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## Integrity of Phosphorus Adsorption in Forested Buffer Strips and Hardwood Forests After Earthworm Invasion

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**Integrity of Phosphorus Adsorption in Forested Buffer Strips and Hardwood Forests After  
Earthworm Invasion**

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**Abstract**

Exotic earthworm invasions have caused the degradation of Vermont's forest floors and changes in the nutrient cycling of forested areas. As climate change progresses, invasions by exotic species are predicted to spread. In Vermont, the most recent species of earthworm to invade are in the genus *Amyntas*. The change in nutrient cycling caused by exotic earthworm invasion will impact the phosphorus cycle in affected ecosystems. Phosphorus runoff is a serious concern for Vermont because it causes eutrophication and consequently algae blooms in Lake Champlain. It is predicted that climate change will increase the intensity of rainstorms which, in turn, will increase nutrient runoff, compounding the problem. This study evaluated if the presence of *Amyntas* spp. earthworms has an impact on the phosphorus adsorption capacity of forested areas, particularly of forested riparian buffers, which help prevent agricultural non-point source pollution. The phosphorus retention capacity was evaluated, as measured by the marginal phosphorus adsorption rate. The earthworm communities present were determined using morphological keys. It was found that the presence of *Amyntas* spp. earthworms did not reduce the phosphorus adsorption capacity of the soil, as initially hypothesized, and that the majority of studied riparian buffers had the ability to retain a significant amount of additional phosphorus.

*Keywords:* Lake Champlain, phosphorus retention, invasive, *Amyntas* spp.

## Introduction

Currently, Vermont is out of compliance with provisions of the Clean Water Act, due to continued phosphorus runoff into Lake Champlain. In many regions of Vermont, phosphorus levels consistently exceed the Total Maximum Daily Load (TMDL) approved by the states of Vermont and New York in 2002. Most of this phosphorus runoff is created by agriculture and homeowners (U.S. Environmental Protection Agency, 2015).

The USDA-NRCS recommends forested riparian buffer strips as a Best Management Practice (BMP) for mitigating phosphorus runoff. Forested riparian buffers have been shown to act as a phosphorus sink, removing as much as 50% of phosphorus leaving agricultural fields (Cooper and Gilliam, 1987; Gilliam, 1994; Simmons et al., 1992; Lawrence et al., 1997). Riparian buffers are effective at reducing nutrient runoff due to the deposition of sediments in these areas, and the subsequent uptake of these nutrients by understory plants. If riparian buffer efficiency decreases due to the presence of earthworms, riparian buffers may become a less efficient management practice for reducing phosphorus runoff, which would have significant implications for the management of phosphorus runoff.

Earthworms were introduced by early European settlers, and are currently spread throughout the entire state of Vermont. Earthworms feed on soil organic matter, mixing the O-horizon with the underlying mineral horizons, reducing the surface organic matter (Dempsey et al., 2011, Burtelow et al., 1998). Few studies conducted have looked at invasive earthworm's impact on forested riparian buffers efficiency. One study did suggest that earthworm presence would increase denitrification in riparian buffers due to the biological changes in the soil (Costello et al., 2009). In fact, many studies on forested riparian buffers fail to control for earthworm presence or absence. Several study sites where forested buffer strip's phosphorus adsorption was quantified (Lyons et al., 1998; Simmons et al., 1992) lacked typical symptoms of earthworm invasions as described by Hale et al. (2006, 2005) at the time of the study (Gorres, personal communication). In the presence of earthworms, these data may not be valid.

In Vermont, there are four distinct ecophysiological earthworm groups. These include:

1. Epigeic earthworms that live near the soil surface and feed on the Oe and Oi horizons.
2. Epi-endogeic earthworms that feed at the soil surface and inhabit the top 20 cm of the mineral soil.
3. Endogeic earthworms that create horizontal burrows in the mineral soil.
4. Anecic earthworms that make deep, vertical burrows into which they translocate litter.

These different ecophysiological earthworm groups impact soil morphology in different ways. *Amyntas* spp. earthworms are an epi-endogeic species of earthworm that leave their castings on the surface of the soil and feed on the top organic horizons and surface soil. It has been found that phosphorus in the upper 0–5 cm of soils was increased by *Amyntas* spp. invasion (Qiu et al., 2017).

Although all 19 species of earthworms currently present in Vermont are invasive, *Amyntas* spp., hailing from eastern Asia, have a particularly significant impact on soil morphology. It was found that in the laboratory setting *Amyntas hilgendorfi* caused a greater increase in concentrations of mineral forms of soil phosphorus than *Lumbricus rubellus*, another epi-endogeic species of earthworm found in Vermont (Greiner et al., 2012).

These changes in soil morphology, coupled with invasive earthworm's impact on the germination and growth of tree seedlings may increase the severity of the problems associated with phosphorus runoff (McCormick, 2007; USDA-NRI Project # 2007-35320-18375). Particularly important for Vermont is that invasive earthworms reduce Sugar Maple (*Acer saccharum*) regeneration (Corio et al., 2009) and understory regeneration in general. As the understory disappears there are likely greater levels of erosion coupled with a decrease in nutrient uptake (Hale et al., 2006, 2005). This allows for increases in nutrient runoff from these forested areas and threatens the integrity of these vital buffers.

In addition, it was found that earthworm castings release four times as much inorganic phosphorus into solution as compared to bulk soil, as measured by water-extractable phosphorus. This indicates that soil phosphorus is more mobile in castings (Sharpley et al. 2002). This could lead to a decrease in the phosphorus adsorption rate of earthworm infested areas. Different

species of earthworms deposit their casting in different layers of the soil; therefore, the earthworm community present could have a significant impact on the level of phosphorus runoff leaving riparian buffer strips. Studies have also found that earthworms may mobilize unweathered soil particles from deeper within the soil, increasing the amount of phosphorus in surface soils (Bohlen et al. 2004). Earthworm invaded riparian buffers may not only become ineffective at retaining nutrient runoff from other sources, but they may actually become a source of pollution themselves.

This study assesses the impact that earthworm communities have on the ability of forested riparian buffers to adsorb phosphorus; more specifically, this study looks at the impact of *Amyntas* spp. as compared to European earthworm species. It also provides more information about the current soil phosphorus levels of riparian buffers in Vermont. It was hypothesized that riparian buffers that have *Amyntas* spp. earthworms present will be more saturated with phosphorus than sites without *Amyntas* spp. present. *Amyntas* spp. earthworms are aggressive epi-endogeic earthworms that feeds on the leaf litter and organic horizon, these earthworms modify the structure of forest soil, mixing the organic horizon with the underlying mineral material. Previous studies have found that carbon cycling was impacted by earthworm species due to the aggregation caused by earthworms (Knowles, 2015). Therefore, it is hypothesized that these earthworms will reduce the phosphorus adsorption capacity of soils due to the increase in mobilized phosphorus and translocation of phosphorus between horizons.

## **Materials and Methods**

### *Site Selection*

Data was collected from six riparian forest sites, three site with *Amyntas* spp. earthworms present and three sites that lacked *Amyntas* spp. earthworms. Within these riparian buffers, samples were collected from the somewhat poorly drained (SPD) and very poorly drained (VPD) areas. Somewhat poorly drained soil is in the transition zone of a riparian forest (Simmons et al., 1992). The very poorly drained soil is in the wetland zone of the forest. These zones are considered independent of each other, therefore they can be statistically compared (Simmons et al., 1992).

The drainage class of each site was determined by examining the occurrence of redoximorphic features. Redoximorphic features are colored patterns created by the reduction of iron or manganese under saturated conditions., which creates a blue or rusty coloration within the soil profile. These features mark seasonal high water tables. Soil pits were dug at each site, the dimensions of which were 30 by 30 cm to a depth of at least 50 cm. The depth of the first redoximorphic feature was measured from the surface of the soil pit, and the drainage class was determined. Redoximorphic features that were found at 20-46cm below the surface indicated very poorly drained soils (VPD), while redoximorphic features first occurring at a depth of 46-91cm indicated somewhat poorly drained soils (SPD).

#### *Identifying Earthworm Communities*

In both the somewhat and very poorly drained areas of each site the composition of the earthworm community was determined. Three areas surrounding each site were examined for earthworms. The leaf litter and the surface of the soil were examined and 30 by 30 cm pits were excavated to a depth of 20 cm. The earthworms found within these areas were identified to species using external characteristics (Reynolds, 1977, Reynolds, 1978). The site was then either determined to be free of *Amyntas* spp. or to be invaded by *Amyntas* spp. earthworms. All sites that were determined to be free of *Amyntas* spp. earthworms did have other earthworm species present. Earthworm density was not accounted since the high densities that *Amyntas* spp. live in may be the reason they have such a significant impact on forest ecosystems. All sites had been invaded by earthworms for at least 3 years at the time of the study.

In addition, the Invasive Earthworm Rapid Assessment Tool (IERAT) was used to classify the extent of the damage to the forest floor at each site (Loss et al., 2013). At each site the amount of leaf litter, presence of middens, and presence of earthworm castings were determined through visual characteristics. The visual characteristics of the forest floor were then used to assess the amount of disturbance caused by earthworm invasion (Figure 4, appendix).

#### *Phosphorus Adsorption*

A composite sample was collected from both the A and B horizons of each site. The

subsamples were collected by randomly selected points within each horizon of the soil pit. The samples were then mixed together in a plastic bag, and shaken to homogenize.

The soil samples were air-dried for three days, then sieved to pass through a 2 mm mesh sieve, and homogenized by shaking in a zip-lock bag. Five concentrations of phosphate solution were used, 0, 1, 3, 5 and 7 mg P/L, to determine the amount of phosphorus adsorption. The phosphorus solutions were prepared with  $\text{NaH}_2\text{PO}_4$  and 0.5 M  $\text{NaNO}_3$ . 15 ml of solution was added to the 1 gram of soil. The vials were then agitated at 50 rpm in the dark at 20° C for 24 hours. The samples were then filtered through an Ahlstrom 642 (9 cm) filter paper. The filtrate was analyzed for soluble reactive phosphorus (SRP) colorimetrically using Lachat QuikChem 800 Series.

Phosphorus adsorption was measured by adding different amounts of phosphorus to the soils. The phosphorus adsorption was then determined by plotting the amount of solution adsorbed versus the amount of phosphorus added. A line was fit to the data. The slope of the line gives the marginal phosphorus adsorption rate. A slope value of 1 would indicate that all of the phosphorus added to the soil was adsorbed, while a value of zero would indicate that none of the added phosphorus was adsorbed onto the soil. Adsorption rates were determined from these soils using the method of Guertal et al. (1991) adjusted by Lyons et al. (1998).

#### *Organic Matter Content (LOI)*

Soil organic matter was calculated through loss on ignition (LOI). 5.000 to 10.000 grams of dried soil, that had been passed through a 2 mm sieve, was weighed and placed into a porcelain crucible with a known weight. The samples were heated in an oven to a temperature of 150° C for 24 hours to remove moisture. Samples were cooled, and reweighed.

The dried samples were heated in a furnace to a temperature 550° C for 6 hours, so that all that would remain would be the mineral portion of the soil. The samples were cooled, and reweighed to calculate the loss on ignition (LOI). The organic matter content was determined based on the pre and post-ignition weights using the following formula:

$$\text{Percent Organic Matter} = \frac{(\text{pre-ignition weight (g)}) - (\text{post-ignition weight (g)})}{(\text{pre-ignition weight (g)})} \times 100$$

### *Statistics*

Treatments were compared at the 10% significance. A Pearson's chi-squared test was used to analyze the relationship between earthworm species and damage class. To examine more specifically the effect of earthworms on phosphorus adsorption, an ANOVA model was used to determine if there was overall an effect of drainage class, soil horizon and earthworm species on adsorption. Since there were only three replicates of the three categorical data there were not enough degrees of freedom to test on all three variables. A line of best fit and r-squared values were calculated to determine the relationship between organic matter content and phosphorus adsorption.

## **Results**

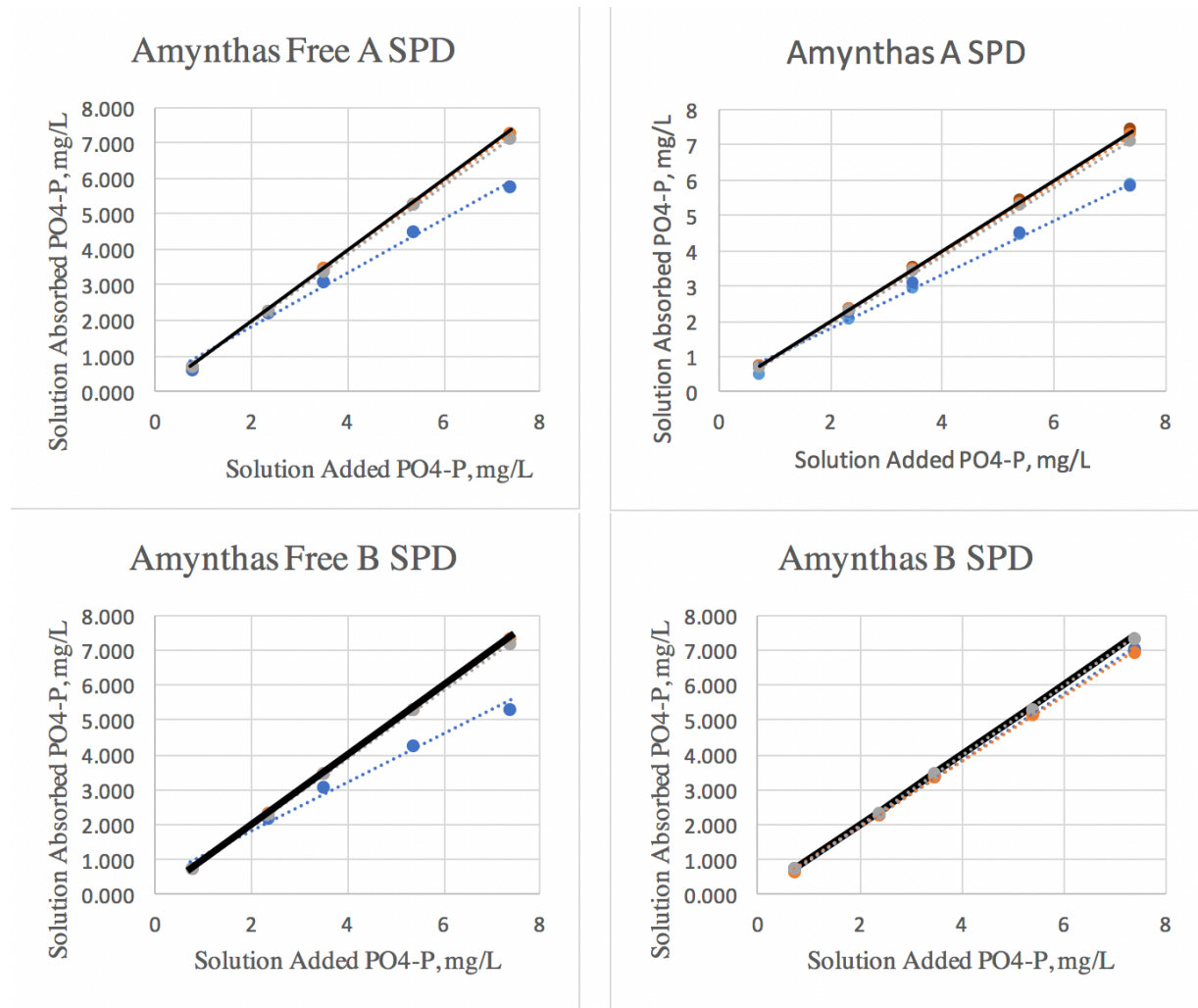
### *Phosphorus Adsorption*

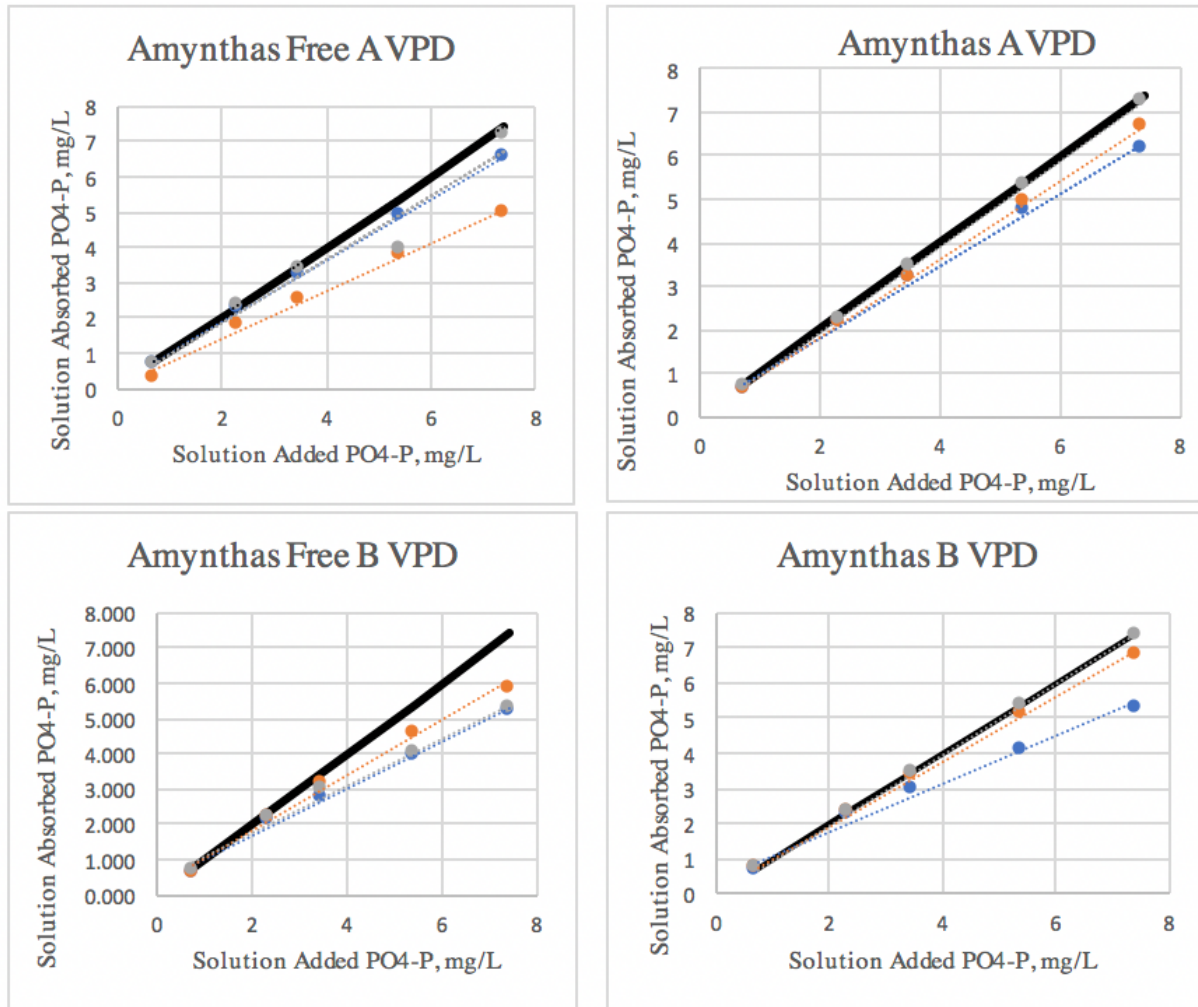
. The rate of phosphorus adsorption was calculated as the slope of the line of best fit (Figure 1). In the graphs a 1:1 line was added for reference; this line represents 100% adsorption. More shallow slopes indicate a lower adsorption capacity.

Phosphorus adsorption was examined for variation by earthworm type, soil horizon, and drainage class (Table 1). The values of the marginal rate of phosphorus adsorption ranged from .6644 to .9993 PO<sub>4</sub>-P mg/L. These values indicate that none of the soils were saturated with phosphorus. No significant difference was observed in the phosphorus adsorption of sites based on earthworm type, soil horizon, or damage class, but at the 0.10 significance level, SPD (somewhat poorly drained) sites had a greater ability to retain phosphorus than VPD (very poorly drained sites (p-value of .0630).

**Figure 1. Phosphorus Adsorption by Earthworm Species, Drainage Class, and Soil Horizon.**

The black line indicates a marginal rate of adsorption of 1 for reference, which would indicate a 100% adsorption rate. Each horizon, drainage class and earthworm species has 3 replicates.





**Figure 2. Adsorption Rate by Variables**

Sample	Adsorption Rate Mean	Standard Deviation	Coefficient of Variation	Range
<i>Amynthus</i> Free	0.827	0.131	0.159	0.664 -0.995
<i>Amynthus</i>	0.897	0.106	0.118	0.671- 0.999
A Horizon	0.869	0.010	0.115	0.6808 -0.985
B Horizon	0.855	0.145	0.170	0.6644 -0.999
SPD Horizon*	0.900	0.110	0.122	0.692 - 0.999
VPD Horizon*	0.824	0.126	0.152	0.664 -0.999

The data was partitioned into different categories; the mean of the adsorption rate (slope of fitted line) was calculated, as was the standard deviation, coefficient of variation, and range for the particular variable. The data was partitioned into earthworm type (*Amynthus* and *Amynthus* Free), soil horizon (A horizon and B horizon) and drainage class (“SPD” somewhat poorly drained and “VPD” very poorly drained).

\*Significant difference at the .10 level

### *Organic Matter*

Organic matter content was determined for each site (Table 2). There was no significant difference in the organic matter content of sites that contained *Amyntas* spp. earthworms as compared to sites that contained European earthworm species. There was also no significant difference in the organic matter content of SPD and VPD sites. As expected, organic matter was significantly higher, at the .05 significance level, in the A horizon as compared to the B horizon of the sites (p-value of .001).

**Figure 3. Organic Matter by Variables**

Sample	Organic Matter Mean	Standard Deviation	Coefficient of Variation	Range
Amyntas Free	4.034	2.647	0.656	1.112 - 8.222
Amyntas	4.036	1.517	0.376	1.761 - 6.744
A Horizon*	5.628	1.744	0.310	3.179 - 8.222
B Horizon*	2.442	0.854	0.350	1.112 - 4.196
SPD Horizon	4.340	2.170	0.486	1.616 - 8.222
VPD Horizon	3.730	2.160	0.579	1.112 - 8.033

The data was partitioned into different categories; the mean of the organic matter content was calculated, as was the standard deviation, coefficient of variation, and range for the particular variable. The data was partitioned into earthworm type (*Amyntas* and *Amyntas* Free), soil horizon (A horizon and B horizon) and drainage class (“SPD” somewhat poorly drained and “VPD” very poorly drained).

\*Significant difference at the .05 level

Furthermore, there was not a significant correlation between the phosphorus adsorption capacity of the soil and the organic matter content. Indicating that there are other factors significantly contributing to the phosphorus adsorption capacity of the soil.

### *Damage Class*

All sites containing *Amyntas* spp. earthworms were classified as a damage class level 5, while the sites containing European earthworm species ranged from a class 3 to 5 (Figure 5, appendix). The sites with *Amyntas* spp. were significantly more likely to be classified as a higher damage class, indicating that *Amyntas* spp. did tend to create greater levels of surface soil disturbance as compared to European species of earthworms.

## Discussion

It was found that both areas with and without *Amyntas* spp. had the capacity to adsorb phosphorus. While this does not hold true for all sites in Vermont, specifically those that apply high levels of cow manure to the surrounding fields, it does suggest that riparian buffer strip soils with earthworms present may still be a significant sink for phosphorus running off agricultural lands. Riparian buffer strips should still be an effective tool for combating phosphorus run-off into Lake Champlain, since they still maintain the ability to adsorb additional phosphorus.

Previous studies have found that epi-endogeic earthworm activity mobilizes unweathered soil particles from deeper horizons, increasing the amount of phosphorus in the surface soils, as compared to anecic earthworm species (Suárez, 2004). It is hypothesized that these differences are caused by the different depth of the soil that these types of earthworms inhabit. Interestingly, there was not a significant decrease in the amount of phosphorus adsorption for the A or B horizons of *Amyntas* infested sites; had phosphorus been translocated from the O to A horizon the increase in the levels of phosphorus may have been indicated by a decrease in the phosphorus adsorption rate of the soils. Furthermore, other studies have shown that earthworm-invaded plots dominated by epi-endogeic species, like *Amyntas* spp., had significantly increased amounts of readily exchangeable phosphorus in the surface soil, which may be more susceptible to leaching (Suárez, 2004). It was also shown, as previously stated, that earthworm castings release four times as much inorganic phosphorus into solution as compared to soil not processed by earthworms (Sharpley et al. 2002). In spite of the fact that both soils with and without *Amyntas* spp. have similar abilities to retain additional phosphorus, more of this phosphorus may be in readily exchangeable forms.

This in combination with the more significant levels of damage to the forest floor, may increase the levels of erosion and phosphorus run-off in areas with *Amyntas* spp. present. Previous studies have found that *Amyntas* spp. reduce the understory seed bank as the earthworms consume the Oi and Oe sub-horizons (Hale et al., 2006). Increased pressure on the remaining vegetation from browsing by deer, further exacerbates the problem (Holdsworth et al., 2007). Vegetation loss leads to a decrease in both sedimentation and nutrient uptake by plants (Dosskey et al., 2010). Due to the decrease in density of understory flora which acts as a physical

barrier to erosion and reduced levels of understory vegetation to uptake nutrients, there is an increased probability of run-off in areas with *Amyntas* spp. present.

*Amyntas* spp. are most commonly disseminated accidentally along with horticultural plantings and thus they are found near horticultural sites. Invasive European earthworms are more likely associated with agricultural sites. This was the case for this study. For this reason, less phosphorus may be received by buffers from horticultural run-off. Land use adjacent to the buffers may confound the effect of earthworms on a soils' phosphorus adsorption capacity in this study. However, regardless of earthworm species (and thus land use) there was not any significant difference in phosphorus adsorption of soils, suggesting that at least this component of the buffer strips is still functioning efficiently.

There was no significant difference in the soil organic matter content of sites that contained *Amyntas* spp. earthworms and those that were populated by European earthworm species. Previous studies had found that *Amyntas* spp. decreased the organic matter content of the O horizon by 36%. This decrease in surface organic matter is caused by earthworm's consumption of soil organic matter from the O-horizon which they mix with the underlying mineral A horizon (Dempsey et al., 2011, Burtelow et al., 1998). There was no difference in organic matter content between the A horizons of soils at sites with *Amyntas* spp. and with European species, suggesting that the European species may also decreased organic matter to the same extent.

Organic matter is an important factor in phosphorus adsorption. Other studies found a negative correlation between phosphorus adsorption and organic matter (OM) for all drainage classes (Lyons et al., 1998). However, here there was no relationship between phosphorus adsorption and soil organic matter. There are seasonal fluctuations in phosphorus adsorption and organic matter. Seasonal fluctuations in the levels of organic matter have been shown to impact the phosphorus retention capacity of soils; higher values were observed in the month of November for all drainage classes, as compared to samples from the month of May (Lyons et al., 1998). Other factors may have played a more significant role in the phosphorus adsorption of the sites; specifically, iron and aluminum were shown to increase the phosphorus retention capacity of soils, in contrast to the reduced retention capacity that is associated with organic matter

content (Lyons et al., 1998). This study shows how one component of riparian buffers efficiency is impacted by invasive earthworms. A more dynamic study into how the other functions of riparian buffers are impacted by invasive earthworms would be useful for developing a more thorough understanding of this system.

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## Appendix

**Figure 4. Invasive Earthworm Rapid Assessment Tool (IERAT)**

Site Name: _____		
Method used to determine location; <input type="checkbox"/> GPS    Other: _____		
Latitude: N ____ . _____ °    Longitude: W - ____ . _____ °		
FF category: (1-5)	<input type="checkbox"/> previous year's litter only;	<u>middens</u> (circle one)
	<input type="checkbox"/> fragmented leaves present, >1yr;	abundant - present - absent
	<input type="checkbox"/> intact, layered ff present;	<u>casts</u> (circle one)
		abundant - present - absent
comments:		

1. Leaf litter greater than one year is present ( $O_i$  and  $O_e$  present).
  - 1a. Yes (go to 2)
  - 1b. No, Leaf litter ( $O_i$ ) is from last fall only (go to 6)
2. Small fragmented relatively undecomposed leaves greater than one year present.
  - 2a. Yes  $O_e$  present (go to 3)
  - 2b. No, Leaf litter ( $O_i$ ) is from last fall only (go to 6)
3. Intact layered forest floor having  $O_i$ ,  $O_e$ ,  $O_a$  layers present, fine roots present in humus ( $O_a$ ) and leaf fragments ( $O_e$ ), no earthworms or earthworm signs present (burrows, castings).
  - 3a. Yes (Classification would be 1)
  - 3b. No (go to 4)
4. Forest floor consists of  $O_i$ ,  $O_e$  with patches of  $O_a$ . Some small earthworms and earthworm signs are present such as small casting in humus ( $O_a$ ) layer, some fine roots but not thick in forest floor.
  - 4a. Yes (Classification would be 2)
  - 4b. No (go to 5)
5. Leaf litter ( $O_i$ ) from previous fall and small fragmented leaves ( $O_e$ ) under intact leaves, no humus, mineral soil and earthworm casting **present** (<50% of forest floor/mineral soil interface upon visual inspection), plant roots absent or rare
  - 5a. Yes (Classification would be 3)
  - 5b. No (go to 6)
6. Mostly intact leaf litter ( $O_i$ ) from the previous fall, mineral soil and earthworm casting **abundant** (>50% of forest floor/mineral soil interface upon visual inspection), plant roots absent, middens absent or present (< 9 middens in a 5 meter radius).
  - 6a. Yes (Classification would be 4)
  - 6b. No (go to 7)
7. No forest floor, no humus or fragmented leaves present, mineral soil and earthworm casting **abundant** (>50% of forest floor/mineral soil interface upon visual inspection), middens **abundant** (> 10 middens in a 5 meter radius).
  - 7a. Yes (Classification would be 5)

**Figure 5. Site Information and Data**

<b><u>Amyntas Free Sample</u></b>	<b><u>Damage Class</u></b>	<b><u>Depth to Redoximorphic Features</u></b>	<b><u>Earthworm Species</u></b>	<b><u>Adjacent Area Use</u></b>
Bio Control Center (SPD)	4	48	<i>Dendrobaena octaedr,</i> <i>Octolaseon cyaneum</i>	Corn Production
Bio Control Center (VPD)	4	14	<i>Lumbricus rubellus,</i> <i>Octolaseon cyaneum</i>	Corn Production
I-89 Field (SPD)	5	46	<i>Apporectodea rosea,</i> <i>Dendrobaena octaedra,</i> <i>Octolaseon cyaneum*</i>	Corn Production
I-89 Field (VPD)	5	25	<i>Apporectodea rosea *</i>	Corn Production
Alfalfa Field (SPD)	4	45	<i>Octolaseon cyaneum,</i> <i>Apportectodea tuberculata</i>	Alfalfa Production
Alfalfa Field (VPD)	4	34	<i>Apporectodea rosea</i>	Alfalfa Production
<b><u>Amyntas</u></b>				
<b><u>Sample</u></b>	<b><u>Damage Class</u></b>	<b><u>Depth to Redoximorphic Features</u></b>	<b><u>Earthworm Species</u></b>	<b><u>Adjacent Area Use</u></b>
Centennial Woods (SPD)	5	45	<i>Amyntas spp.</i>	Gardens
Centennial Woods (VPD)	5	11	<i>Amyntas spp.</i>	Gardens
Hort Farm (SPD)	5	42	<i>Amyntas spp.</i>	Gardens
Hort Farm (VPD)	5	12	<i>Amyntas spp.</i>	Gardens
Audubon Center (SPD)	5	45+	<i>Amyntas spp.</i>	Recreation
Audubon Center (VPD)	5	8	<i>Amyntas spp.</i>	Recreation

**Figure 6. Sample Slopes (Marginal Rate of Phosphorus Adsorption)**

<b><u>Sample</u></b>	<b><u>Slope PO<sub>4</sub>-P, mg/L</u></b>
<i>Amynthas</i> Free #1 (SPD) A	0.963
<i>Amynthas</i> Free #1 (SPD) B	0.9767
<i>Amynthas</i> Free #1 (VPD) A	0.9017
<i>Amynthas</i> Free #1 (VPD) B	0.6739
<i>Amynthas</i> Free #2 (SPD) A	0.7623
<i>Amynthas</i> Free #2 (SPD) B	0.6915
<i>Amynthas</i> Free #2 (VPD) A	0.8707
<i>Amynthas</i> Free #2 (VPD) B	0.6644
<i>Amynthas</i> Free #3 (SPD) A	0.9573
<i>Amynthas</i> Free #3 (SPD) B	0.9952
<i>Amynthas</i> Free #3 (VPD) A	0.6808
<i>Amynthas</i> Free #3 (VPD) B	0.7824
<i>Amynthas</i> #1 (SPD) A	0.7714
<i>Amynthas</i> #1 (SPD) B	0.9332
<i>Amynthas</i> #1 (VPD) A	0.9034
<i>Amynthas</i> #1 (VPD) B	0.9162
<i>Amynthas</i> #2 (SPD) A	0.8043
<i>Amynthas</i> #2 (SPD) B	0.956
<i>Amynthas</i> #2 (VPD) A	0.8352
<i>Amynthas</i> #2 (VPD) B	0.671
<i>Amynthas</i> #3 (SPD) A	0.9944
<i>Amynthas</i> #3 (SPD) B	0.9993
<i>Amynthas</i> #3 (VPD) A	0.9854
<i>Amynthas</i> #3 (VPD) B	0.9992
<b>Range</b>	<b>0.6644- 0.9993</b>

**Figures (7-12) Raw Adsorption Data***Amyntas* Free: Site 1

<b>Sample Name</b>	<b>Standard PO4-P, mg/L</b>	<b>Solution PO4-P, mg/L</b>	<b>Adsorbed PO4-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.083	0.646
	2.34	0.134	2.206
	3.49	0.416	3.074
	5.4	0.933	4.467
	7.39	1.600	5.790
<b><u>B Horizon SPD</u></b>	0.729	0.042	0.687
	2.34	0.133	2.207
	3.49	0.416	3.074
	5.4	1.090	4.310
	7.39	2.040	5.350
<b><u>A Horizon VPD</u></b>	0.729	0.043	0.686
	2.34	0.089	2.251
	3.49	0.213	3.277
	5.4	0.496	4.904
	7.39	0.879	6.511
<b><u>B Horizon VPD</u></b>	0.729	0.107	0.622
	2.34	0.205	2.135
	3.49	0.726	2.764
	5.4	1.460	3.940
	7.39	2.210	5.180

*Amynthas* Free: Site 2

<b>Sample Name</b>	<b>Standard PO4-P, mg/L</b>	<b>Solution PO4-P, mg/L</b>	<b>Adsorbed PO4-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.033	0.696
	2.34	0.041	2.299
	3.49	0.0582	3.432
	5.4	0.095	5.305
	7.39	0.124	7.266
<b><u>B Horizon SPD</u></b>	0.729	0.027	0.702
	2.34	0.030	2.311
	3.49	0.036	3.454
	5.4	0.052	5.348
	7.39	0.055	7.335
<b><u>A Horizon VPD</u></b>	0.729	0.383	0.346
	2.34	0.516	1.824
	3.49	0.963	2.527
	5.4	1.67	3.730
	7.39	2.4	4.990
<b><u>B Horizon VPD</u></b>	0.729	0.0943	0.635
	2.34	0.134	2.206
	3.49	0.386	3.104
	5.4	0.754	4.646
	7.39	1.53	5.860

*Amyntas* Free: Site 3

<b>Sample Name</b>	<b>Standard PO4-P, mg/L</b>	<b>Solution PO4-P, mg/L</b>	<b>Adsorbed PO4-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.062	0.667
	2.34	0.068	2.272
	3.49	0.101	3.389
	5.4	0.153	5.247
	7.39	0.314	7.076
<b><u>B Horizon SPD</u></b>	0.729	0.040	0.689
	2.34	0.046	2.294
	3.49	0.058	3.432
	5.4	0.113	5.287
	7.39	0.192	7.198
<b><u>A Horizon VPD</u></b>	0.729	0.042	0.687
	2.34	0.050	2.290
	3.49	0.102	3.388
	5.4	1.430	3.970
	7.39	0.205	7.185
<b><u>B Horizon VPD</u></b>	0.729	0.0454	0.684
	2.34	0.167	2.173
	3.49	0.54	2.950
	5.4	1.38	4.020
	7.39	2.08	5.310

*Amynthas*: Site 1

<b>Sample Number</b>	<b>Standard PO<sub>4</sub>-P, mg/L</b>	<b>Solution PO<sub>4</sub>-P, mg/L</b>	<b>Adsorbed PO<sub>4</sub>-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.275	0.454
	2.34	0.313	2.027
	3.49	0.602	2.888
	5.4	0.993	4.407
	7.39	1.520	5.870
<b><u>B Horizon SPD</u></b>	0.729	0.0417	0.687
	2.34	0.0879	2.252
	3.49	0.11	3.380
	5.4	0.192	5.208
	7.39	0.342	7.048
<b><u>A Horizon VPD</u></b>	0.729	0.124	0.605
	2.34	0.165	2.175
	3.49	0.322	3.168
	5.4	0.633	4.767
	7.39	1.21	6.180
<b><u>B Horizon VPD</u></b>	0.729	0.0652	0.664
	2.34	0.123	2.217
	3.49	0.53	2.960
	5.4	1.3	4.100
	7.39	2.135	5.255

*Amyntas: Site 2*

<b>Sample Number</b>	<b>Standard PO<sub>4</sub>-P, mg/L</b>	<b>Solution PO<sub>4</sub>-P, mg/L</b>	<b>Adsorbed PO<sub>4</sub>-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.24	0.489
	2.34	0.29	2.050
	3.49	0.698	2.792
	5.4	1.16	4.240
	7.39	1.67	5.720
<b><u>B Horizon SPD</u></b>	0.729	0.0525	0.677
	2.34	0.0649	2.275
	3.49	0.137	3.353
	5.4	0.255	5.145
	7.39	0.493	6.897
<b><u>A Horizon VPD</u></b>	0.729	0.117	0.612
	2.34	0.148	2.192
	3.49	0.252	3.238
	5.4	0.497	4.903
	7.39	0.723	6.667
<b><u>B Horizon VPD</u></b>	0.729	0.042	0.687
	2.34	0.062	2.278
	3.49	0.130	3.360
	5.4	0.283	5.117
	7.39	0.600	6.790

*Amyntas: Site 3*

<b>Sample Number</b>	<b>Standard PO<sub>4</sub>-P, mg/L</b>	<b>Solution PO<sub>4</sub>-P, mg/L</b>	<b>Adsorbed PO<sub>4</sub>-P, mg/L</b>
<b><u>A Horizon SPD</u></b>	0.729	0.0379	0.691
	2.34	0.040	2.300
	3.49	0.042	3.448
	5.4	0.0572	5.343
	7.39	0.0737	7.316
<b><u>B Horizon SPD</u></b>	0.729	0.040	0.690
	2.34	0.041	2.300
	3.49	0.043	3.447
	5.4	0.043	5.358
	7.39	0.045	7.345
<b><u>A Horizon VPD</u></b>	0.729	0.037	0.692
	2.34	0.084	2.256
	3.49	0.047	3.443
	5.4	0.100	5.300
	7.39	0.143	7.247
<b><u>B Horizon VPD</u></b>	0.729	0.0396	0.689
	2.34	0.041	2.299
	3.49	0.044	3.446
	5.4	0.044	5.356
	7.39	0.045	7.345