decline in resources for these earthworms and this may account for subsequent biomass loss.

Conclusions

Failure to demonstrate direct earthworm mortality due to fire does not mean that fire does not directly impact earthworms. Further study into the validity of the use of fire in controlling exotic, invasive earthworm species is warranted. Direct effects were not observed in this study, possibly because of an incomplete combustion of surface litter and subsequent heating of surface soil. What little literature exists on responses of below-ground species to fire does not cover any possible direct effects of fire and should be explored more. Post-fire effects on earthworms however, were seen giving credence to the use of fire in abatement of exotic, invasive earthworm distribution. Though expansion in the study of post-fire effects is also warranted, it is suggested that invasive, epigeic earthworm species are not successful after fire due to a lack of food resources or habitat alteration (e.g. loss of surface litter). This, and other future studies, should be used to incorporate fire into policy decisions and land management strategies.

References

Bohlen, P. J., P. M. Groffman, et al. (2004). Ecosystem Consequences of Exotic Earthworm Invasion of North Temperate Forests. Ecosystems. **7:** 1-12.

Bohlen, P. J., D. M. Pelletier, et al. (2004). Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests. Ecosystems. **7:** 13-27.

Bossyut, H., J. Six, et al. (2005). "Interactive effects of functionally different earthworm species on aggregation and incorporation and decomposition of newly added residue carbon." Geoderma **130**(2006): 14-25.

Callaham, M. A., J. M. Blair, et al. (2003). "Macroinvertebrates in North American tallgrass prarie soils: effects of fire, mowing and fertilization on density and biomass." Soil Biology and Biochemistry **35**(2003): 1079-1093.

Callaham, M. A., D. D. Richter, et al. (2006). Long-term land-use effects on soil invertebrate communities in Southern Piedmont soils, USA. European Journal of Soil Biology. **42:** S150-S156.

Choromanska, U. and T. H. DeLuca (2001). "Prescribed Fire Alters the Impact of Wildfire on Soil Biochemical Properties in a Ponderosa Pine." Soil Science Society of America Journal **65**(1): 232.

Choromanska, U. and T. H. DeLuca (2002). "Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects." Soil Biology & Biochemistry **34**(2): 263.

De Ronde, C., Ed. (1990). Impact of prescribed fire on soil properties- comparison with wlidfire effects. Fire In Ecosystem Dynamics. The Hague, The Netherlands, SPB Academic Publishing.

DeBano, L. F., D. G. Neary, et al., Eds. (1998). Fire's Effects on Ecosystems. New York, John Wiley and Sons.

Fisk, M. C., T. J. Fahey, et al. (2004). Earthworm Invasion, Fine-root Distributions, and Soil Respiration in North Temperate Forests. Ecosystems. **7:** 55-62.

Fragoso, C., P. Lavelle, et al. (1999). Earthworm communities of tropical agroecosystems: origin, structure and influences of management practices. Earthworm Management in Tropical Agroecosystems. P. Lavelle, L. Brussard and P. F. Hendrix. New York, CABI Publishing**:** 27-55.

Hale, C. M., L. E. Frelich, et al. (2006). "Changes in harwood forest plant communities in response to European Earthworm invasions." Ecological Society of America **87**(7): 1637-1649.

Hale, C. M., L. E. Frelich, et al. (2005). "Effects of European Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA." Ecosystems **8**: 911-927.

Hendrix, P. F. (2006). "Biological invasions belowground-earthworms as invasive species." BIological Invasions **8**: 1201-1204.

Hendrix, P. F. and P. J. Bohlen (2002). "Exotic Earthworm Invasions in North America: Ecological and Policy Implications." Bioscience **52**(9): 801-811.

Hendrix, P. F., M. A. Callaham Jr., et al. (2008). "Pandora's Box Contained Bait: The Global Problem of Introduced Earthworms." Annual Reviews of Ecological and Evolutionary Systematics **39**: 593-613.

Jegou, D., Cluzeau, D., Balesdent, J. and Trehen, P. (1998). "Effects of four ecological categories of earthworms on carbon transer in soil." Applied Soil Ecology **9**: 249-255.

Jones Jr., J. B. (1990). "Universal soil extractants: their composition and use." Communications in Soil Science and Plant Analysis **21**(13-16): 1091-1101.

Keeley, J. E. (1991). "A Continent on Fire." Natural History **100**(4): 82-84.

Keeley, J. E. and C. J. Fotheringham (2001). Historic Fire Regime in Southern California Shrublands. Conservation Biology, Blackwell Publishing Limited. **15:** 1536-1548.

Keeley, J. E., C. J. Fotheringham, et al. (2005). Factors affecting plant diversity during post-fire recovery and succession of mediterranean-climate shrublands in California, USA. Diversity & Distributions, Blackwell Publishing Limited. **11:** 525-537.

Keeley, J. E., C. J. Fotheringham, et al. (2006). DEMOGRAPHIC PATTERNS OF POSTFIRE REGENERATION IN MEDITERRANEAN-CLIMATE SHRUBLANDS OF CALIFORNIA. Ecological Monographs. **76:** 235-255.

Mabuhay, J., Y. Isagi, et al. (2006). Wildfire effects on microbial biomass and diversity in pine forests at three topographic positions. Ecological Research, Springer Science & Business Media B.V. **21:** 54-63.

Mabuhay, J. A., N. Nakagoshi, et al. (2003). Microbial biomass and abundance after forest fire in pine forests in Japan. Ecological Research, Springer Science & Business Media B.V. **18:** 431-441.

Mehlich, A. (1953). Determination of P, K, Na, Ca, Mg and NH4. S. T. Division. Raleigh, NC, North Carolina Department of Agriculture.

Parr, C. L. and S. L. Chown (2003). Burning issues for conservation: A critique of faunal fire research in Southern Africa. Austral Ecology, Blackwell Publishing Limited. **28:** 384-395.

Rhoades, C., T. Barnes, et al. (2002). Prescribed Fire and Herbicide Effects on Soil Processes During Barrens Restoration. Restoration Ecology, Blackwell Publishing Limited. **10:** 656-664.

Steinberg, D. A., R. V. Pouyat, et al. (1997). "Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient." Soil Biology and Biochemistry **29**(3/4): 427-430.

USDA and NRCS. (2008). "Official Soil Series Description." Retrieved February 10, 2008, from http://soils.usda.gov/technical/classification/osd/index.html.

Viro, P. J., Ed. (1974). Effects of Forest Fire on Soil. Fire and Ecosystems. New York, Academic Press.

Zorn, M. I., C. A. M. Van Gestel, et al. (2008). Flooding responses of three earthworm species, Allolobophora chlorotica, Aporrectodea caliginosa and Lumbricus rubellus, in a laboratory-controlled environment. Soil Biology & Biochemistry. **40:** 587-593.

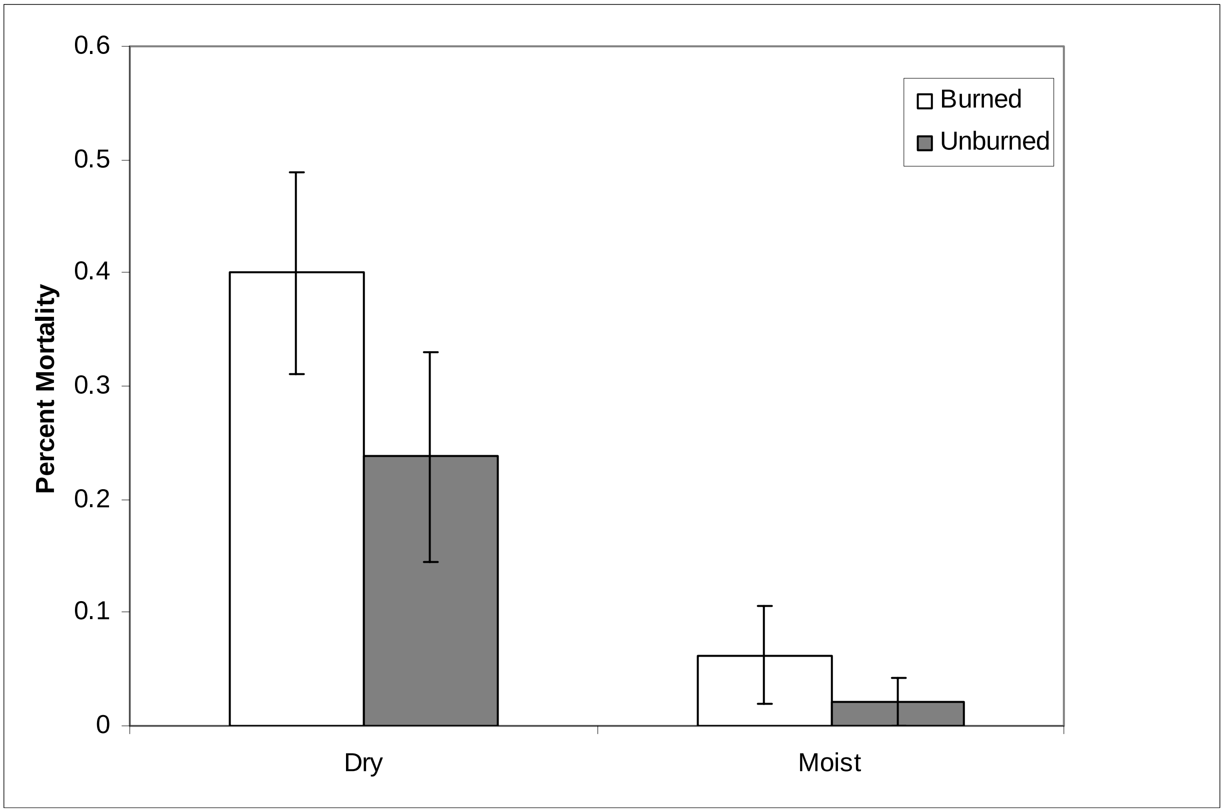

Fig 3.1. Direct earthworm mortality (± SE) immediately after a simulated prescribed fire experiment.

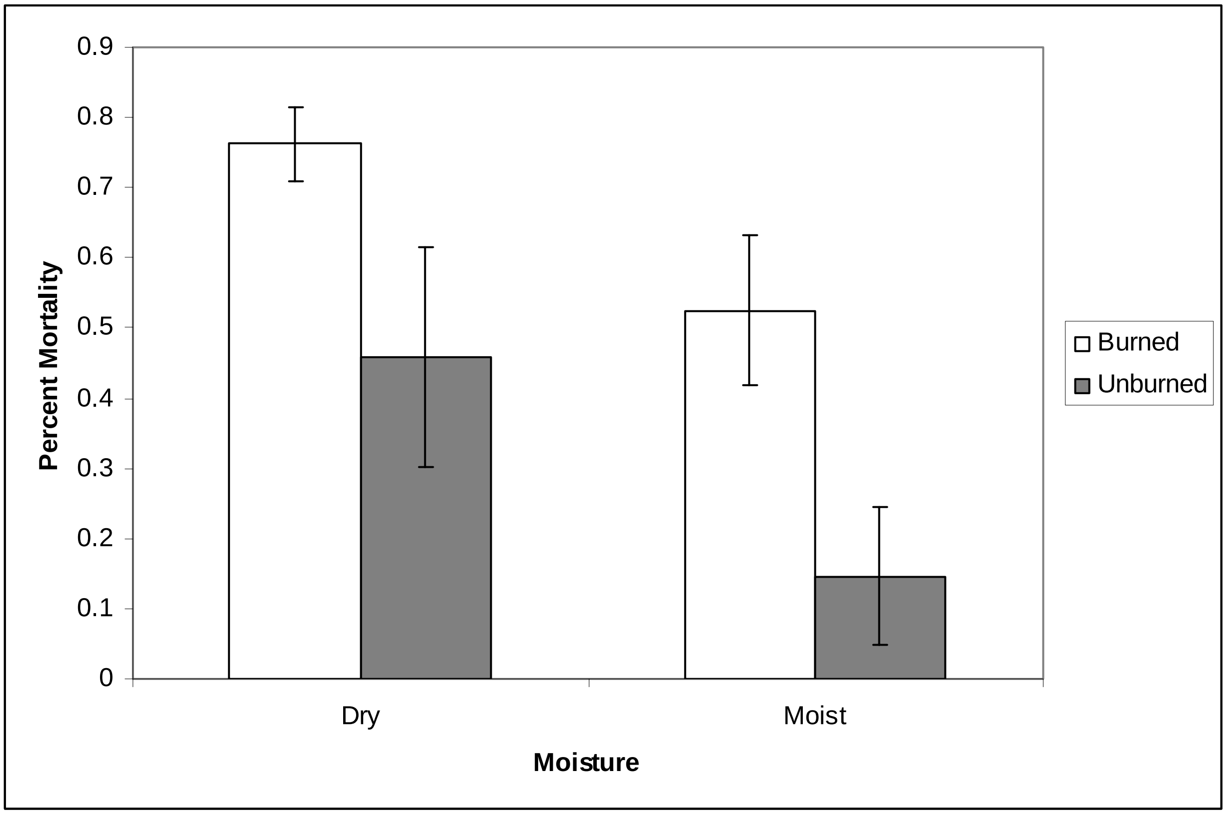

Figure 3.2. Direct earthworm mortality (± SE) four weeks after combustion in the simulated prescribed fire experiment.

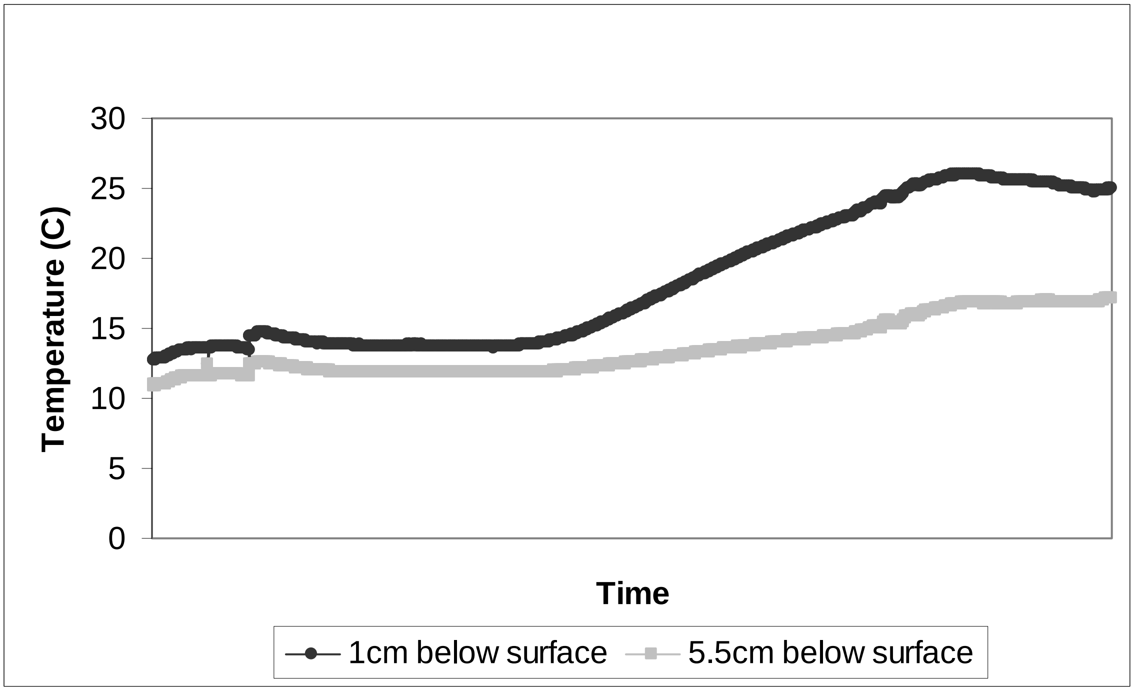

Fig. 3.3. Mean temperatures below the soil surface in microcosms during the simulated prescribed fire experiment. Total length of time for temperature data is approximately one hour and is scaled accordingly.

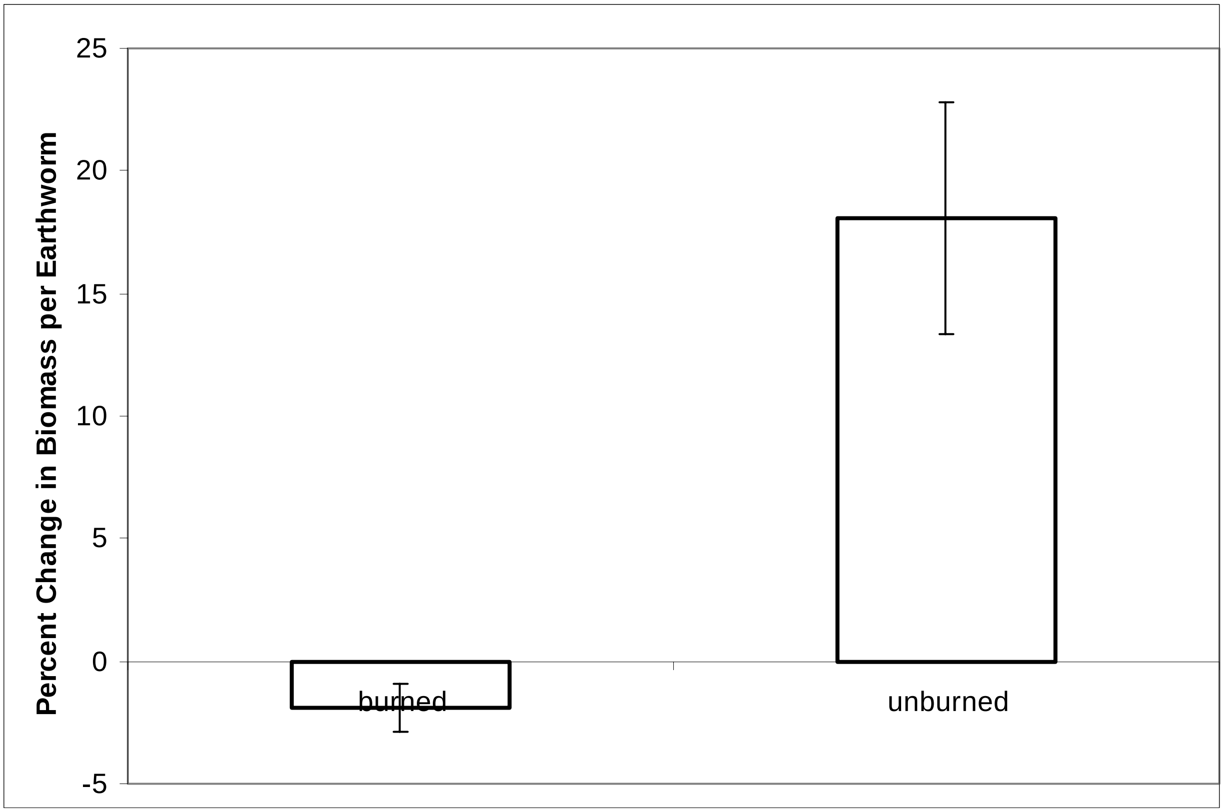

Fig. 3.4. Mean percent change in biomass (± SE) per earthworm after four weeks in the post-wildfire survival experiment.

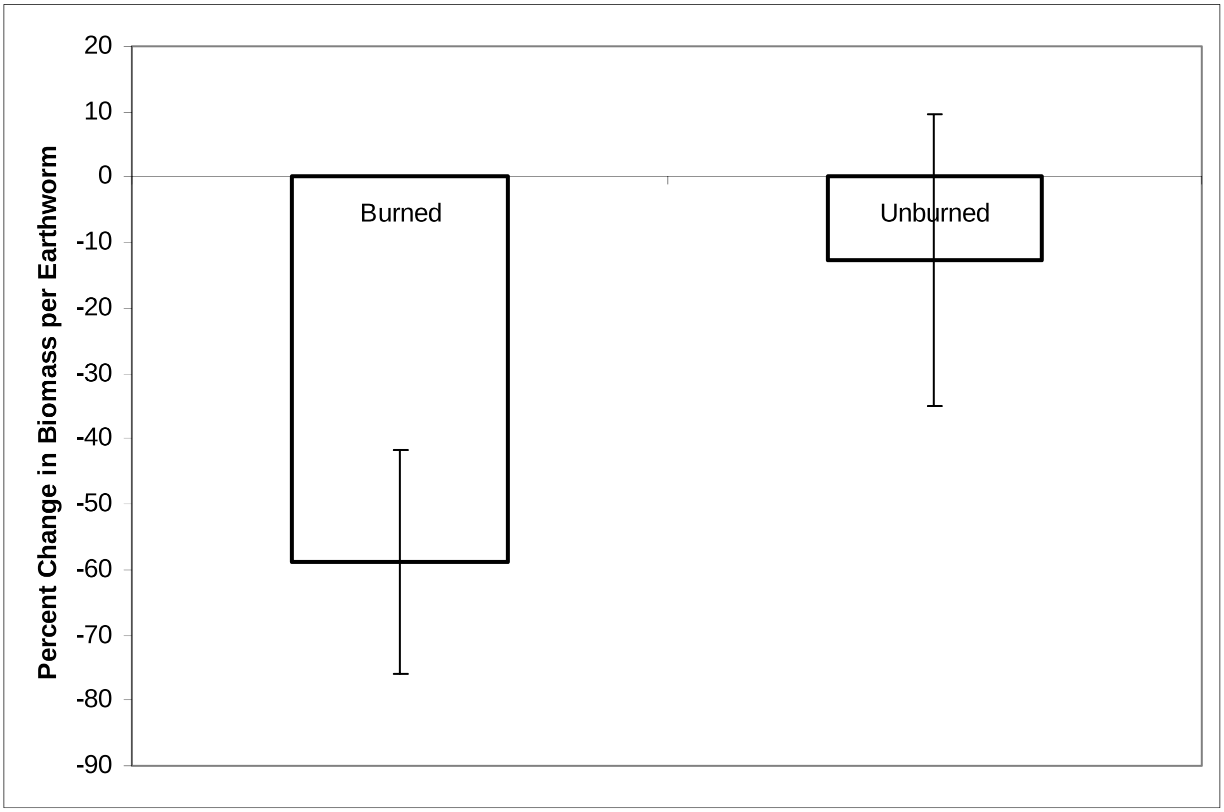

Fig. 3.5. Mean percent change in biomass (± SE) per earthworm after eight weeks in the post-wildfire survival experiment.

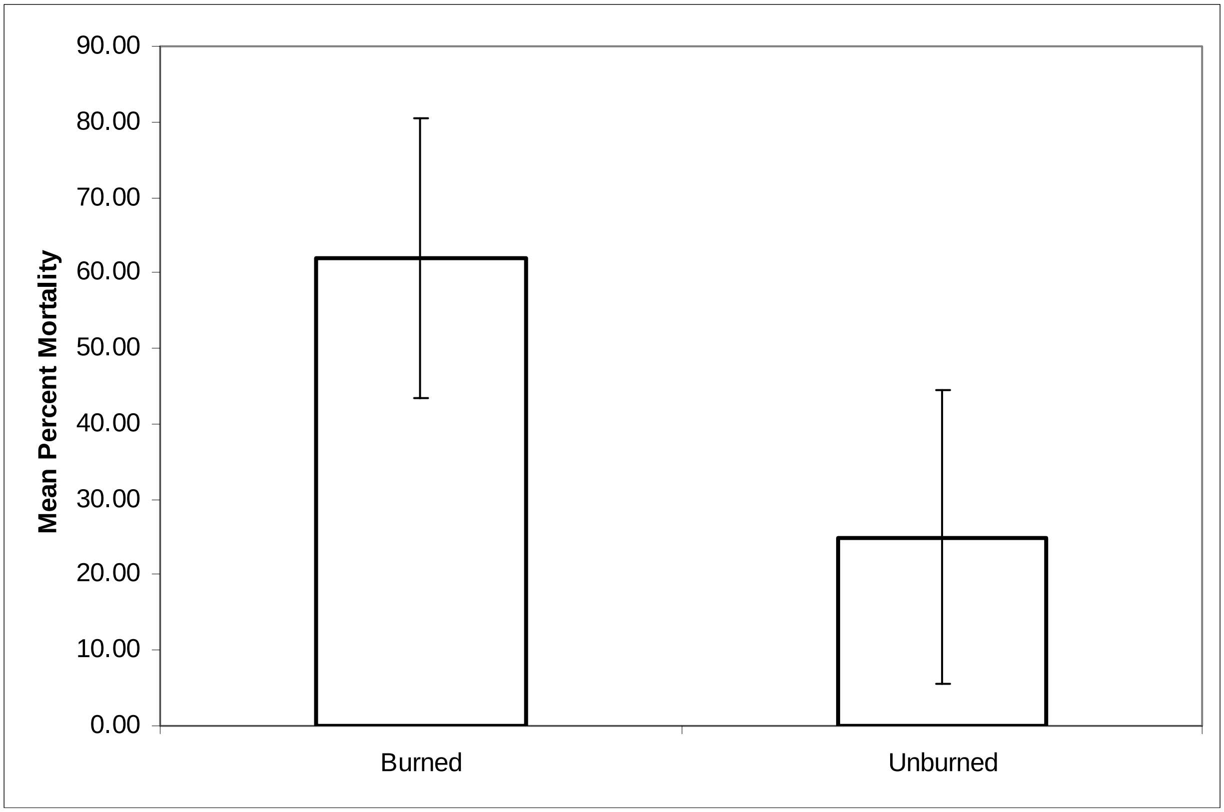

Fig. 3.6. Mean mortality (± SE) after eight weeks in the post-wildfire survival experiment.

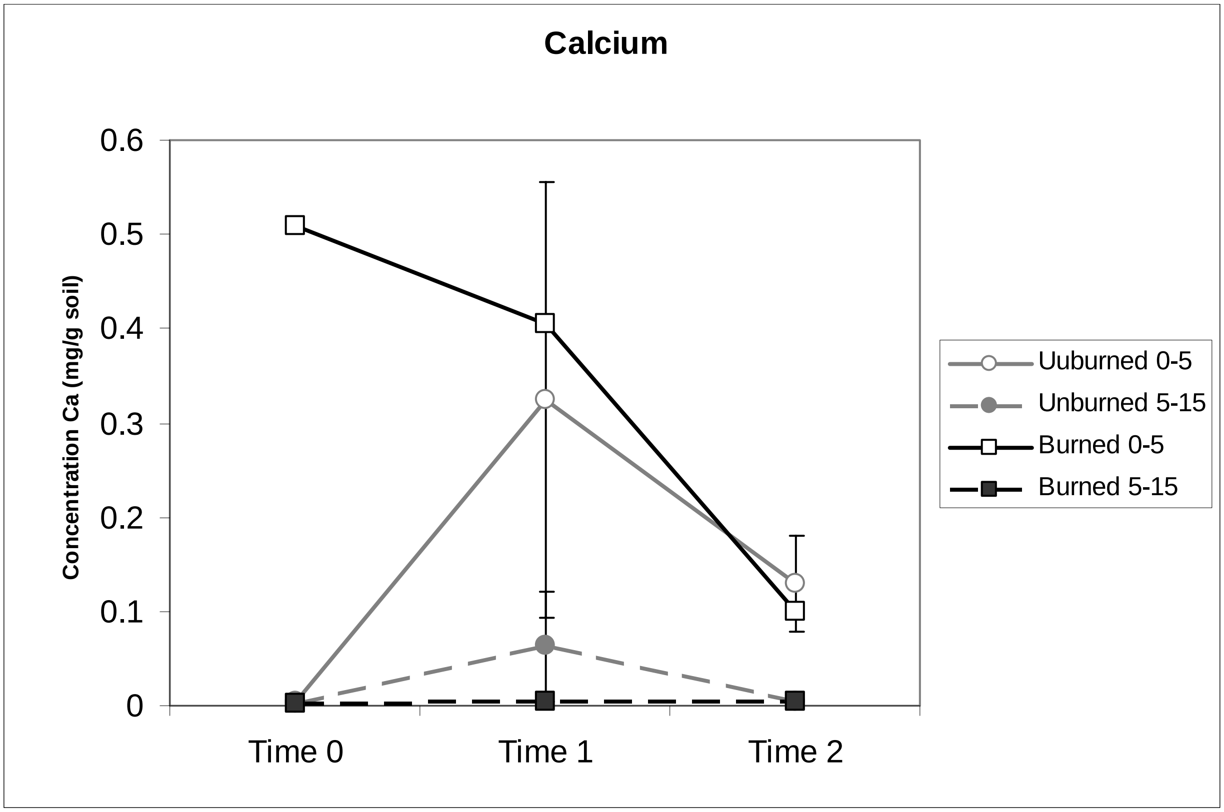

Fig. 3.7. Concentrations of Ca (± SE) for burned and unburned microcosms in the post-wildfire survival study at two different depth ranges (0-5 cm; 5-15 cm) for three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) in the post-wildfire survival experiment

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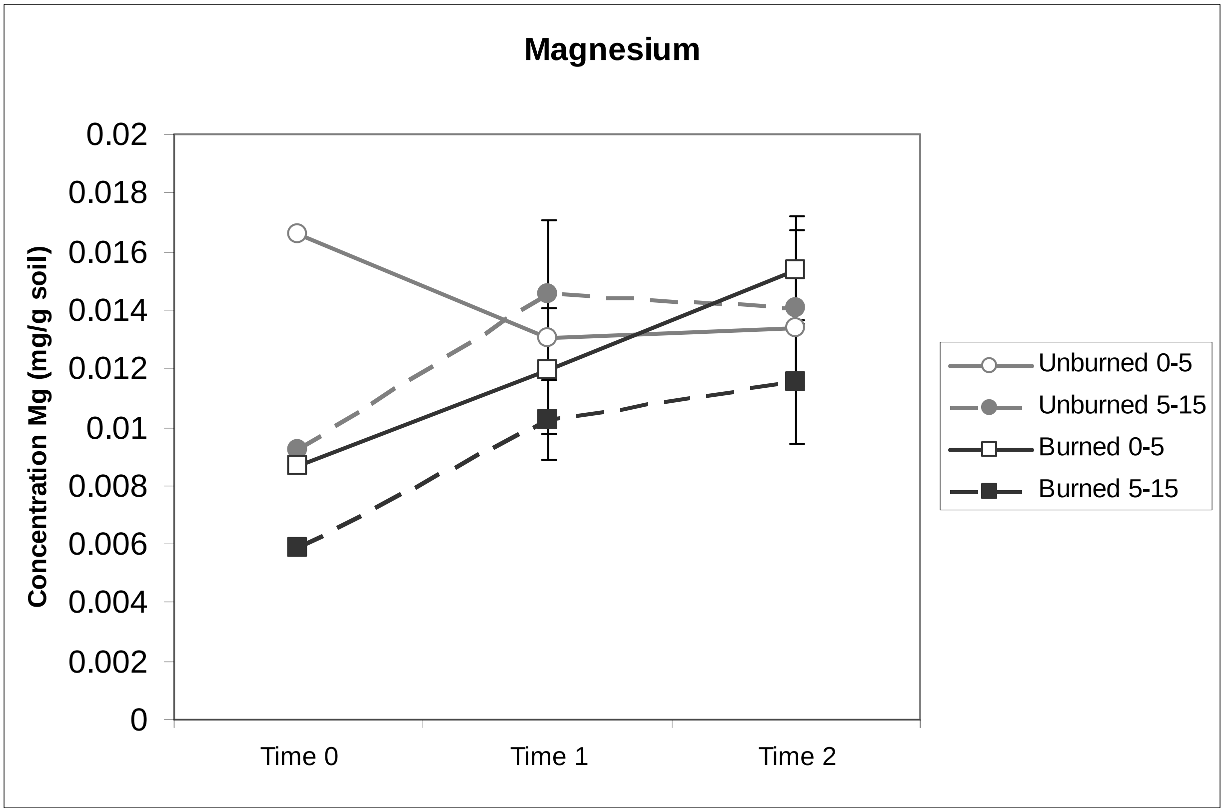

Fig. 3.8. Concentrations of Mg (± SE) for burned and unburned microcosms in the post-wildfire survival study at two different depth ranges (0-5 cm; 5-15 cm) for three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) in the post-wildfire survival experiment.

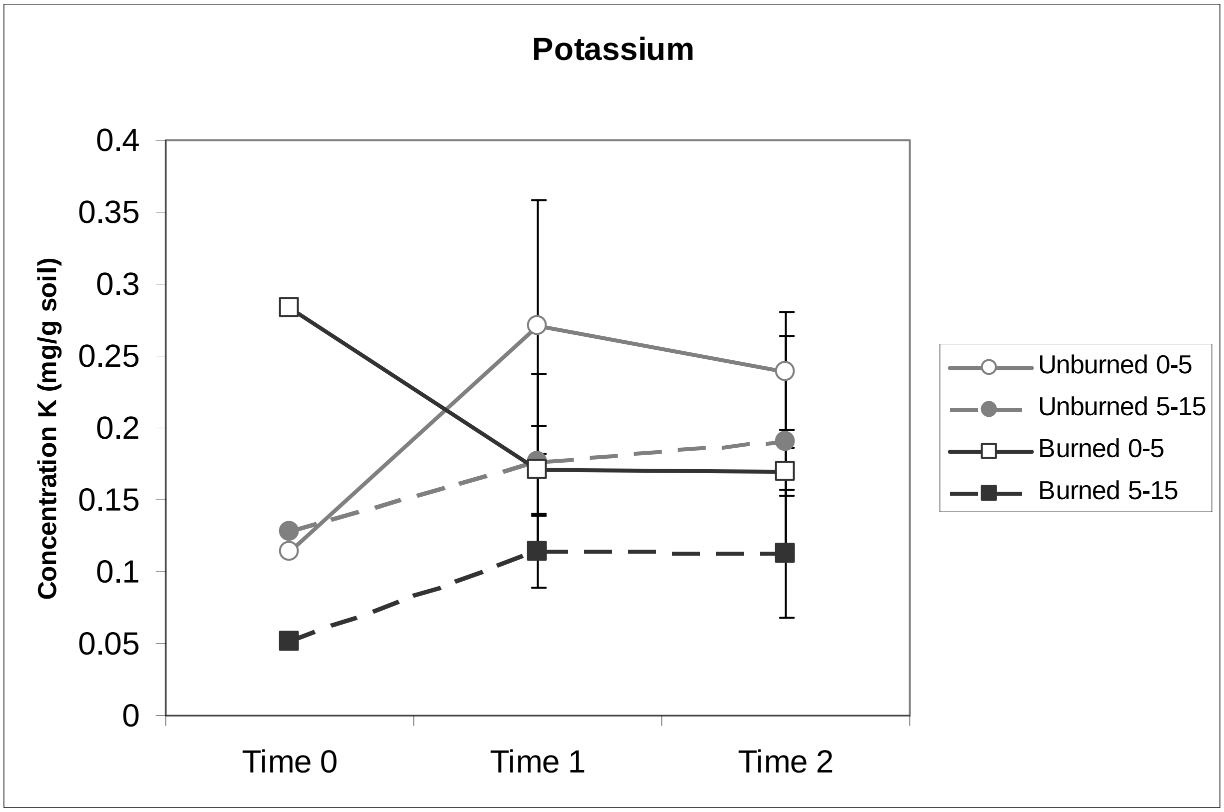

Fig. 3.9. Concentrations of K (± SE) for burned and unburned microcosms at two different depth ranges (0-5 cm; 5-15 cm) for three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) in the post-wildfire survival experiment.

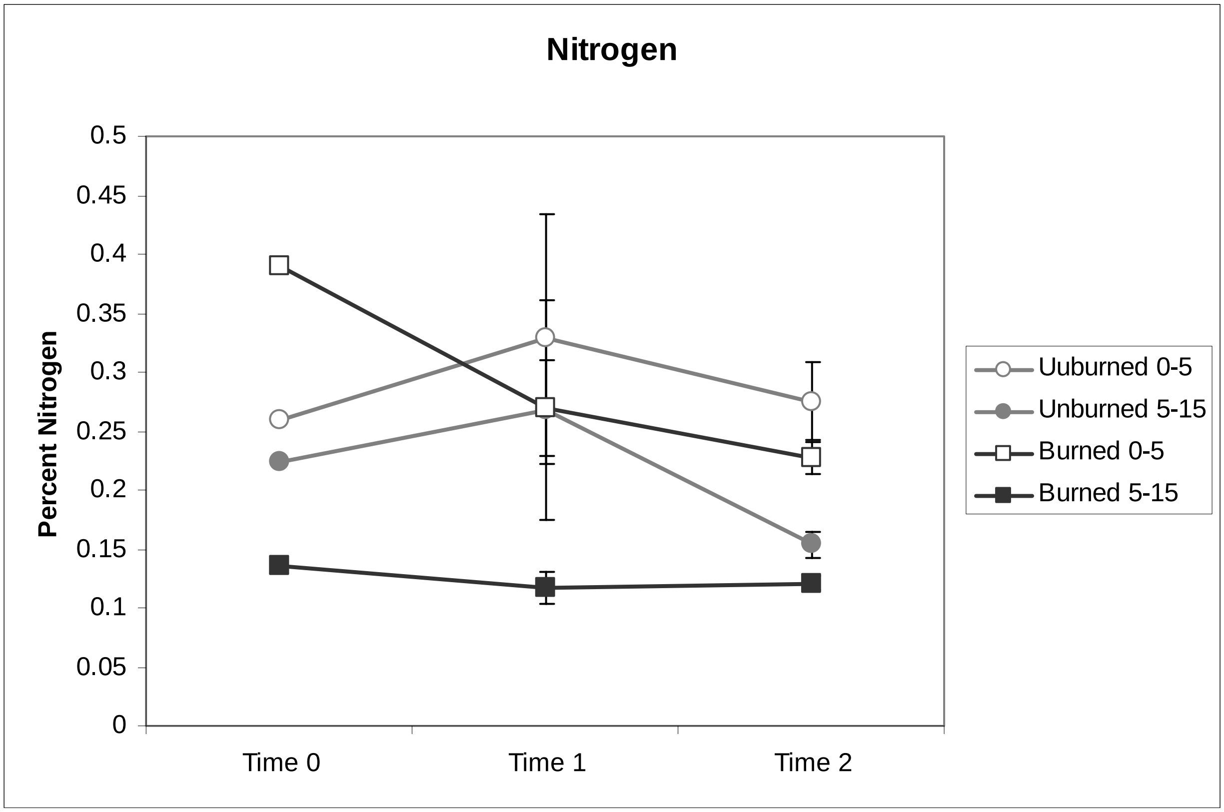

Fig. 3.10. Percent N (± SE) at three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) at two different depth ranges (0-5 cm; 5-15 cm) in the post-wildfire survival experiment.

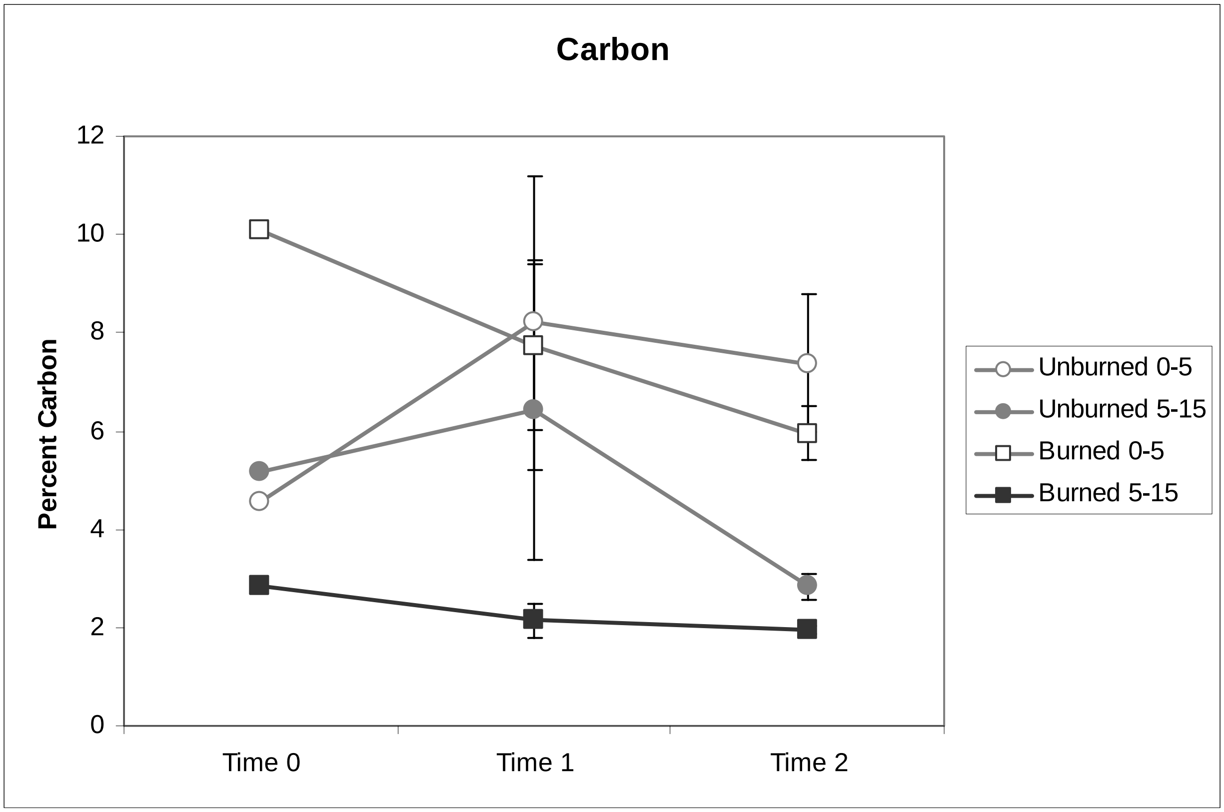

Fig. 3.11. Percent C (± SE) at three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) at two different depth ranges (0-5 cm; 5-15 cm) in the post-wildfire survival experiment.

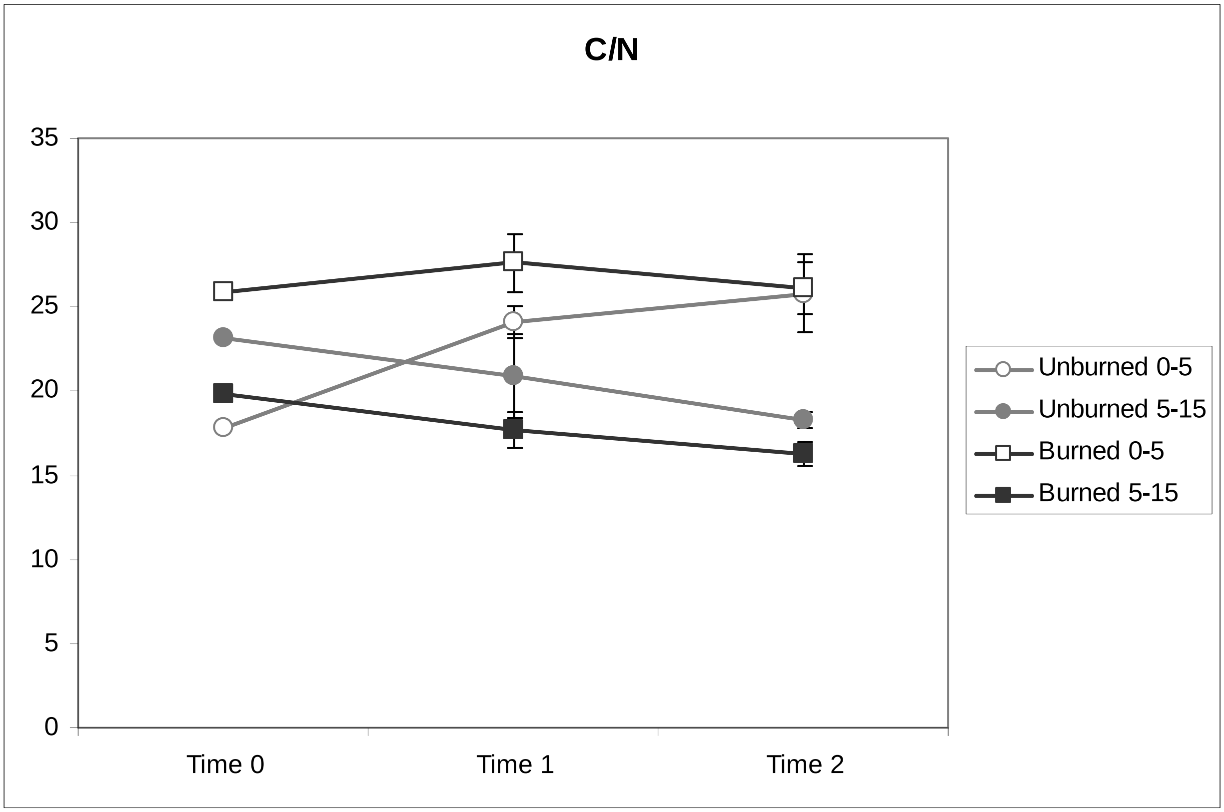

Fig. 3.12. C/N ratios (± SE) at three different sampling times (Time 0= initial; Time 1= after four weeks; Time 2= after eight weeks) at two different depth ranges (0-5 cm; 5-15 cm) in the post-wildfire survival experiment.

Chapter 4

Conclusions

Effects of Temperature on Hatchling Emergence in Amynthas agrestis Cocoons

Results from the manipulation of *Amynthas agrestis* cocoons using temperature were somewhat expected. In general, temperature was expected to affect the hatching rate of cocoons. However, it was thought that *A. agrestis* cocoons would be adapted to higher temperatures for hatching due to their subtropical origins. It was not expected that 10˚C would be the temperature at which optimal emergence would occur. Given the significance of hatching at this temperature (p=0.02), *A. agrestis* cocoons are more adapted for colder temperatures than previously thought.

Since significantly different rates of hatching were seen at 10˚C (p=0.02), this is the optimal temperature cue for *Amynthas agrestis*. Cocoons over-winter in the organic horizons of the soil and experience temperatures at this level or lower. In a natural setting, once soil temperatures reached 10˚C for a period of time, they again warm. This would lead to emergence of hatchlings and was demonstrated in the limited-cold incubations as cocoons held at 10˚C hatched approximately six weeks after incubation. This occurred in every case, the exception being that cocoons held at 10˚C for 60 days emerged a week earlier (Fig. 1.4). Under field conditions, cocoons would presumably not experience 60 days of temperatures at 10˚C and after being subjected to this temperature for a longer period of time, embryonic development would be more rapid once temperatures were raised.

Timing of hatching was also important as cocoons held at 10˚C took longer to hatch (Fig. 1.2). Hatching did not occur until after 17 weeks of incubation and ceased by the 21st week. This is a very short time period for emergence and is biologically significant when considered in the context of invasion biology. Before death, *Amynthas agrestis* is likely to produce large numbers of cocoons to ensure success (e.g. as in Table 1.1). Once temperatures drop during winter months and a warming is experienced in spring months, cocoons hatch at approximately the same time. This information could be useful to prevent or inhibit the invasional success of *A. agrestis*. If cocoons can be destroyed before hatching, future population numbers can be significantly decreased. Cocoon removal by either mechanical, human or burning methods could be employed in order to decrease population numbers of juvenile *A. agrestis*. Further investigation into these methods is warranted.

Limited-cold manipulations again showed 10˚C was the optimal temperature for emergence as only one cocoon from any other incubation temperature (5 or 15˚C) hatched. Cocoons held for 60 days at 10ºC had a higher rate of hatching (50%) than any other combination of incubation temperature and time interval. This demonstrates that length of colder temperatures is important and could impact future generations and preventative methods. For example, if an unseasonably warm winter occurs, perhaps cocoons of *A. agrestis* will have less success, which could also be important in the face of global climate change as mean annual temperatures are expected to increase. This could lead to future invasions in a poleward direction for *Amynthas agrestis* into habitats where they are not presently known to occur (Hendrix et al. 2008).

Conclusions

In order to fully understand invasion biology, life history traits must be fully explored. Little is known about life history traits for many exotic invasive earthworm species. As knowledge of these characteristics increases, so shall the understanding of the invasional success of many Asian species of earthworm and how they are able to invade areas that have been previously invaded by European species and areas that are seemingly pristine habitats. We have demonstrated that external temperature cues affect hatching rates of *Amynthas agrestis* cocoons and that 10ºC is the optimal temperature at which hatching occurs. Knowledge of the life history strategies of Asian invaders will be important in unlocking the mystery of their invasional success if not the key itself. Knowing that temperature plays such a crucial role in cocoon emergence of *A. agrestis* will give insight into how other *Amynthas* species are able to survive away from their places of origin. It will also present a chance to prevent or inhibit future invasions.

The Use of Fire on Exotic, Invasive Earthworm Species

Simulated Prescribed Fire Experiment

No direct effect of fire on earthworm mortality was immediately seen in the simulated burn portion of this study; mortality was not significantly different between burned and unburned cores. However, there was an effect of moisture on earthworm survival (regardless of fire treatment) which is not surprising. Recent studies have shown that the epigeic earthworm species *Lumbricus rubellus* is much more tolerant of high moisture conditions and has much greater success in high moisture conditions than in lower moisture conditions (Zorn et al. 2008). What was surprising was the fact that little to no direct mortality was seen due to fire. Microcosms at field capacity moisture that experienced fire had a six (± 4 standard error) percent rate of mortality whereas microcosms that did not experience fire had two (± 2 standard error) percent mortality (Fig. 3.1) but they were not significantly different from one another (p= 0.39). Samples with low moisture content were also not significantly different from one another (p= 0.15) with low moisture microcosms that experienced fire having a 40 (± 8 standard error) percent mortality compared to a 23 (±9 standard error) percent mortality in microcosms that were not burned (Fig 3.1). These trends suggest a higher overall mortality for burned cores but significance was not detected due to large variances in mortality.

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References

Choromanska, U. and T. H. DeLuca (2001). "Prescribed Fire Alters the Impact of Wildfire on Soil Biochemical Properties in a Ponderosa Pine." Soil Science Society of America Journal **65**(1): 232.

Choromanska, U. and T. H. DeLuca (2002). "Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects." Soil Biology & Biochemistry **34**(2): 263.

De Ronde, C., Ed. (1990). Impact of prescribed fire on soil properties- comparison with wlidfire effects. Fire In Ecosystem Dynamics. The Hague, The Netherlands, SPB Academic Publishing.

DeBano, L. F., D. G. Neary, et al., Eds. (1998). Fire's Effects on Ecosystems. New York, John Wiley and Sons.

Hendrix, P. F., M. A. Callaham Jr., et al. (2008). "Pandora's Box Contained Bait: The Global Problem of Introduced Earthworms." Annual Reviews of Ecological and Evolutionary Systematics **39**: 593-613.

Mabuhay, J. A., N. Nakagoshi, et al. (2003). Microbial biomass and abundance after forest fire in pine forests in Japan. Ecological Research, Springer Science & Business Media B.V. **18:** 431-441.

Parr, C. L. and S. L. Chown (2003). Burning issues for conservation: A critique of faunal fire research in Southern Africa. Austral Ecology, Blackwell Publishing Limited. **28:** 384-395.

Rhoades, C., T. Barnes, et al. (2002). Prescribed Fire and Herbicide Effects on Soil Processes During Barrens Restoration. Restoration Ecology, Blackwell Publishing Limited. **10:** 656-664.

Viro, P. J., Ed. (1974). Effects of Forest Fire on Soil. Fire and Ecosystems. New York, Academic Press.

Zorn, M. I., C. A. M. Van Gestel, et al. (2008). Flooding responses of three earthworm species, Allolobophora chlorotica, Aporrectodea caliginosa and Lumbricus rubellus, in a laboratory-controlled environment. Soil Biology & Biochemistry. **40:** 587-593.