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Research Article

The Effect of Earthworm (*Lumbricus terrestris* L.) Population Density and Soil Water Content Interactions on Nitrous Oxide Emissions from Agricultural Soils

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Earthworms may have an influence on the production of N_2O, a greenhouse gas, as a result of the ideal environment contained in their gut and casts for denitrifier bacteria. The objective of this study was to determine the relationship between earthworm (*Lumbricus terrestris* L.) population density, soil water content and N_2O emissions in a controlled greenhouse experiment based on population densities (90 to 270 individuals m^{-2}) found at the Guelph Agroforestry Research Station (GARS) from 1997 to 1998. An experiment conducted at considerably higher than normal densities of earthworms revealed a significant relationship between earthworm density, soil water content and N_2O emissions, with mean emissions increasing to 43.5 g $ha^{-1}day^{-1}$ at 30 earthworms 0.0333 m^{-2} at 35% soil water content. However, a second experiment, based on the density of earthworms at GARS, found no significant difference in N_2O emissions (5.49 to 6.99 g $ha^{-1}day^{-1}$) as a result of earthworm density and 31% soil water content.

1. Introduction

The presence of earthworms can be seen as an added benefit to many agricultural systems since earthworms contribute greatly to the overall physical properties of agricultural soils [1]. Previous studies in sole cropping systems have focused on the ability of earthworms to facilitate soil mixing and the decomposition of organic matter, which is especially important in agricultural systems [2–4]. Earthworms also affect soil properties, by increasing soil porosity and decreasing bulk density and through bioturbation and cast deposition on the soil surface [1]. Earthworm activity stimulates mineralization of N in residues, which promotes the availability for plants and microorganisms of inorganic forms of N from plant material [1, 5].

However, increased earthworm populations might increase the production of nitrous oxide (N_2O) emissions from agricultural soils. Over 50% of *in situ* N_2O emissions, in some soils, could be a result of earthworm activity [6]. Recent research suggests that, globally, earthworms could be producing up to 3×10^8 kg of N_2O annually [6]. Conventional agricultural practices, which aim to encourage earthworm populations due to their positive influence on soil properties are the highest anthropogenic sources of N_2O emissions. On a global scale, annual emissions of N_2O were 16.2 Tg in 2004 [7], and as a result, earthworms could be responsible for nearly 2% of global emissions.

One reason for this is that the earthworm gut is an ideal environment for denitrification [8–10]. Using microsensors, Horn et al. [9] determined that the earthworm gut is anoxic and contains copious carbon substrates for microorganisms and is therefore ideal for N_2O production. Denitrification is enhanced when the earthworm ingests denitrifier bacteria with organic matter [1, 8–10]. When gaseous N_2O is produced, it is able to escape the permeable epidermis of the earthworm and diffuses from the soil surface [9].

At the Guelph Agroforestry Research Station (GARS) in Guelph, Ontario, Canada, Price and Gordon [11] found that earthworm density was greater in a Tree-Based Intercropping (TBI) system than in a conventional agricultural monoculture. A TBI system is defined as "an approach to land use that incorporates trees into farming systems and allows for the production of trees and crops or livestock from the same piece of land in order to obtain economic, ecological, environmental and cultural benefits" [12]. These systems incorporate leaf litter and increase soil water content, which could encourage higher earthworm populations compared to sole cropping systems. In turn, this could increase the overall volume of the earthworm gut, thereby facilitating denitrification and higher N_2O emissions from a TBI system. Price and Gordon [11] also speculated that the reason earthworm densities were higher in the intercropped system compared to the conventional monoculture was because earthworms move to an area with a lower soil temperature, which in turn are areas that also have higher soil water content.

Currently, very little information exists on the influence that earthworm density has on N_2O emissions from agricultural soils, and specifically those potentially associated with a TBI system. The objective of this study was to investigate the relationship, if any, between N_2O flux, earthworm density, and gravimetric soil water content, taking into account the earthworm densities calculated by Price [13] in the TBI and monoculture systems located at GARS and using the most common earthworm species found in GARS, the common nightcrawler (*Lumbricus terrestris* L). It was hypothesized that N_2O flux would be higher as earthworm density and soil water content increased.

2. Materials and Methods

2.1. Study Design. The first experiment was conducted in the Science Complex Phytotron at the University of Guelph, Guelph, Ontario, Canada. The purpose of the first experiment was to determine the optimal soil water content for earthworm activity resulting in the highest N_2O emissions. The experiment was a two factorial, completely random design with four replications for a total of 64 experimental units. The first factor was earthworm density (see below) and the second factor was gravimetric soil water content (15%, 25%, 35%, and 45%).

Soil was collected from GARS and homogenized using a 2 mm sieve. The soil is sandy loam in texture with an average pH of 7.2 [14]. A leaf litter mixture composed of silver maple (*Acer saccharinum* L.) and poplar (*Populus spp.*) leaves was also collected from GARS, dried at 60°C for one week, and mixed into the homogenized soil to achieve a soil organic matter content of approximately 3%. Four kilograms of the soil and leaf litter mixture was then put into each of the 5 L polypropylene mesocosms, equipped with an airtight lid and rubber septum for sampling. The lids were only placed on the mesocosms at the time of N_2O sampling. The surface area of each mesocosm was $0.033\,m^2$.

Earthworm density was calculated based on data collected in the spring of 1997 from GARS by Price [13]. The three earthworm densities included high, medium, and low earthworm densities, representing populations found 0 m, 3 m, and 6 m from the tree row in a TBI system, respectively. However, these values were tripled in order to ensure the detection of N_2O for the purpose of finding optimal soil water content and also to represent an earthworm invasion where populations could initially be very high and decline over time [15]. These values were 30, 20, and 10 earthworms per 4 kg of soil or $0.033\,m^2$, for the high, medium, and low treatments, respectively, and a control with no earthworms. *L. terrestris* were purchased from Kingsway Sports (Guelph, Ontario, Canada). Earthworms were counted and weighed prior to being added to the mesocosms.

Prior to adding the earthworms, each mesocosm was fertilized with urea (46-0-0, N-P-K), which represented the N fertilizer requirement for corn planted at GARS ($215\,kg\,ha^{-1}$). Deionized water was applied to each mesocosm for one week prior to adding the earthworms in order to achieve the desired gravimetric soil water content for each treatment. A small hole in the bottom of each mesocosm allowed for proper drainage. During the course of the experiment, soil water content was maintained by weight. The mesocosms were weighed every day for the entire course of the experiment and deionized water was added to bring each mesocosm to the desired water content.

The mesocosms were placed in a greenhouse with a constant air temperature of 20°C and monitored light conditions of 16/8 hr cycles. Soil temperature was monitored using Priva soil temperature sensors (Priva North America Inc., Vineland Station, Ontario, Canada) to ensure a constant soil temperature of approximately 20°C. N_2O sampling technique and calculations will be explained in the following section.

A second experiment was conducted from February 2009 to March 2009 in the Science Complex Phytotron at the University of Guelph. Experiment 2 was a completely random design with four replications for a total of 16 experimental units. A control with no earthworms and earthworm densities of 9 (high), 6 (medium), and 3 (low) earthworms per mesocosm were used for a total of four treatments. The high, medium, and low density treatments were calculated based on actual densities found by Price [13] at GARS representing an earthworm density adjacent to the tree row, 3 m from the tree row, and 6 m from the tree row in a TBI system, respectively; a control with no earthworms was also included.

Optimal gravimetric soil water content was determined in Experiment 1 and was found to be 31%. This soil water content treatment was held constant for all four earthworm density treatments over the duration of the experiment. Methods for soil preparation, maintaining gravimetric soil water content, and monitoring temperature were the same as in Experiment 1.

2.2. Sampling Procedure. At the time of N_2O sampling, the airtight lid was placed onto each mesocosm and a 30 mL air sample using a 26-gauge needle and syringe was taken at $t = 0$, 30, and 60 min to calculate N_2O flux over an hour. Air samples were deposited into 12 mL Exetainers (Labco Limited, United Kingdom) and analyzed using a SRI Model 8610C Gas Chromatograph (Torrance, California, USA) at

Environment Canada (Burlington, Ontario, Canada). N_2O samples were taken once a week for four weeks beginning at 10:00 AM.

A soil sample was taken from each mesocosm, both before the addition of earthworms and after the last week of sampling. This was done to measure the initial and the final nitrate ($NO_3{}^-$), ammonium ($NH_4{}^+$), and total inorganic N (TIN) concentrations to determine if there was a change over the course of the experiment. Soil samples were stored in the freezer until analysis. N content was measured following a 2N KCl extraction [16], and samples were run through an Astoria 2-311 Analyzer (Astoria-Pacific Inc., Oregon, USA). Measurements of soil inorganic and organic carbon (C) were also done for initial and final C content using a Leco C determinator (Leco Corporation, St Joseph, MI, U.S.A.). However, results for soil N and C are not reported here and are part of a larger study.

2.3. N_2O Flux Calculation. N_2O flux was calculated using the ideal gas law; the molar volume of N_2O at 0°C and 1 atm is 44.0128 L/mol. The N_2O flux was adjusted for air temperature and pressure using the following formula:

$$\text{Flux adjustment} = 44.0128\,\text{L}\,\text{mol}^{-1} * \frac{[(273.16^\circ\text{K}+T^\circ\text{C})]}{273.16^\circ\text{K}} * \frac{(1013.2\,\text{hPa})}{P\,\text{hPa}}, \qquad (1)$$

where T is the air temperature and P is the air pressure on the day of sampling. These values were taken into consideration because a temperature greater than 0°C increases molar volume and, air pressure that is greater than atmospheric decreases molar volume.

The volume of the mesocosm was then converted to mol of air and multiplied by the slope of the flux determined by hourly measurement. This value was then used to calculate the flux in μmol m^{-2} s^{-1}:

$$\text{Flux}\ (\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}) = \frac{\left(S\,\text{nmol}\,\text{mol}^{-1}\ \text{s}^{-1}\right)(M\,\text{mol})}{X\,\text{m}^2}, \qquad (2)$$

where S represents the slope of the line (N_2O concentration at each measurement interval over one hour), M is the molar volume of the air in the mesocosm, and X represents the area of the mesocosm. This value was then converted into kg of N_2O ha^{-1} day^{-1}:

$$\begin{aligned}\text{Flux}\left(\text{g ha}^{-1}\text{day}^{(-1)}\right) &= \left(F\mu\text{mol}\,\text{m}^{-2}\ \text{s}^{-1}\right)(1.0 * 10^{-9}\,\text{mol})\\ &\quad * \left(44.0128\,\text{L}\,\text{mol}^{-1}\right)(10000\,\text{m}^2)\\ &\quad * (86400\,\text{s})(1000\,\text{g}),\end{aligned} \qquad (3)$$

where F is the flux calculated from (2).

Some of the flux values were negative as a result of a sink of N_2O being created rather than the N_2O being emitted through the soil surface during the extraction period from the mesocosms, which created negative flux values [17]. Therefore, a value of 100 was added to all flux values in order to complete statistical analyses and maintain positive values since the statistical program could only read positive values. The final flux values following analysis were then subtracted by 100 to present actual flux values in the following sections.

2.4. Statistical Analysis. All statistical tests were conducted using SAS v.9.1 (SAS Institute, Cary, NC, USA) at an error rate of $\alpha = 0.05$. An analysis of variance (ANOVA) using repeated measures in the PROC MIXED function was used to compare the effects of earthworm density and N_2O flux according to soil water content treatment to determine the variance in initial and final earthworm biomass between moisture treatments, as well as mortality rates between moisture treatments in Experiments 1 and 2. A response surface design using the PROC RSREG function [18] was applied to data from Experiment 1 to determine the optimal range levels of earthworm density and soil water content for the production of N_2O over ranges for these parameters that were not part of the original experimental design. The optimal soil water content found through RSREG was then applied to Experiment 2.

3. Results

3.1. N_2O Emissions. The earthworm density and soil water content interaction on N_2O emissions was significant ($P = .0457$). Mean N_2O emissions ranged from 0.54 g ha^{-1} day^{-1} from the 15% moisture and no earthworm density treatment to 43.5 g ha^{-1} day^{-1} from the 35% moisture and high earthworm density treatment as illustrated in Figure 1. Patterns did exist in emissions, where N_2O emissions were highest at the high density across all moisture treatments and lowest in the mesocosms with no earthworms across all moisture treatments. The extent of emissions across all of the moisture treatments was high > medium > low > control. Emissions due to moisture were 35% > 25% > 45% > 15% across all earthworm densities except when earthworm density = 0, where emissions were 35% > 25% > 45% = 15%. Emissions were only significant at the high density and 25% and 35% soil water content treatments, as well as the medium density and 35% soil water content treatment compared to the rest of the treatments.

Over the course of the experiment, N_2O emissions only increased at 45% soil water content, where emissions were highest in the last week of sampling compared to the first week at all density treatments (Figure 2). At 15% and 25% soil water content, emissions peaked at week three and week two, respectively, and declined by week four. In the 25% moisture treatment, emissions had a significant peak at 56.6 g ha^{-1} day^{-1} at high density in week two compared to 1.5 g ha^{-1} day^{-1}, 3.2 g ha^{-1} day^{-1}, and 3.6 g ha^{-1} day^{-1} in the control, low, and medium densities, respectively. An outlier did exist in the 25% moisture and high density treatment during week two, but when left in, it did not significantly change the result. However, it may explain the peak in emissions during week two at the high density treatment.

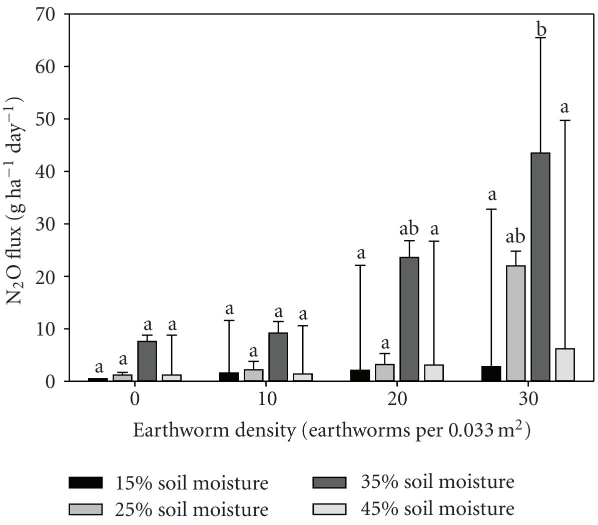


Figure 1: The relationship between N_2O emissions, earthworm density per 0.033 m^2, and gravimetric soil water content (**P** = **.0457**, SE = 4.07, 5.29, 6.10, and 4.52 for 15%, 25%, 35%, and 45%, resp.). Bars with same letter indicate no significant difference between treatments at **P** = **.05** according to Tukey-Kramer means adjustment.

Table 1: Mean earthworm mortality in the low, medium, and high earthworm densities according to θ_g (%) treatment.

	Mortality Rate (%)		
θ_g (%)	Low[§]	Medium	High
15	2.0 a[†]	11.0 a	15.6 a
25	2.0 a	2.5 a	5.0 a
35	0.0 a	8.5 a	18.3 a
45	0.0 a	6.0 a	10.0 a
SE	0.6	0.6	1.0
P value	.1994	.2139	.0571

[†] Within columns, means followed by the same letter are not significantly different according to Tukey-Kramer means adjustment (0.05).

[§] Low, medium, and high refer to densities of earthworms per 0.033 m^2: 10, 20, and 30, respectively.

N_2O emissions declined over the course of the experiment in all densities at 35% moisture except in the high density treatment where emissions were the highest in week two at 69.6 g ha^{-1} day^{-1}.

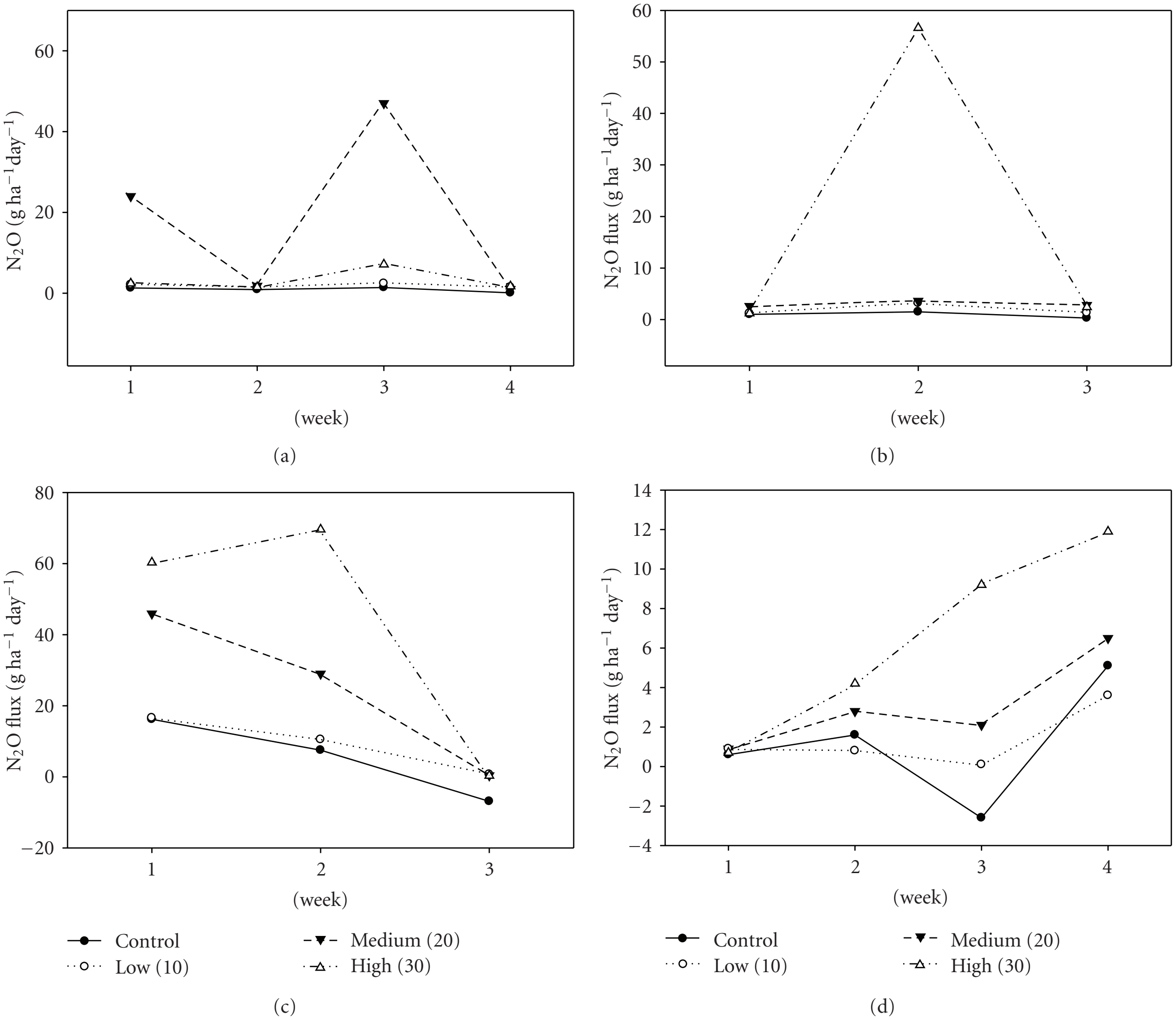
A response surface regression indicated that the lowest N_2O emissions would occur at soil water content of 15% and an earthworm density of 13 earthworms per 0.033 m^2, whereas the highest emissions would occur at a soil water content of approximately 31% and an earthworm density of 30 individuals per 0.033 m^2 as seen in Figure 3. The lowest and highest emissions correspond to −1.7 and 22.3 g ha^{-1} day^{-1}, respectively. These numbers represent emissions within the treatment range of the experiment. Emissions at soil water content or earthworm density outside of the treatment range can be determined using the equation found in the caption for Figure 3.

3.2. Earthworm Mortality and Biomass. Mortality rates were not significantly different between moisture treatments within the density treatments (Table 1). There was very little mortality in the low-density treatment across all soil moisture treatments. Mean mortality rates in the medium density treatment ranged from 3% to 11%, the highest mortality rate occurring in the 15% moisture treatment and the lowest in the 25% moisture treatment. Mean mortality in the high-density treatment ranged from 5% to 18%, the highest mortality rate occurring in the 35% soil moisture treatment and the lowest occurring in the 25% moisture treatment.

The difference in the initial and final earthworm biomass was significant according to soil water content across all earthworm density treatments as seen in Table 2. The largest increase in biomass in the low density treatment also occurred at 35% soil water content. The largest increase in biomass in the medium density treatment occurred at 35% soil water content where the final earthworm biomass was significantly higher than the initial biomass. Earthworm biomass declined in the 15% soil water content treatment due to a mortality rate of 11%; however, this decline was not significant. The highest increase in earthworm biomass over the course of the experiment occurred at 25% soil water content in the high density treatment; however, this increase was not significant. There was also a decline in earthworm biomass over the course of the experiment in the 15% and 35% soil water content treatment due to high mortality rates in the high density treatment; however this decline was not significant.

3.3. N_2O Emission at 31% Gravimetric Soil Water Content. Based on the gravimetric soil water content of 31% found in the response surface in Experiment 1, there was no significant difference in N_2O flux across all earthworm densities (P = .8085). Mean N_2O flux over the duration of the experiment was 6.99, 5.49, 6.36, and 5.63 g ha^{-1} day^{-1} for the control, low, medium, and high earthworm densities, respectively. There was also no significant difference in mean N_2O flux according to the week by density interaction (P = 0.7611, SE = 2.37 for the control, SE = 2.05 for low, medium, and high earthworm density). However, at all earthworm densities, N_2O flux peaked at week two and then declined below week one levels at week three.

3.4. Earthworm Mortality and Biomass at 31% Soil Water Content. Earthworm survival was 100% in the low and medium density treatments and 95% in the high density treatment. Initial and final earthworm biomass was significantly different across all earthworm densities. Earthworm biomass in the low density treatment increased from 12.6 g at the start of the experiment to 27.1 g at the end (P = .0007) as seen in Table 3. In the medium density treatment the initial earthworm biomass was 28.6 g and increased to 44.4 g by the end of the experiment (P = .0003). In the high density treatment, earthworm biomass increased from 36.9 g to 74.2 g by the completion of the experiment (P = .0001).



(a) (b) (c) (d)

Figure 2: N_2O flux over the entire course of the experiment according to the control, low, medium, and high earthworm density at (a) 15% ($P = .1398$), (b) 25% ($P = .3912$), (c) 35% ($P = .2451$), and (d) 45% ($P = .0685$) gravimetric soil water content.

4. Discussion

Overall, emissions were highest at the 25% and 35% soil water content treatments and the lowest emissions were seen at 15% and 45% soil water content. Bertora et al. [19] found similar results with the presence of earthworms, where emissions increased significantly over the course of their experiment at 25% soil water content up to 62 days, when emissions began to decrease. N_2O emissions were significantly higher at 25% than at the lower moisture treatments (19%, 12.5%) where emissions were not significant.

Conversely, at 35% moisture, there was a downward trend in emissions over the course of the experiment, except at the high density where N_2O flux peaked at 69.6 g ha^{-1} day^{-1} in week two with a significant decline in emissions in week three. This could mean that earthworms may only be able to tolerate high soil water content for a limited time. Therefore, the high earthworm mortality in this treatment could have occurred toward the end of the experiment, which could explain the decline in N_2O flux following week two. However, the 45% soil water content treatment also contradicts optimal soil water content for earthworm activity. N_2O emissions gradually increased across all earthworm densities at 45% soil water content showing that *L. terrestris* may have been adapting to the soil conditions. Increases in emissions were gradual and did not reach levels found at 25% and 35% soil water content, but mortality rates were lower, but not significant, compared to mortality rates at 35% soil water content showing some tolerance. El-Duweini and Ghabbour [20] also reported soil water content tolerance levels, but for two Australian species, *Allolobophora caliginosa* and *Metaphire californica*, to be 20%–45% and 35%–55%, respectively in a clay soil.

Earthworm mortality was the highest in the 35% moisture treatment, at the highest earthworm density even though emissions were significantly higher than at any other treatment combination. Dymond et al. [15] reported an initial earthworm invasion of 2,621 individuals m^{-2} of

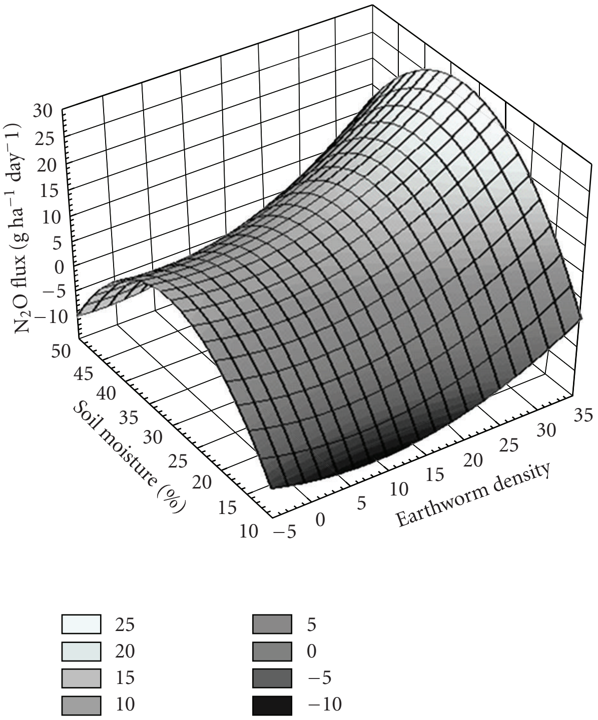


Figure 3: A response surface regression showing the relationship between N_2O flux (kg ha^{-1} day^{-1}), gravimetric soil water content (%), and earthworm density (number of earthworms 0.033 m^{-2}). Equation of the line is $36.7186 - (0.36143 * D) + (3.1095 * M) + (0.0174 * D * D) + (0.00810 * M * D) - (0.0518 * M * M)$ ($R^2 = 0.17$, $P \leq .0001$), where D is earthworm density and M is gravimetric soil water content.

Table 2: Mean initial and final earthworm biomass in the low, medium and high earthworm densities according to θ_g (%) treatment.

	Density Treatment Biomass (g)		
θ_g (%)	Low[§]	Medium	High
15 Initial	40.88 a[†]	86.97 a	130.55 abc
15 Final	50.92 a	83.85 a	121.48 abc
25 Initial	43.08 a	86.97 a	125.85 abc
25 Final	52.19 a	101.95 b	150.85 c
35 Initial	47.20 a	103.60 b	145.63 ac
35 Final	89.94 b	123.30 c	144.50 ac
45 Initial	33.28 c	70.29 a	103.90 b
45 Final	55.59 a	75.60 a	117.10 ab
SE	2.69	4.02	5.54
P value	<.0001	.0420	.0323

[†] Within columns, means followed by the same letter are not significantly different according to Tukey-Kramer means adjustment (0.05).

[§] Low, medium, and high refer to densities of earthworms per 0.033 m^2: 10, 20, and 30, respectively.

Table 3: Mean initial and final biomass in the low, medium, and high earthworm densities at 31% gravimetric soil moisture content.

	Density Treatment Biomass (g)		
	Low[§]	Medium	High
Initial	12.6 a[†]	28.6 a	36.9 a
Final	27.1 b	44.4 b	74.2 b
SE	1.94	1.94	1.94
P value	.0007	.0003	.0001

[†] Within columns, means followed by the same letter are not significantly different according to Tukey-Kramer means adjustment (0.05).

[§] Low, medium, and high refer to densities of earthworms per 0.033 m^2: 3, 6, and 9, respectively.

Dendrobaena octaedra into a northern Alberta pine (*Populus sp.*) and aspen (*pinus sp.*) forest. This population dropped to 76 individuals m^{-2} within just a few years as a result of competition for resources. High competition could have been the reason for the drastic decline in emissions in the high density treatment at 35% soil water content and lower mortality in the medium (9%) and low (0%). Another reason for the decline in emissions after week two could be due to the ability of high earthworm populations to speed up residue decomposition [19]. Organic matter is more palatable to earthworms at higher soil water content; therefore, ingestion of organic matter is enhanced. Organic matter turnover could have been enhanced at the 35% moisture and high density combination by week two resulting in a decrease in preingested organic matter and a decline in earthworm activity.

The gravimetric soil water content treatments of 15%, 25%, 35%, and 45% are approximately equivalent to a water-filled pore space (WFPS) of 30%, 55%, 75%, and 100%. It is generally accepted that denitrification rates are optimal between a WFPS between 60% and 100%, where N_2O is the primary product between 60% and 90% [21]. Above 90% N_2 is the dominant product [21], which could be the reason for lower N_2O flux measurements in the 45% soil water content, where the WFPS was 100%. The highest N_2O flux occurred at 35% soil water content or 75% WFPS, which is within the range of optimal denitirification rates. Furthermore, nitrification rates are highest between 45% and 75% [21]. The product of nitrification is $NO_3{}^-$, a primary input for denitrification. This means that in the 35% soil water content treatment, both nitrification and denitrification were optimal, which may have contributed to the significantly higher N_2O flux compared to the 15% and 45% soil water content treatments.

N_2O emissions could be lower at dryer soil water contents as a result of earthworm diapause or aestivation. In this state, earthworms will decrease their activity to prevent water loss from the body [2]. Ingestion of soil and organic matter content would decrease, thereby limiting microbial activity in the earthworm gut and reducing emissions. The same occurs at high moisture contents and could explain the lower N_2O emissions at the 45% moisture treatment in this study.

Perrault and Whalen [22] found that earthworm burrowing length decreased in wetter soils, which would indicate a decrease in earthworm activity. However, wetter soils caused an increase in the ingestion of organic matter compared to dryer soils. Leaf litter is more palatable to earthworms when wetted, and as a result ingestion is increased. This could explain higher emissions in the 25% and 35% moisture treatments compared to 15% soil water content, as well as the decrease in earthworm biomass in the medium and high densities at 15% soil water content. Earthworms would ingest higher carbon substrates at these moisture contents, which would in turn provide energy for denitrifying bacteria found in the earthworm gut and increase N_2O production.

Earthworm surface casting also increases in wetter soils, which provides another ideal environment for denitrification. Earthworm casts contain higher populations of denitrifying bacteria compared to mineral soils due to higher amounts of carbon substrates, and as a result, higher N_2O emissions are produced [23]. Elliot et al. [24] found that denitrification was higher in earthworm casts than surrounding mineral soil. Denitrification rates from earthworm casts ranged between 0.2–0.9 $\mu g\,N\,g^{-1}$ during the fall compared to 0.05–0.3 $\mu g\,N\,g^{-1}$ from the soil within the same time period. This indicates that a portion of the emissions from this experiment could be due to increased surface casting in the 25% and 35% moisture treatments at the high density treatments.

Trends in N_2O emissions according to earthworm density did occur. The high, medium, and low density treatments represent 9.1×10^5, 6.1×10^5,and 3.0×10^5 earthworms ha^{-1}, respectively. Emissions consistently increased as earthworm density increased in all moisture treatments. However, emissions were only significantly higher at the medium and high densities in the 25% and 35% soil water content treatments (**Figure 1**). Frederickson and Howell [25] found no relationship between earthworm density and N_2O emissions in large-scale vermicomposting beds. However, in a subsequent laboratory experiment, emissions were correlated with earthworm density at five earthworm density treatments ($R^2 = 0.76$).

The reason for this may be a result of an increase in the ingestion of organic matter and, with that, denitrifier bacteria at higher earthworm densities; therefore, denitrification may occur at faster rates than in soils with lower earthworm densities. Denitrification occurs at higher rates in the earthworm gut due to the anoxic environment and sufficient supply of carbon for denitrifier bacteria compared to soil homogenates [6, 26, 27]. An increase in earthworm density results in an increase in this ideal environment of earthworms for denitrifier bacteria and therefore, could increase emissions. The number of denitrifier bacteria is also higher in the earthworm gut and surface castings than outside soil homogenates [8]. These authors calculated that there were 256-fold more denitrifier bacteria in the earthworm gut of *L. rubellus* than in the surrounding soil where the earthworms were found. This indicates that an increase in earthworm density also increases the number of denitrifier bacteria in the gut of the earthworms facilitating higher N_2O emissions as could be the case in this study.

Another reason why N_2O emissions were highest at the high density earthworm treatments could have been a result of an increase in the microbial biomass pool and subsequent increase in respiration causing lower O_2 levels in the soil. Groffman et al. [28] found that in areas with the presence of earthworms, microbial biomass was significantly higher in the mineral soil compared to areas without the presence of earthworms. In turn, Fisk et al. [29] discovered that this increase in microbial biomass due to the presence of earthworms increased respiration rates by 20% compared to areas without earthworms. Therefore, O_2 levels will decline providing a more ideal environment for denitrification to occur and subsequent gaseous N losses. However, even though microbial biomass may increase with earthworm presence, a subsequent increase in mineralization and nitrification rates may not occur. Bohlen et al. [30] and Groffman et al. [28] found that mineralization and nitrification rates in the soil did not differ significantly in plots with and without earthworms. They speculated that earthworms facilitated a C-sink in the soil and subsequently created an N-sink, preventing the increase in N mineralization and nitrification rates in the soil. This could mean that the majority of the N_2O released from the mesocosms was attributed to the presence of earthworms and earthworm gut, rather than denitrification occurring in the surrounding soil, since ${NO_3}^-$ concentrations may have been low due to low nitrification rates.

In Experiment 2, N_2O emissions were not significantly different across earthworm population densities; however, the results were consistent to what was found in Experiment 1. N_2O flux in Experiment 2 across all earthworm densities (0, 3, 6, and 9) was in the same range as emissions in Experiment 1 between the control and low density earthworm treatments (0 and 10). This was expected since the earthworm densities used in Experiment 2 were within the range of the control and low density treatments in Experiment 1, and there were no significant differences in emissions between the control and low density treatments in Experiment 1. No significant differences in N_2O flux occurred even with significant differences in initial and final biomass between density treatments. This shows that even with an increase of approximately 3.0×10^5 earthworms ha^{-1} from zero earthworms, there would be no significant corresponding change in emissions between a TBI and sole cropping system, like the systems found at GARS. This could be a result of other compounding factors such as soil water content, soil temperature, residual soil N and C, and land management practices, which could all mask the earthworm effect on denitrification.

The same general trend of N_2O emissions occurred over time as in the 35% soil water content treatment in Experiment 1, where emissions hit a peak at week 2 and declined at week 3 to levels the same or lower than at week 1. This cannot be explained by earthworm mortality since earthworm mortality was insignificant or did not occur in Experiment 2 compared to Experiment 1. However, since soil water content of 31% was found to be optimal for earthworm activity, this may have sped up organic matter decomposition [18] between weeks 1 and 2 leaving the less palatable lignin compounds, thereby slowing earthworm activity between weeks 2 and 3.

5. Conclusion

A relationship was found between earthworm density, gravimetric soil water content, and N_2O flux in Experiment 1. As earthworm density increased, N_2O flux also increased; however, flux was only significantly higher in the high density treatment at 25% soil water content and at both the medium and high earthworm densities at 35% moisture. This could be attributed to optimal gravimetric soil water content for earthworm activity between 25% and 30%, which closely corresponds to the 31% moisture value reported by the response surface analysis in which emissions were also the highest.

Experiment 2 showed no relationship between earthworm density and N_2O emissions, which was expected because the earthworm densities used in Experiment 2 are within the range of the control and low density treatments used in Experiment 1 in which there was no significant difference in N_2O emissions. As a result, the results found here would only have implications in a TBI system where earthworm populations were triple to what is found at GARS. However, earthworms prefer environments with higher organic matter content and soil water content, both of which are present in a TBI system. This could result in higher emissions indirectly related to earthworm population as TBI systems have more favourable environments to earthworms. However, N_2O emissions as a result of the presence of earthworms could be dependent on the proximity of earthworm to the tree row, as well as the species of trees present in the TBI system.

The results of this study are important to consider when deciding on the implementation of agricultural practices to reduce N_2O emissions and also the invasion of earthworms into areas previously void of earthworms. The benefits that are normally seen from earthworms in agricultural systems may be masked by their influence on facilitating the production of N_2O and in turn, climate change.

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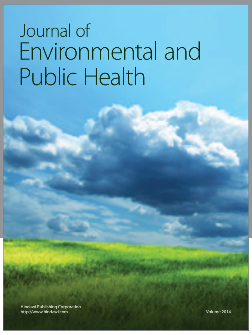

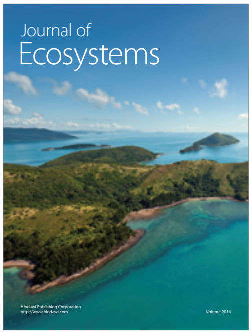

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