Forest Ecology and Management manuscript

Timeline and goal dates:

Methods and results - 20171217

Hypothesis and predictions (last paragraph of introduction) and Discussion - 20171221

Introduction - 20171227

Abstract, title and first draft - 20171229

To remember:

-Think about where you are going before you write

-Stick to the point and be concise

-Write the last sentence (or paragraph) of the introduction first

REFER TO HARPER AND SEMLITSCH (2007) FOR POSSIBLE MODEL TO FOLLOW FOR METHODS LAYOUT

Material and methods

Provide sufficient details to allow the work to be reproduced by any independent research. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

what to call pens? enclosures? terrestrial mesocosm?

2. Methods

2.1 Study area

Research was conducted within three mixed hardwood forest stand blocks in northeast Ohio. These forest stands were selected because they have been managed for acidified soil since the fall of 2009 as part of an (ecosystem)-level pH experiment. Each of the three forest stands consist of three 800 m2 (30 x 40 m) plots that have been managed via the application of lime-stone (Hi-Ca lime) to elevate soil pH. An additional three untreated plots are located within each forest stand.

2.x Study design

We established three 1m2 sampling locations within each study plot. Sampling locations were randomly assigned one of three types: no enclosure, enclosure with no toads, or enclosure with toads. These three sample locations were generally placed within 2 m of each other along the perimeter of a given study plot. We built enclosures using silt construction fencing that measured approximately 1 meter in height. The bottom of the fencing was buried into the soil and further reinforced with garden staples to prevent toads from escaping. Screen mesh was attached to the top of the fencing and ran along the entire 1m2 perimeter to further prevent toads from escaping and to discourage other animals from potentially colonizing the enclosure. Enclosure construction was completed between 26 April and 25 May 2017 at all three forest plots.

2.x American toad

We collected three clutches of American toad egg masses on 18 April, 2017 from both the Case Western Reserve University’s Squire Valleevue and Valley Ridge Farm (hereafter “CWRU Farm”; Hunting Valley, Cuyahoga County , OH) and the Holden Arboretum (Kirtkland, Lake County, Ohio). Toad egg masses were brought to the CWRU Farm, and each egg mass/clutch was placed into an individual nursery box within a 1,000 L aquatic mesocosom. Once tadpoles reached free swimming stage, we haphazaerdly selected 50 tadpoles from each nursery box and randomly assigned them to one of six 1,000 L aquatic mesocosms based on their population of origin (i.e. CWRU Farm or Holden Arboretum). Aquatic mesocosms were stocked with 10 g rabbit chow and \_\_\_\_ (g?) of leaves from the CWRU Farm. Mesocosms were covered with tightly fitted lids made of 60% shade cloth to prevent colonization by other organisms. Tadpoles were left in mesocosms until completion of metamorphosis (45-52 days).

Newly metamorphosed toads were removed from aquatic mesocosms and placed in enclosures. We placed four toads into each enclosure that was assigned a toad treatment. Toads were weighed immediately following removal from the aquatic mesocosm on the same day they were placed into their respective terrestrial enclosures. Toad mass in enclosures at the initiation of the study period (i.e. mass at metamorphosis) did not differ between forest treatments (*p*=0.40) or among forest stands (*p*=0.26). Average mass at metamorphosis was 0.28 g for toads placed into treated plots and 0.29 g for toads placed into untreated plots. Average mass at metamorphosis was 0.28 g, 0.29 g, and 0.3 g for toads placed into plots at the Farm, Pierson Creek, and Schoop Forest, respectively. Toads from the Holden Arboretum source population reached metamorphosis up to one week prior to toads from the CWRU Farm, and all toads were placed into Holden Arboretum enclosures between 8 and 12 June 2017. All toads from the CWRU Farm source poplulation were placed into enclosures at the CWRU Farm on 15 June 2017.

Toads remained in their respective enclosures for 90 days. On day 25, 60, and 90 each enclosure was searched by one observer (DAD) for five minutes by searching the surface of the leaf litter and gently searching under leaves. All toads captured were weighed before being released back into their pen on days 25 and 60. On day 90 all captured toads were euthanized in MS-222 and preserved in formalin. A second day of searching of each enclosure was conducted the following day (day 91) and an exhaustive search of all leaf litter was conducted on day 92 to ensure that all toads were located. No additional toads were located during these follow-up surveys.

2.x Invertebrate sampling

Invertebrates were sampled twice during the study period, both immediately prior to the introduction of toads into encolosures and immediately following removal of toad. A dual sampling approach was taken in order to assess the invertebrate community both before and after toad presence. The first sampling was conducted in the 24 hours preceding the placement of toads into enclosures. A second sampling was conducted on day 91 (i.e. immediately following removal of toads from the enclosures). Invertebrates were sampled within each of the 54 1m2 sample locations during both sampling periods by placing four 500 mL pitfall traps into the ground so tht each of the four traps were located within one of the four corners of the sampling location. Traps were filled with approximately 60mL of 95% ethanol and left in place for 24 hours (generally from 12:00-12:00). We removed traps after 24 hours and pooled the contents into one sample per 1m2 sample location. Samples contents were later identified to order or to family using a dissecting microscope. Abundances of invertebrate groupings were quantified for each pooled sample. Additionally, Aranaea and Coleoptera were measured to the nearest mm. These measurements were later used in analyses to group possible prey items into size classes.

2.x Soil pH sampling

Average soil pH within each 400 m2 treated and untreated plot in a given forest stand is determined annualy by Holden Arboretum research staff; however, we were interested in measuring pH soil at the microhabitat (i.e. 1m2 sample location) scale. We collected three soil core sub-samples (depth ca. 5cm, diameter ca. 5cm) from each sample location in order to determine soil pH. Sub-samples were homogenized, and we measured pH by adding 6 ml of deionized water to 3 g of homegeneized soil from each sample location to create an aqueous mixture (i.e. a 2:1 water:soil mixture). This mixture was allowed to homogenize for 5 minutes before pH measurements of the aqeuous solution were taken. pH was measured using an  ExTech ExStik II EC500 pH meter. All pH measurements were taken within 24 h of collection.

2.x Statistical analysis

Results

Results should be clear and concise.

3. Results

3.1 Soil pH

3.2 American toad survival and growth

3.3 Invertebrate abundance