Greenhouse Gas Emissions From Aggregates of a Mesocosm Soil Worked By *Lumbricus rubellus* and *Amynthas agrestis*

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INTRODUCTION

Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide concentrations in the atmosphere all have increased by 42, 140 and 12%, respectively since the 19th century (IPCC, 1995). Increased greenhouse gas concentrations are in part due to human activities. However, some mitigation may occur by sequestration into less available compounds in the environment (Denman et al. 2007). Earthworms have recently been implicated in increased greenhouse gas emissions from soils (Lubbers et al. 2013). Earthworms change the microbial community of the soil at least in the drilosphere (Savin et al. 2004). Concomitant with these changes are aggregate-scale changes in pore structure that increase CO_2 emissions (Gorres et al., 2001). In this study, we were interested in how aggregate scale greenhouse gas emission, nitrate and calcium concentrations, pH were affected by earthworm species and ammonium addition. We hypothesized that additional ammonium may be quickly nitrified by the community left by the earthworms and thus affect nitrate and calcium concentrations.

Figure 1. Pot experiment with soils excavated from spodosol soils under a mixed hardwood/softwood forest .

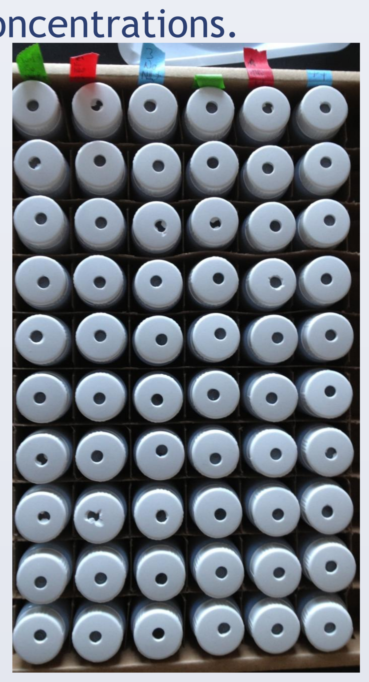


Figure 2. Mesocosm sampling and sealed vials.

All earthworms are exotic to the Northeastern USA and are assumed to change soil structure so that the biodiversity of native understory vegetation is diminished (Hale et al. 2008). There is good evidence that N and C cycles are affected by these invasions. Little is known about base cations and we chose to measure calcium because we found it to be more available at invaded forest sites. We thought that ammonium additions would stimulate nitrification and thus potentially N_2O emissions and potentially increase available Ca concentrations to buffer the soil. We measured soil Ca, pH, NO_3^--N, and NH_4^+-N as well as greenhouse emissions from each replicate aggregate set.

MATERIAL AND METHODS

The A, E and B horizons of a spodosol soil under a mixed hardwood/softwood forest were used to construct mesocosm to observe the calcium-earthworm-plant interaction for *L.rubellus* (L), *A. agrestis* (A) and a control treatment with no earthworms (N) (Fig. 1). 2 g of soil aggregates (earthworm casting) were taken from these mesocosms of the plant-soil experiment and transferred to haeadspace vials (Fig. 2). We had two factors: earthworm species and addition/no addition of ammonium, signified as (+) and (-). Treatments are listed in Table 1. The samples were divided into 3 sets. One set for the initial analysis, the second set after 7 days and the third set after 14 days. Ammonium-N was added to half the soils in sets 2 and 3 to achieve 200 µg NH_4^+-N l^{-1} content. For greenhouse gas analyses, the vials were sealed tightly with a rubber septum and incubated for 16 hours. After measurement of CO_2, N_2O and CH_4 with Shimadzu GC, soils were subjected to other measurements. For pH and Ca measurements 7.5 ml distilled water were added and left for 2 hours and then measured with micro-electrode connected pH meter instrument. To measure extractable inorganic N (NO_3^- + NH_4^+) after extraction with KCl 2.5 ml 2 M KCl was added to soils and measured colorimetrically with Lachate instrument.

OBJECTIVES

Objectives of this study was to see;

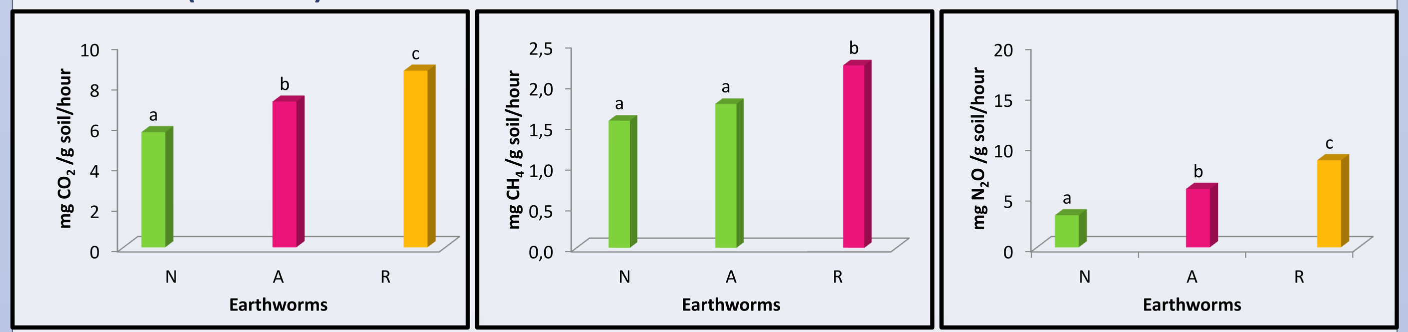
- whether nitrification affects pH,
- whether Ca is involved in buffering acidity,
- whether addition of NH_4^+-N accelerates acidification and at which rate,
- how will the greenhouse gas emission be,
- to reach to a consideration of microbial activity from release of CO_2 and
- to see the effect of NH_4^+-N addition on N_2O release

In relation with the earthworm species.

RESULTS

At the beginning of the experiment *Amynthas* castings showed significant difference from others with higher NH_4 content and lower Ca and pH levels ($p<0.05$ ANOVA). Initially, NO_3 contents were similar among treatments ($p<0.05$ ANOVA). But at the two later dates, NO_3 concentrations were greater for earthworm treatments for (-) ammonium treatments. When NH_4 was added, *L. rubellus* treatments had greater NO_3 concentrations. CO_2 and N_2O results showed significant difference among each other with the sequence *Rubellis* (R) > *Amynthas* (A) > control (N) while only R had a significant higher methane release ($p<0.05$ ANOVA)(Figure 3).

Figure 3. CO_2, CH_4 and N_2O Emmission Prior to Incubation for N (Control), A (*Amynthas*) and R (*Rubellus*) Earthworm



At the end of first week of incubation earthworms had significantly greater CO_2 and methane release at (-) sample set ($p<0.05$) (Figure 4). Ammonium addition was significantly different on methane release (Figure 4). *L. rubellus* with ammonium addition caused significantly greater N_2O emissions. At the end of second week of incubation, earthworms castings had significantly greater CO_2 release ($p<0.05$) (Fig. 5) for (+) and (-) applications.

Figure 4. CO_2, CH_4 and N_2O Emmission After 7 d Incubation for N (Control), A (*Amynthas*) and R (*Rubellus*) Earthworm and Ammonium Addition (+) (-) Applications

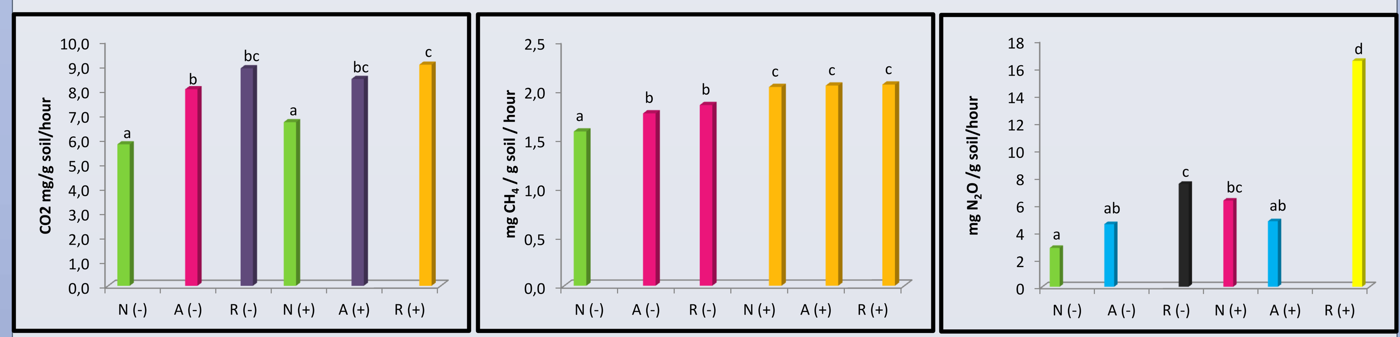
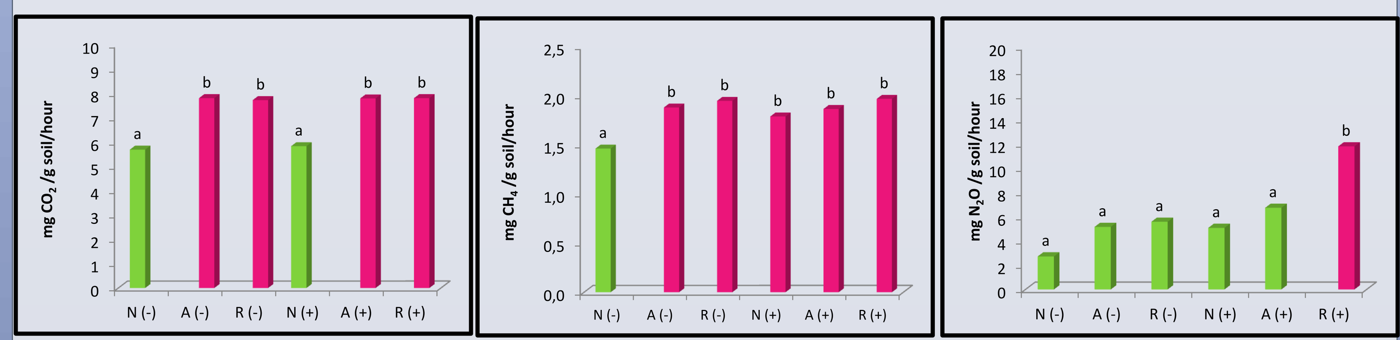


Figure 5. CO_2, CH_4 and N_2O Emmission After 14 d Incubation for N (Control), A (*Amynthas*) and R (*Rubellus*) Earthworm and Ammonium Addition (+) (-) Applications



Lower pH values in aggregates in earthworm soils than in the N(+) treatment (p(F) < 0.001) may have been caused by nitrification as NO_3^--N concentrations in the earthworm treatments were greater than in the N(-) treatment (p(F) < 0.032). Greater water soluble Ca concentrations in the earthworm treatments could not buffer the pH differences. N_2O and CO_2 emissions were consistently greater for the earthworm treatments in both NH_4^+-N treatments with the exception of A(+) which emitted significantly less N_2O than either the L(+) or N(+). For CO_2 in soils without NH_4^+-N additions, worm type and pH contributed significantly to CO_2 predictions ($p<0.0001$). For treatments with NH_4^+-N additions, model predictions ($p<0.0096$) were mainly influenced NH_4^+-N concentrations. For N_2O without NH_4^+-N additions ($p< 0.0001$), earthworm type was the only significant contributor to predictions. For N_2O emissions with NH_4^+-N additions ($p<0.0001$), NH_4^+-N concentrations, NO_3^--N concentrations and earthworm type were significant contributors to the predictions.

Table 1 : Summary of treatments

Earthworm	Ammonium addition incubation	Measurement Period	Replication (n)	Total
Control (no earthworm)	No addition	Initial	10	30
Amynthas agrestis	No addition		10	
Lumbricus rubellis	No addition		10	
Control (no earthworm)	No addition	After 7 days	10	60
	Added		10	
Amynthas agrestis	No addition		10	
	Added		10	
Lumbricus rubellis	No addition		10	
	Added		10	
Control (no earthworm)	No addition	After two weeks	10	60
	Added		10	
Amynthas agrestis	No addition		10	
	Added		10	
Lumbricus rubellis	No addition		10	
	Added		10	

We reached proportionally higher levels of relations between NH_4 cc and each greenhouse gas contents especially at control and *A. agrestis* treatments but almost no correlation could be detected for *L. rubellus* after NH_4 addition (Figure 6). There also was a significant correlation between Ca and NO_3 concentration (r^2 =0.528, Figure 7). The relations between Ca and greenhouse gas contents were not high (r^2 values between 0.100 and 0.150).

Figure 6: NH_4-CO_2 relation along incubation period at N, A and R worm data set.

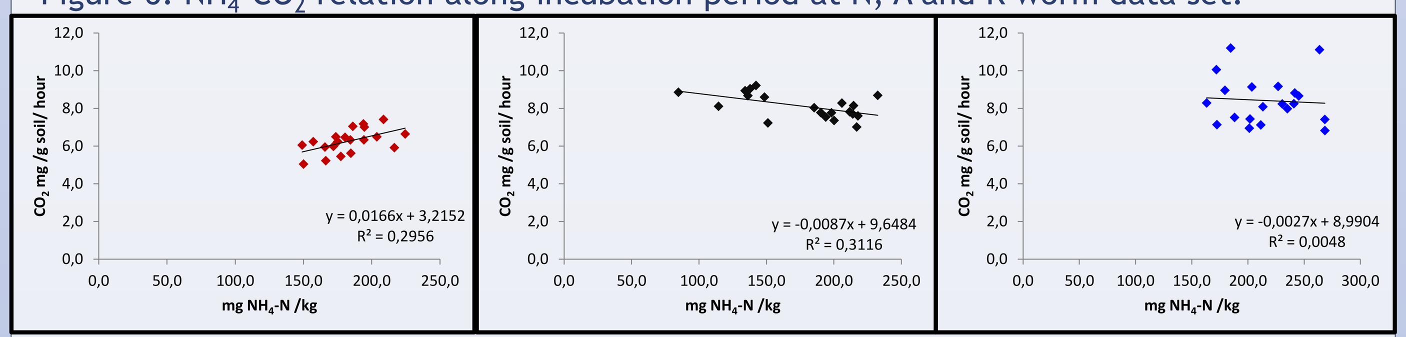
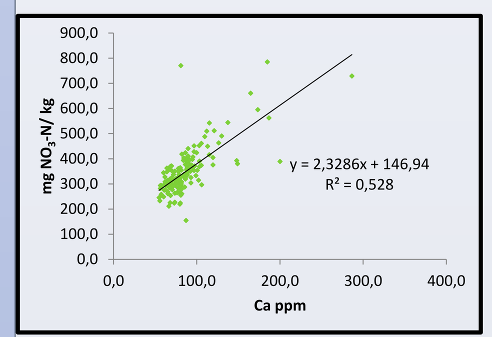


Figure 7: Relation between Ca to NO_3 concentrations.



Pooling all three time points, our results suggest that NH_4 concentration had a significant effect on CO_2, CH_4 and N_2O release especially for the no earthworm control revealing r^2 values 0.297, 0.146 and 0.675 respectively while NO_3 cc did not show good correlation with any greenhouse gas at all worm treatments.

DISCUSSION & CONCLUSION

Difference of ammonium concentrations among initial values were attributed to the applications along pot experiment. We had expected to see increased pH with ammonium addition as the effect of nitrification was buffered by Ca release (Figure 7). The net effect was that there was lower pH in earthworms soils despite of the increase Ca concentrations. For pH our results were different from some of our field studies and those of others that report an increase in pH when earthworms are present. Our lab study differed in that we looked at aggregate scale effects where microsite effects may override the effect of inter-aggregate soil solution. Our lab study also differed in that the microbial community was separated from the earthworms potentially suggesting that the earthworm needs to "renew" its effect on the community.

Our findings on greenhouse gas emissions resemble those of others (Lubbers et al., 2013). Aggregates taken from earthworm treatments have greater emissions than aggregates from the control soil. Methane is a notable exception after NH_4 was added. Also N_2O emissions from *L. rubellus* aggregates responded more to NH_4 additions than the two other treatments.

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ACKNOWLEDGEMENTS

We would like to thank to Joel Tilley for his contribution at the analysis at Lachate instrument in the laboratories of Plant and Soil Science department in University of Vermont. We are thankful to Vermont Agriculture Experiment Station (VT AES) for their contributions to our study.Thankful to Istanbul University Scientific Research Projects office by supporting H. Barış Tecimen visit to US with the project number 18262 and 35545.

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