Influence of *Amynthas agrestis* and *Lumbricus rubellus* on Soil and Atmospheric C and N

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Abstract

Many studies of invasive species focus on the resultant change in ecological interactions due to addition/loss from the trophic structure and resulting change in energy flow. Environment-organism interactions are important to understand due to increase anthropogenic introductions and climate change. In many Northeastern states, exotic earthworms have invaded forest soils, thereby bringing changes to forest floor ecology and chemistry. A 90 day mesocosm study was undertaken to examine the effects of earthworm invasion on C and N dynamics. Two epi-endogeic invasive earthworm species Lumbricus rubellus and Amynthas agrestis were selected for study. Greenhouse gas production by total mesocosm and soil were monitored. Gas flux measurements on 11 dates indicate both worm species increase CO_2 and N_2O emitted from mesocosm system as well as soil. Mesocosm total C and N (mass balance) indicate significantly less N but no change in C between treatments and control. This indicates a disruption of denitrification by earthworm invasion that results in increased N_2O emissions, a potent greenhouse gas. Also, gross soil C measurement on a short time scale may be insufficient to estimate changes to a system in the long term. Conclusively, small organisms can have a substantial impact when habitual behavior is continued en masse.

Introduction

For the past 13 millennia, the humid temperate forests in the Northeastern US developed in the absence of earthworms (reference). The result of this absence has seen the development of forest floors with thick O and well distinguished mineral horizons. With the arrival of European settlers, so began the introduction of earthworms (a new trophic level) into this region. In the last 5 centuries, many more species have gained a foothold and modified ecosystems in very pronounced ways (reference).

Earthworm ontogeny is varied; however there are common functions and invasion outcomes. Generally, worms can be classified into three ecological groups: anecic, epigeic, and endogeic. Epigeic worms live within the surface litter, endogeic worms construct horizontal burrows in the top mineral horizon of the soil and anecic worms construct deep, vertical burrows. Though life histories may vary, earthworms consume or/and mix organic material which is then mineralized and distributed through the soil profile (Darwin, 1882). This action serves to modify the soil properties (CEC, pH, nutrient distribution, soil structure) that can have a major impact on microbes and vegetation. Notably, there is a loss or major decrease in surface organic horizons which serves as both a seed bank and habitat for micro and meso fauna.

Many Northeastern Forests have reached a saturation (or equilibrium) point; destabilizing these systems can have profound effects (EPA). Carbon storage of forest soils can conservatively be in the area of 100 metric tons per hectare. With the invasion of earthworms, forest floor dynamics can be modified in a very profound manner.

The objective of this work is to quantify and compare epigeic invaders *A. agrestis* (Görres et al., 2012) and *L. rubellus* (reference) on greenhouse gas production and resultant total and soil C and N. Though these species have similar life histories, they originate on opposite sides of the globe and exhibit characteristically different behaviors (estivation, autotomy), so investigating ecosystem effects may show variable effects.

Material and Methods

Materials

Soil was collected from Camel's Hump State Park (Duxbury, VT). The area was selected for absence of invasive earthworms whilst vegetation and over story were consistent with Northern Hardwood Forest, a commonly invaded biotype (Thompson et al.). Leaf litter, A and B horizon was collected and transported to UVM soils lab. Leaf litter was macerated and sieved to 7.5 mm while soil was sieved at 2 mm to remove non-soil particles.

Naturalized *Amynthas agrestis* and *Lumbricus rubellus* earthworms were collected from Jericho Research Forest (Jericho, VT-University of Vermont). Earthworms were acclimated to laboratory temperature and humidity for one week while being fed ad libitum.

Mesocosms consisted of glass housing with impermeable base and restricted egress sealable lid (to prevent earthworm escape).

Method

A two way factorial design was conceived with A horizon as one factor and worm treatment as second factor. To each mesocosm homogenized B horizon soil was added (1100 +/- 5 g). To half of the mesocosms, homogenized A horizon soil was added (50.00 +/-0.05g). To all mesocosms, homogenized leaf litter was added (10.00 +/-0.05g). Mesocosms were randomized and then assigned to worm treatment. Earthworm treatments consisted of none (control) or two individuals of *A. agrestis* or *L. rubellus*. Each treatment was replicated 5 times for a total of 30 mesocosms.

Mesocosms were held at standard laboratory temperature (19-21 °C) under ambient daylight for 90 days. Soil moisture was monitored by total mass throughout the study and average loss from control treatments was added back by simulated dew deposition.

Analysis Methods

Data collection included water loss, interval and evolution of CO_2, N_2O and CH_4 and quantification of total C and N. Mesocosms were weighed (Metler-Toledo) every 3 days and water replaced at loss rate of controls to simulate stable dew/precipitation. At establish dates, mesocosms were sealed for 30 minutes and gas sampled at three intervals to determine system flux rate. On four dates, equivalent soil samples were collected from surface of mesocosm and sealed in glass vials for 12 hours with subsequent quantification of gases. All gas measurements were performed using a Shimadzu GC-17 Gas Chromatograph equipped with FID and ECD. Total C and N were quantified in duplicate for all soil layers, leaf litter, worms and gas sampled soils (Thermo C N analyzer). Mesocosm totals were determined by mass balance with subtraction of earthworm contribution.

Statistical Analysis

All analysis was performed using SAS 9.3 (SAS Institute, Cary, NC). For mesocosm gas evolution, PROC MIXED analysis was performed where fixed effects are horizon, earthworm and time while mesocosm number is random. SLICE function was performed where interaction term was significant to determine significance of simple effects. DDF estimate was set to Kenward-Roger as dates were not evenly spaced. A simple two way crossed factorial ANOVA was performed for mesocosm total C and N, cumulative water loss and vial gas evolution. All data sets were examined for normality and equal variance by univariate procedures.

Results

Gas

Mesocosm gas flux was significantly influenced by earthworms. CO_2 flux was greater for earthworm additions but not A-horizon addition (Figure 1, Table 1). Earthworm*Time interaction was split by SLICE function, which showed all dates were significant except for day 33 and 60. N_2O flux was also significantly increased by earthworm addition but not A- horizon presence/absence (Figure 2, Table 2).

Soil C and N

Total mesocosm N but not C was influenced by earthworm presence. Total mesocosm C did not vary significantly amongst worm treatment Figure 3, Table 3). Total mesocosm N was significantly higher in earthworm addition mesocosm, despite correction for addition by worm (Figure 4, Table 4). In both cases, A-horizon treatments showed higher C and N as A-horizon soil addition would have included significant additions of both elements.

To also be included in near future, water loss, vial gas evolution and rates per C and N and remaining mass balance fractions

Discussion

These data support the hypothesis that earthworms are ecosystem engineers that have influence on not only trophic interactions but also environmental abiotic properties.

Most commonly, forest floor structure changes have been observed in both field and laboratory studies. A microcosm study with simulated *Acer/Fagus* soil indicated an increased loss of leaf litter to mineral horizons with addition of earthworms (Hale 2008). Ecosystems studies in temperate hardwood forests have consistently indicated decreased O horizon thickness and modified forest floor structure (Bohlen 2004, Groffman 2004). Notably at invasion fronts of northern hardwood forests, disappearance of the O horizon, increased depth of the A horizon, increase in bulk density and decrease in fine root mass have been observed (Hale et al, 2005, Lawrence et al, 2003). As indicated above (too follow), surface leaf litter remaining at the end of the study period was significantly less in the presence of earthworms than control. Implications of decreased litter mass and cover increases the susceptibility to invasion by exotic plant species (Belote, 2009). This, tied to chemical soil changes may also contribute to changes in the plant community with particularly great shifts in observed understory community (Eisenhauer et al, 2009 and Addison, 2008). Conclusively, invasive earthworms make way for invasive plants.

A number of studies have examined the effect of earthworms on C and N respiration from soils. Examination of anecic earthworm *L. terrestris* found increased C mineralization in casts and burrow soil compared to bulk soils (Görres et. al, 1997). This effect was also seen in conjunction with endogeic earthworms where increased C respiration was maintained (Whalen, 2008). Changes in soil structure (i.e. porosity) can have an effect on aeration and microenvironments for other organisms (Gorres and Amador, 2010). Consistent with results seen here, increased soil respiration may be attributed to enhanced activity of soil community due to earthworm effects on increasing available nutrients through organic matter integration to mineral soil. Increased aeration of soil may lead to aerobic respiration as it proceeds more efficiently, shifting resource equilibrium point.

System total C results hint at consistency with other studies. An ecosystem study by Bohlen et al., found that total carbon pools were reduced by earthworm invasion. Though not seen here, the increased carbon loss from the system may have been significant if the study was carried out for a longer period of time. As well, C:N ratio decreased , which can have a significant effect on biota (Bohlen 2004). Though not shown above, C:N ratio was lower in the earthworm treatments, supporting the current observation that earthworm engineered systems vary in this regard.

Inconsistent results revolve around N fractions. Though total system N was found to be less here, an ecosystem study found increased biomass C and N, suggesting an ecosystem may find a new equilibrium point with earthworm invasion (Groffman 2004). Alternatively, active biota could be immobilizing N as biomass, due to the increased microbial activity. This would fit with the observation that total system N may be decreased at a lesser rate and support the conclusion that C:N ratios are decreased in earthworm invaded systems.

Conclusion

Earthworm invasion into forest ecosystems can lend to destabilization of the system and may have profound effects on soils and forests in general to buffer/sequester C and N. Increases in potent greenhouse gas emission from invaded soils raise concern for current climatic models. But while mesocosm studies are good at laying groundwork for further studies, up scaling will be necessary to determine in these results are applicable to full scale systems.

Figure 1: Average CO_2 flux rate from mesocosm. Results shown are for earthworm effect, where L. rubellus (1), Control (0) and A. agrestis (-1). Bars indicates quartile, n=10.

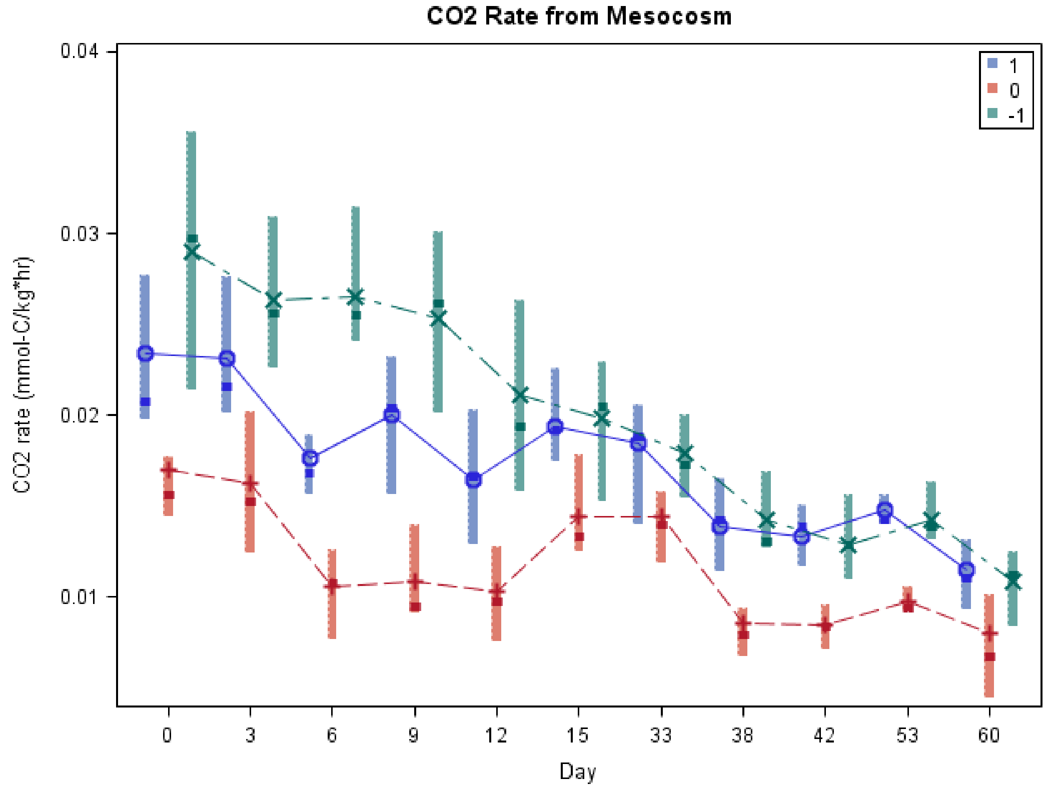

Table 1: Mesocosm CO_2 ANOVA table

Effect	Num DF	Den DF	F Value	Pr > F
A-horizon	1	24.1	2.86	0.1037
Earthworm	2	24.1	21.74	<.0001
A-hor*Earthworm	2	24.1	0.46	0.6365
Time	10	233	49.89	<.0001
Time*A-hor	10	233	2.60	0.0052
Time*Earthworm	20	233	5.45	<.0001
Time*A-hor*Worm	20	233	0.66	0.8643

Figure 2: Average N_2O flux rate from mesocosm. Results shown are for earthworm effect, where L. rubellus (1), Control (0) and A. agrestis (-1). Bars indicates quartile, n=10.

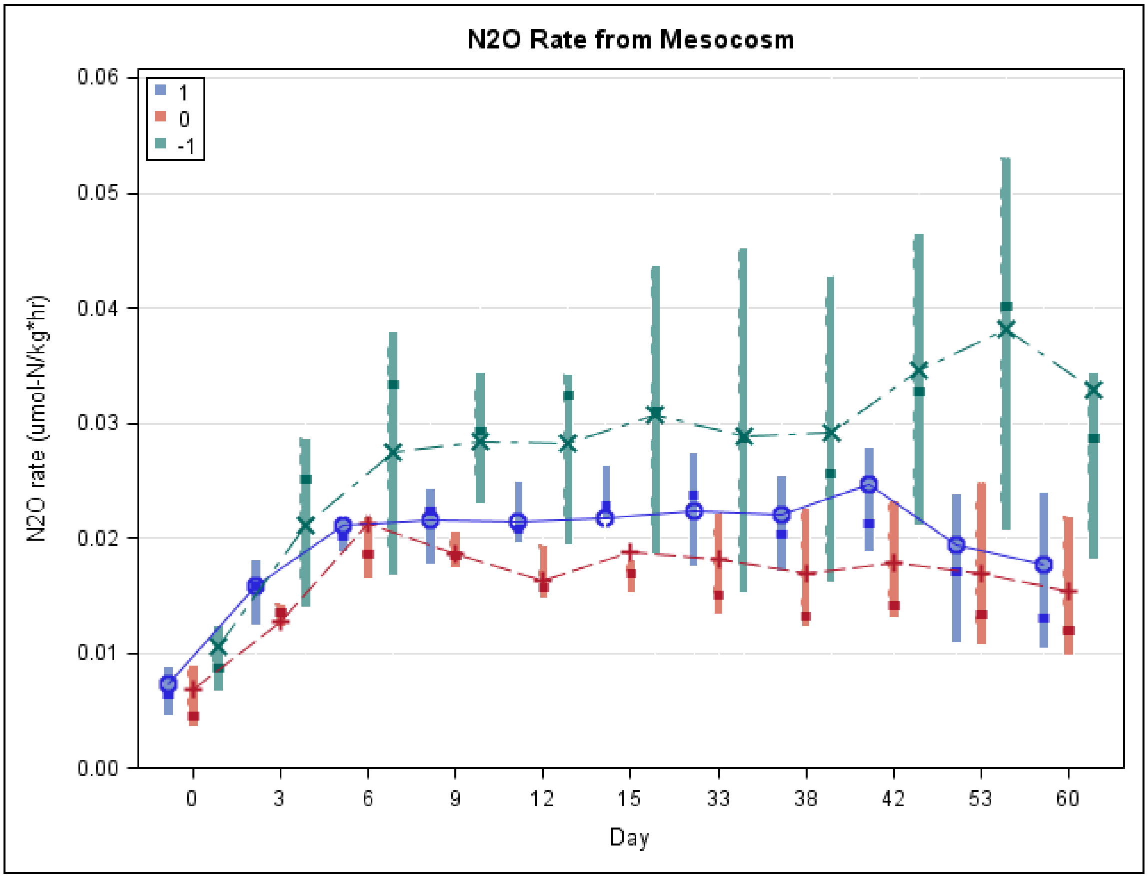

Table 2: Mesocosm N_2O ANOVA table

Effect	Num DF	Den DF	F Value	Pr > F
A-horizon	1	24.1	0.14	0.7120
Earthworm	2	24.1	5.20	0.0132
A-hor*Earthworm	2	24.1	0.27	0.7688
Time	10	228	10.70	<.0001
Time*A-hor	10	228	1.79	0.0643
Time*Earthworm	20	228	1.29	0.1852
Time*A-hor*Worm	20	228	1.39	0.1273

Figure 3: Total mesocosm C. Treatments are L. rubellus (1), Control (0) and A. agrestis (-1). Bars indicates quartile, n=5.

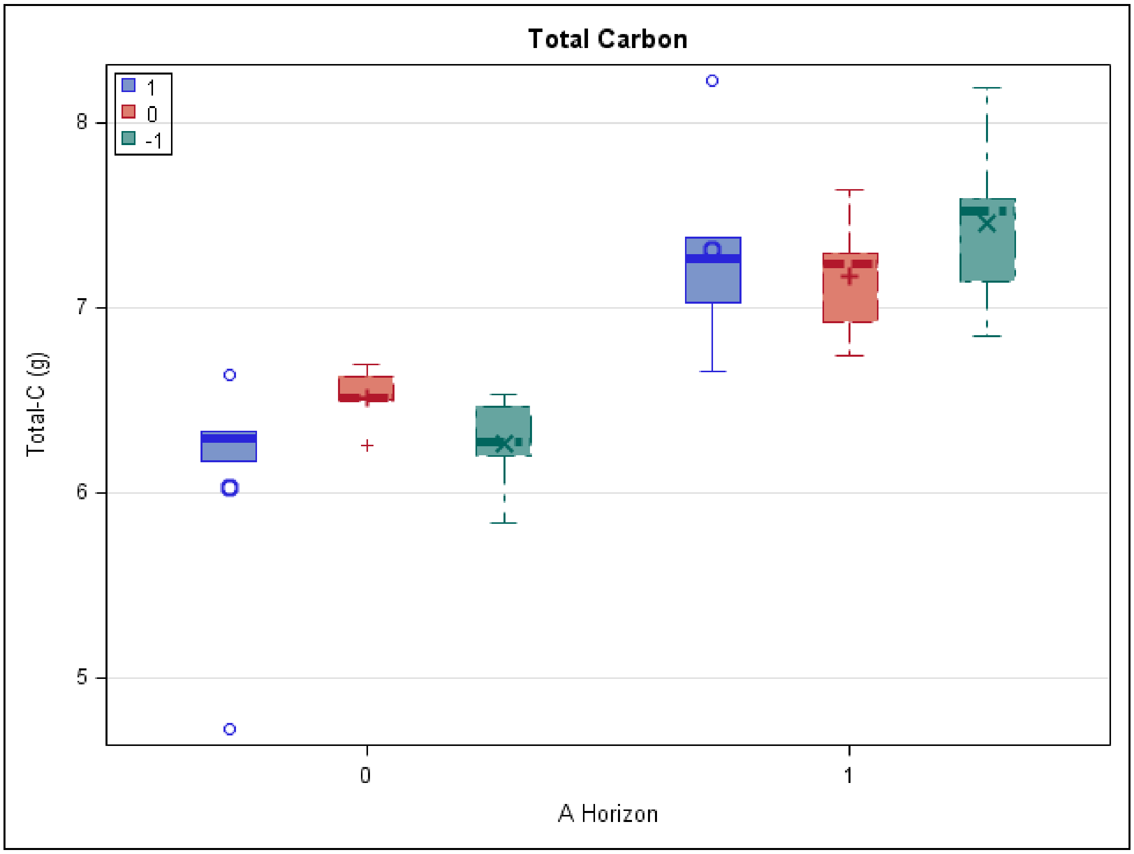

Table 3: ANOVA total C

Effect	NumDF	DenDF	F Value	Pr> F
A-horizon	1	24.1	35.1423	<.0001
Earthworm	2	24.1	0.4747	0.6278
A-hor*Worm	2	24.1	1.2665	0.3000

Figure 4: Total Mesocosm N. Treatments are L. rubellus (1), Control (0) and A. agrestis (-1). Bars indicates quartile, n=5.

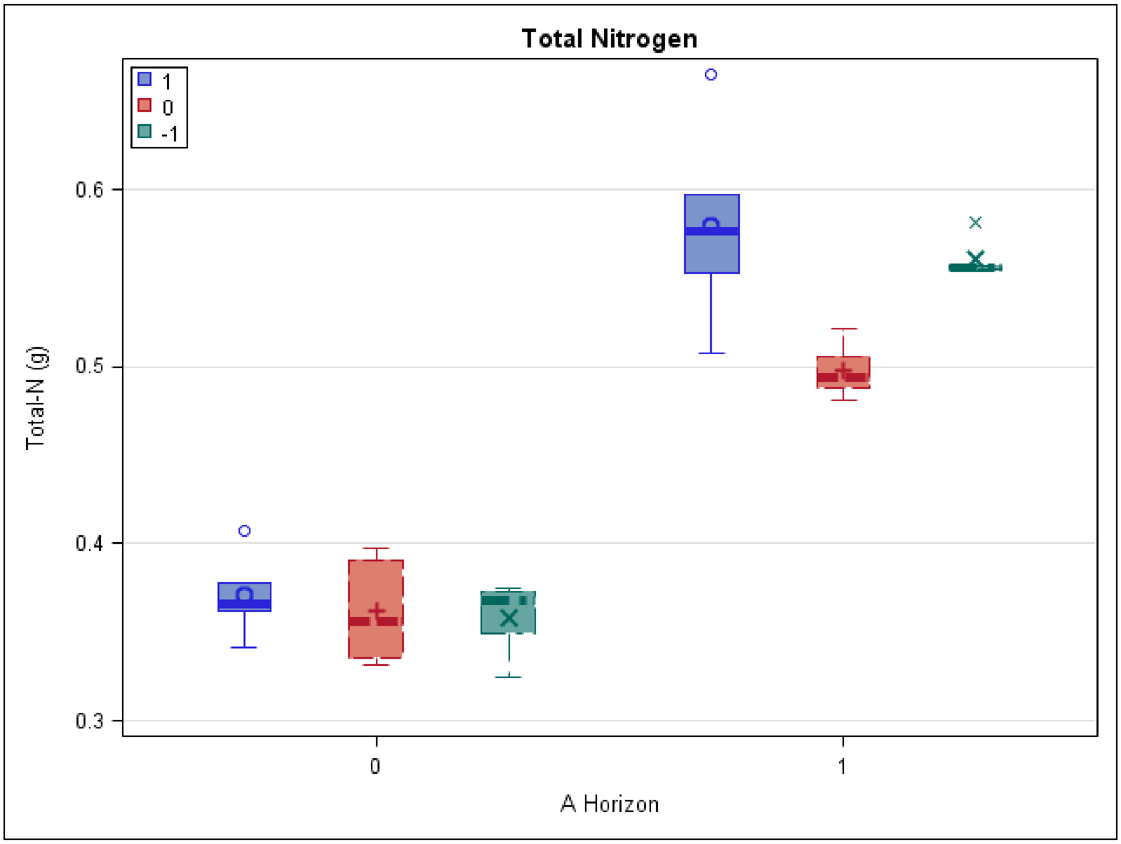

Table 4: ANOVA total N

Effect	Num DF	DenDF	F Ratio	Pr > F
A-horizon	1	24.1	283.5438	<.0001
Earthworm	2	24.1	4.3792	0.0239
A-hor*Worm	2	24.1	3.1036	0.0633

References

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