

# Exotic earthworms dispersion through protected forest areas and their potential impacts on nitrous oxide production

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**Abstract** It is generally accepted that human activities are responsible for the dispersal of exotic earthworms in northeastern North America. We know little, however, about the relative effects of concurrent human activities on the structure of these earthworm communities in protected forest areas, nor on their impacts on soil biological activities. Our first objective was to infer the relative importance of recreational fishing and road traffic on the structure of Lumbricidae communities in Mont-Tremblant National Park, the oldest conservation area in the province of Quebec, Canada. Our second objective was to test the relationship between earthworm species abundances and soil properties related to microbial and nitrogen dynamics. We sampled earthworm communities around 61 lakes, which included 23 heavily-fished lakes and 20 non-fished lakes located near roads, as well as 18 non-fished lakes located in remote areas of the park. Our results revealed that fishing and proximity to roads both have a positive effect on the abundance of earthworms, as does the soil pH. Fishing activities had a greater effect than road proximity on the abundance and diversity of earthworm communities, notably on the abundance of the anecic species *Lumbricus terrestris*. To assess at a finer scale the effects of earthworm community structure on soil microbial and

nitrogen dynamics, we collected and analyzed soils from 47 sampling points around two lakes with high earthworm densities. Exploratory redundancy analysis found a negative correlation between epigeic and anecic earthworm species, with the former correlating positively to microbial biomass and the latter correlating positively to nitrification and denitrification. Confirmatory path analysis established a positive indirect effect of *Lumbricus terrestris*, the preferred fishing bait, on potential soil nitrous oxide emissions. We conclude that the human-mediated dispersion of earthworms in the most pristine ecosystems of Quebec affects ecosystem functioning and thus requires a review of current policies regarding the use of live-bait by fishermen.

**Keywords** Exotic earthworm survey · Forest soil properties · Human-mediated dispersion · *Lumbricus terrestris* · Northern temperate forests · Soil nitrous oxide production

## Introduction

It is generally recognized that earthworms in Eastern Canada did not survive the Wisconsin glaciation, which receded about 10,000 years ago (McKey-Fender et al. 1994). Consequently, earthworm species that are currently found in this region were introduced by European settlers over the course of recent centuries

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(Gates 1982). Today, most agricultural fields in the province of Québec are colonized by exotic earthworm species, where their effects on nutrient cycling (Eriksen-Hamel and Whalen 2007) and soil structure (Blouin et al. 2013) have been amply described. In recent decades, earthworms have also been reported in forest ecosystems located north and east of Québec's agricultural belt (Moore et al. 2009; Drouin et al. 2016), as well as in forest ecosystems of neighbouring regions of Canada and the U.S. (McLean and Parkinson 1997; Burtelow et al. 1998; Hale et al. 2006). This has generated interest for the human factors driving the dispersal of earthworms into northern temperate forests (Sackett et al. 2012), as well as their impacts on the integrity of these ecosystems (Drouin et al. 2014; Groffman et al. 2015; Drouin et al. 2016).

Marinissen and van den Bosch (1992) estimated that the natural rate of earthworm dispersal does not exceed 5–10 m year<sup>-1</sup>. It is thus likely that earthworms are introduced into new habitats via human activities (Hale 2008). For example, humans can voluntarily introduce earthworms into gardens in order to improve compost quality (Edwards and Bohlen 1995), or on contaminated sites as agents of soil restoration (Butt et al. 1995). Humans are also responsible for involuntary introductions, for example with the importation of soil-containing material for agricultural purposes (Hendrix and Bohlen 2002). In northern temperate forests of eastern North America, it is believed that voluntary introductions may result from anglers discarding non-used live bait near boat launches and in adjacent areas (Keller et al. 2007; Cameron et al. 2007; Kilian et al. 2012). Involuntary introductions, on the other hand, may occur when earthworms and earthworm cocoons get lodged in tire treads and are later released along roadsides in wooded areas (Cameron et al. 2008). Little is known, however, on the relative importance of these two mechanisms in contributing to the abundance and diversity of earthworm communities in forest ecosystems.

Earthworms are known to affect forest soil properties in many ways. Their feeding activities involve comminution of leaf litter that accelerates its decomposition, thereby causing the loss of the forest floor (Alban and Berry 1994; Suarez et al. 2006), a change of soil microbial community structure and function (Li et al. 2002; McLean et al. 2006), and a lower retention of key soil nutrients (Bohlen et al. 2004a; Sackett et al. 2013; Resner et al. 2014; Ewing et al. 2015). Little is known,

however, on the effects of exotic earthworm communities on the emission of greenhouse gases from forest soils. In agricultural soils, earthworms have often been reported to stimulate nitrous oxide (N<sub>2</sub>O) emissions (Giannopoulos et al. 2010; Kuiper et al. 2013; Marhan et al. 2015), which has a global warming potential 300 times higher than CO<sub>2</sub> (IPCC Intergovernmental Panel on Climate Change 2013). Earthworms are known to exacerbate N<sub>2</sub>O emissions in agricultural soils via their stimulatory effects on two processes, nitrification and denitrification (Bremner 1997; Wrage et al. 2001; Kool et al. 2011). Their burrowing activities may increase nitrification by aerating the soil and stimulating microbial growth, resulting in a positive effect on ammonium oxidation (Edward and Bohlen 1995; Blair et al. 1997). They may also enhance denitrification, as their guts and casts are hot-spots for anaerobic denitrifying bacteria (Horn et al. 2003; Drake and Horn 2006). However, it is uncertain whether nitrification, and by extension denitrification, would be stimulated by earthworms in forest soils. The lower soil pH (Paavolainen and Smolander 1998), the presence of plant derived inhibitory metabolites (Clein and Schimel 1995) and the lack of fertilizer N may all preclude the open cycling of mineral N and the possibility of N<sub>2</sub>O emissions.

Here, we report on a study in which we extensively surveyed earthworm communities along roadsides and near lakes that had either been open or closed to recreational fishing. We tested the relative importance of roadsides and recreational fishing in controlling earthworm abundance and community composition. We hypothesized that areas near boat launches around lakes with recreational fishing would constitute hot-spots for exotic earthworms, especially *Lumbricus terrestris*, which is a preferred bait for anglers (Kilian et al. 2012). As an extension to this study, we intensively sampled earthworm communities and measured soil microbial biomass and nitrogen (N) dynamics, including potential nitrification and denitrification, in a sampling grid around two lakes with recreational fishing.

## Materials and methods

### Study site

The study was conducted within the boundaries of Mont-Tremblant National Park (46°28'39"N,

74°17'26"W), a 1510 km<sup>2</sup> area located ca. 150 km northwest of Montreal (Quebec, Canada). Sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britt.) and balsam fir (*Abies balsamia* (L.) Mill.) are the principal late successional tree species. The park lies at ca. 500 m above sea level within the geological range of the Canadian Shield and contains over 400 lakes distributed in three watersheds. The nearby weather station in Ste-Agathe-des-Monts reports daily average temperatures of  $-13.1$  °C in January and  $+17.8$  °C in July. Average annual rainfall is 1175 mm of which 31% falls as snow.

Prior to its creation in 1895, Mont-Tremblant National Park had no permanent human settlements. In the first half of the 20th century, commercial logging was allowed as well as fishing and hunting by members of a few exclusive hunting and fishing clubs. Public access to a few restricted areas of the Park was granted in 1958, and all logging activities ceased in 1977. Since 1972, the number of daily fishing permits issued for each lake in the Park has been compiled in a registry.

#### Experimental design and field sampling

In summer 2013, we sampled earthworms adjacent to 61 lakes (see map—Fig. 1), each surrounded by a narrow belt of conifers near the water's edge, transitioning to mixed hardwood forests moving upslope. Among these, 23 lakes were located near roads and had been assigned numerous fishing permits over many years (i.e. up to >30,000 in past 40 years); 20 lakes occurred near roads and had seldom (i.e. <100) or never been assigned fishing permits; 18 lakes occurred in remote areas of the park (i.e. at least 500 m from the closest road) and had never been open to recreational fishing. We randomly sampled these three categories of lakes over time because earthworm sampling efficiency may change with fluctuations in climate and resource availability.

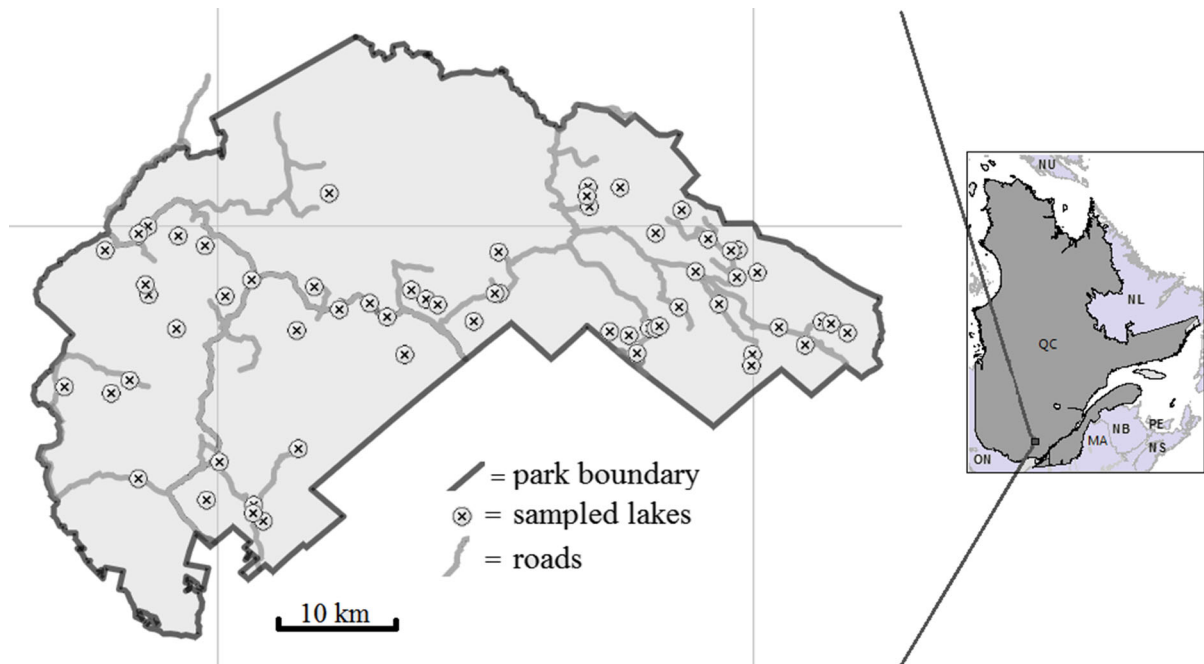
At each of the 61 lakes, we sampled earthworms at four locations: (1) in the coniferous belt near the water's edge, either beside a boat launch or in an area suitable for a boat launch; (2) 20 m upslope from the first sampling point, in the mixed deciduous forest; (3) 200 m upslope from the first point (i.e. perpendicular to the water's edge), also in the mixed deciduous forest; (4) 200 m from the first sampling point, in the coniferous belt near the water's edge. This design allowed us to disentangle the effects of dominant tree

type from the effects of proximity to human activities (i.e. in the case of lakes located near roads). At each sampling point, we collected earthworms in four quadrats (25 cm × 25 cm) located 1 m apart. We removed the forest floor from each quadrat and sifted this material by hand to extract epigeic specimen. We then applied 4 L of a dry mustard solution (10 g L<sup>-1</sup>) in each quadrat to extract endogeic and anecic species over a 15 min period (Lawrence and Bowers 2002). Finally, we obtained three containers of earthworms at the Park's Visitor Center, which were destined to be sold to anglers. All earthworms from each sampling point as well as those sold at the Visitor Center were placed in jars containing 70% isopropanol and brought back to the laboratory for counting and identification. At each sampling point of each lake, we also collected a bulk sample of organic forest floor F-layer material as well as a bulk sample of the underlying mineral soil up to a depth of 10 cm.

In order to evaluate the effects of earthworms on soil microbial and N dynamics, we intensively sampled the shores of two lakes in summer 2014. Based on the extensive earthworm survey of 2013, the land adjacent to these two lakes harboured a relatively high abundance and diversity of earthworms. By intensively sampling soils around only two nearby lakes, this design ensured that differences between soil properties would be mainly ascribed to differences in local earthworm community structure as opposed to site specific differences in parent material, texture, CEC, drainage and other static soil properties. For each of the two lakes, we established a sampling grid of approximately 2500 m<sup>2</sup> with the first corner of the grid located 20 m upslope from the boat launch and subsequent sampling points occurring at ca. 10 m intervals (i.e. 47 sampling points total). At each sampling point, we collected a 20 cm × 20 cm sample of organic forest floor F-layer material as well as a sample of the underlying mineral soil up to a depth of 10 cm. Earthworms were then collected at each sampling point, as described for the extensive earthworm survey of 2013. Soil samples were transported in coolers under ice packs and placed in a cold room (4 °C) until analyzed.

#### Earthworm species identification

Earthworms from both surveys (2013 and 2014) were soaked 24 h in a 10% formaldehyde solution to fix their tissues and then returned into jars containing isopropanol. Species identification of mature



**Fig. 1** Geographic location of Mont-Tremblant National Park within the province of Quebec (*right*), and location (⊗) of the 61 sampled lake within the park (*left*); *pale grey lines* represent roads

individuals was accomplished with a binocular light microscope based on the key developed by Reynolds (1977). Juvenile individuals without clitella remained unidentified.

#### Soil analyses—2013 survey

The bulk soil samples from the 2013 survey (i.e. 61 lakes,  $N = 244$ ) were dried at 101 °C to determine moisture content. Soil pH was then measured using a standard hydrogen electrode (soil:water = 1:10 for organic soil, 1:2 for mineral soil). Total C and N content were determined by high temperature combustion followed by gas analysis using a Vario Macro CN Analyser (Elementar GmbH, Hanau, Germany). The textural class of each mineral soil sample was estimated by a tactile assay developed by Saucier (1994), which we first calibrated against the hydrometer method (Bouyoucos 1962).

#### Soil analyses—2014 survey

Soil subsamples from the two intensively sampled lakes ( $N = 47$ ) of the 2014 survey were analyzed for moisture content, pH in water, total C and N as well as

textural class as described for soil samples from the 2013 survey.

Soil basal respiration was measured in field moist soil subsamples by weighing 5 g of organic and 15 g of mineral soil (dry wt. equiv.) into 125 mL sampling vessels. The vessels were covered to minimize moisture loss and left 5 days at room temperature to allow microbial respiration to acclimate. The vessels were then flushed with ambient air for 5 min. and sealed with air-tight lids equipped with rubber septa. Exactly 6 h later, a 2 mL aliquot of gas was taken from the headspace and injected into a model Varian 431-GC gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with a thermal conductivity detector and He as the carrier gas.  $\text{CO}_2$  concentrations were adjusted for fluctuations in room temperature using Ideal Gas Laws and assuming  $Q_{10} = 2$ .

Soil microbial biomass was measured by substrate induced respiration (Anderson and Domsch 1978), as modified by Bradley and Fyles (1995). Briefly, field moist soil samples were removed from the cold room and left 4 h to acclimate to room temperature. Subsamples of organic (5–10 g dry wt. equiv.) and mineral (20–25 g dry wt. equiv.) soil were then

weighed into 125 mL sampling vessels and mixed with 500 mg of a 3% glucose-talc mixture. Sampling vessels were left undisturbed for 100 min. in order to attain maximum initial soil respiration rates. Vessels were then flushed for 5 min. with ambient air using an aquarium pump and sealed with air tight lids equipped with septa. After 30 additional minutes, a gas sample was removed from the headspace and analyzed for CO<sub>2</sub> as described above. Substrate induced respiration rates were converted to microbial biomass using equations derived by Anderson and Domsch (1978).

Potential ammonification and nitrification rates were estimated by aerobic soil incubations. Briefly, field moist soil subsamples (15–20 g) were weighed into 500 mL Mason jars, covered with polyethylene films to prevent moisture losses while allowing gas exchanges, and left to incubate at room temperature for 30 days. Soils were then extracted in a 1.0 N KCl solution and extracts were analyzed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentration on an Astoria II auto-analyzer (Astoria-Pacific, Clackamas, OR) using respectively the Berthelot and Griess-Ilosvay methods (Mulvaney 1996).

Potential denitrification rates were measured using the acetylene reduction assay. Briefly, field moist soil subsamples of organic soil and mineral soil (respectively 5 and 20 g dry wt. equiv.) were mixed with 5 mL of KNO<sub>3</sub> solution (500 ppm N) as well as 250 mg of a glucose-talc (1:8) mixture. The treated soil subsamples were then transferred into 125 mL sampling vessels and sealed with airtight lids equipped with rubber septa. The headspace of each sampling vessel was injected with 10 mL of acetylene (C<sub>2</sub>H<sub>2</sub>) and incubated 18 h at room temperature. A 5 mL aliquot of gas from the headspace of each sampling vessel was then sampled and injected into a model Varian CP 3800 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with an electrical conductivity detector and He as carrier gas. The measured concentration of ethylene (C<sub>2</sub>H<sub>4</sub>) gas in each sample was used as a relative measurement of potential denitrification.

### Statistical analyses

For the 2013 survey, earthworm abundance at each sampling location was calculated as the average of four quadrats, whereas earthworm abundance at each lake was calculated as the sum of earthworm

abundance at the four sampling locations. We first performed a Chi square test to determine whether the abundance of earthworms was distributed randomly across the three categories of lakes. Subsequently, we tested for the effects of lake category, soil pH, total C and N, and soil texture on earthworm abundances. Here we used negative binomial regressions in order to correct for over-dispersion in earthworm counts across the 61 lakes. The identity of each lake was included as a random variable in these models to avoid pseudo-replication bias. We used the backward stepwise procedure (Harrell 2001) in order to find the most parsimonious model in which all variables were statistically significant. Using only data from the 28 lakes where earthworms were found, we applied a nested model to test the effects of sampling location within lakes, and the effects of soil properties on earthworm abundances. Tukey HSD tests were used to identify significantly different group means.

In order to explore the relationships between earthworm community composition and soil variables, data from the 2014 survey were first analyzed by multivariate redundancy analysis (RDA) (van den Wollengerg 1977) using the *vegan* package of R statistical software (R Development Core Team; <http://www.R-project.org>). All soil properties and earthworm species abundances were standardized prior to RDA. Based on the RDA output (see Results), we further tested for direct and indirect correlations between endogeic + anecic earthworm species and potential denitrification using Pearson's correlation coefficient as well as confirmatory path analysis (Shipley 2009). For our path analysis, we tested all independence relationships using Fisher's C test as implemented in the *shipley.test* function of R statistical software. The *SEM* function in the *lavaan* R package was then used to calculate path coefficients and correlations with direct and indirect effects on the final path. Since variables were not normally distributed, we selected the *MLM* (Maximum Likelihood Method) estimator (Shipley 2002) for more robust standard error estimations.

### Results

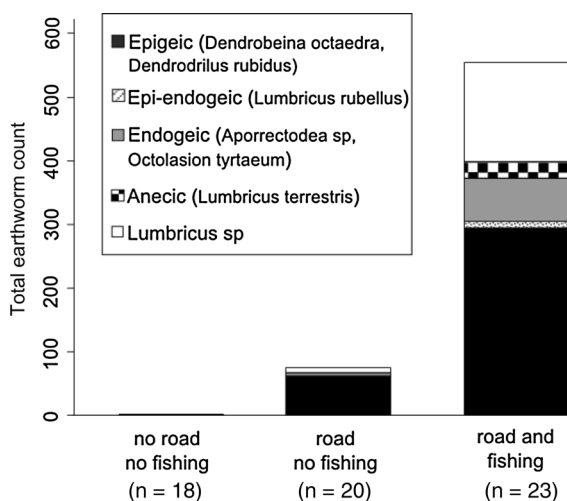
For the extensive survey conducted in 2013, Chi square analysis confirmed that the abundance of earthworms was not distributed randomly across the



three categories of lakes ( $\chi^2 = 8.58$ ,  $P = 0.015$ ). More specifically, earthworms were seven times more abundant at lakes with abundant fishing permits than at lakes near roads without fishing permits (Fig. 2). Furthermore, nearly 50% of earthworms near lakes with fishing permits were classified as either endogeic or anecic, whereas the epigeic species *Dendrobaena octaedra* comprised about 85% of earthworms around lakes near roads without fishing permits. A single earthworm specimen of a rare species, *Dendrodriilus rubidus*, was found at one of the remote lakes without fishing, and we detected no earthworms at the 17 remaining lakes in this category. As for the earthworms that we bought at the Park's Visitor Center, all specimen were from the anecic species *L. terrestris*.

Negative binomial regressions confirmed that the number of fishing permits per lake was significantly ( $P = 0.028$ ) and positively related to earthworm abundance, along with soil pH ( $P < 0.001$ ). When analyzing only the 28 lakes where earthworms were found, negative binomial regressions revealed a significant ( $P < 0.001$ ) effect of sampling location on earthworm abundances (Fig. 3). A *post hoc* Tukey test revealed that earthworms were more abundant near the boat launch, either under conifer or deciduous forest cover, than at 200 m away from the boat launch, either perpendicular or parallel to the shoreline.

For the intensive survey conducted in 2014, RDA revealed that earthworms explained 16% of the variance in soil properties along the first two principal

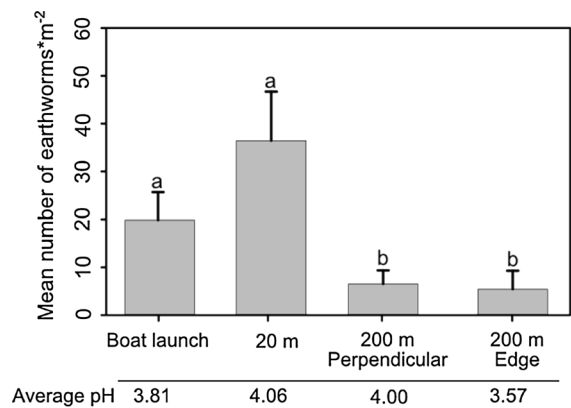


**Fig. 2** Stacked bar plot showing the total number of earthworms, sorted by species, recorded within each category of lake

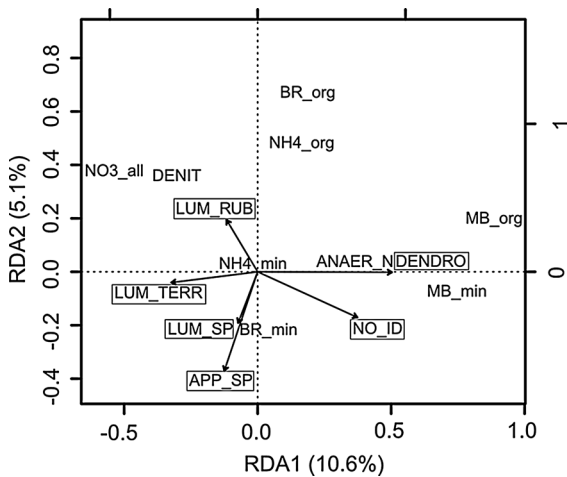
component axes (Fig. 4). The RDA bi-plot revealed a strong negative correlation between the abundance of the epigeic species *D. octaedra* and the abundance of endogeic and anecic species. *D. octaedra* correlated positively with soil microbial biomass, whereas endogeic and anecic species correlated positively with net nitrification and potential denitrification rates. Pearson's correlation coefficient revealed a slightly non-significant ( $P = 0.064$ ) correlation between the abundance of endogeic + anecic species and potential denitrification. However, exploratory path analysis found a significant causal pathway between *L. terrestris* and potential denitrification via the forest floor C/N ratio as well as via nitrification rates in both the forest floor and mineral soil (Fig. 5).

## Discussion

Taken collectively, our survey data provide strong evidence that human activities play a crucial and important role in the dispersal of earthworms in natural mixedwood forests of Quebec. Our results also establish that recreational fishing has a greater potential for spreading exotic earthworms in the forests of Mont-Tremblant National Park than car traffic does. Moreover, recreational fishing results in greater



**Fig. 3** Mean number of earthworms ( $m^{-2}$ ) and average soil pH at each sampling location within the 28 lakes where earthworms were found. Sampling locations are (1) beside boat launch under coniferous canopy, (2) 20 m upslope from boat launch under mixed deciduous canopy, (3) 200 m upslope from boat launch, under mixed deciduous canopy, (4) 200 m from boat launch near water's edge, under coniferous canopy. Bars with different lower case letters differ significantly ( $P < 0.05$ ) according to Tukey's HSD test; vertical lines = 1 S.E.



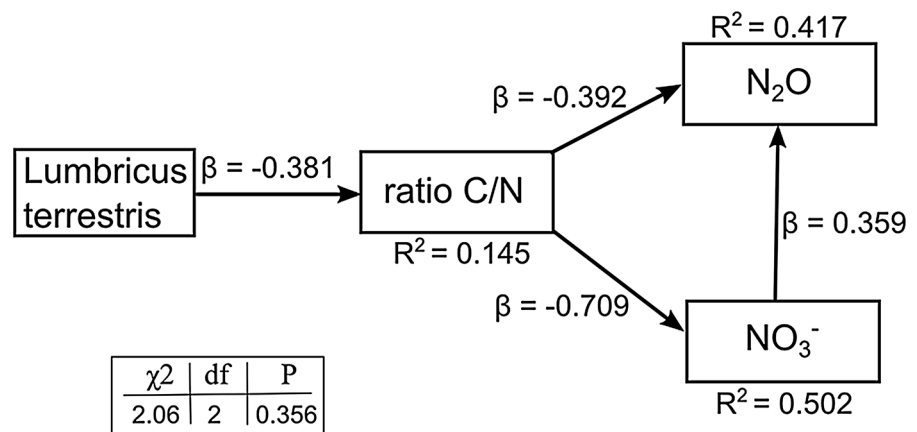
**Fig. 4** Ordination biplot resulting from redundancy analysis using earthworm species’ abundances as independent variables, and soil microbial and nitrogen measurements as response variables. Earthworm species (shown in frames) are as follows: LUM\_RUB = *Lumbricus rubellus*; DENDRO = *Dendrobaena octaedra*; NO\_ID = unidentified juveniles; APP\_SP = *Apporectodea* sp.; LUM\_SP = *Lumbricus* sp.; LUM\_TERR = *Lumbricus terrestris*. Soil properties are: BR\_min and BR\_org = basal respiration rate in mineral and organic soil layers; MB\_min and MB\_org = microbial biomass in mineral and organic soil layers; NH4\_min and NH4\_org = aerobic ammonification rate in mineral and organic soil layers; NO3\_all = aerobic nitrification in the combined mineral and organic soil layers (shown as one because they overlap on the biplot); DENIT\_min = potential denitrification in the mineral soil layer. The first two principal components explained 16% of the total variance in soil properties

earthworm species diversity and proportionately more endogeic and anecic species. It is noteworthy that *L. terrestris* was the only species detected from bait samples bought at the Park’s Visitor Center, and that

this species was only found adjacent to lakes with frequent fishing. According to several studies (e.g. Holdsworth et al. 2007; Corio et al. 2009; Sackett et al. 2012), the ecological impacts of endogeic and anecic species, especially *L. terrestris*, are greater than those of epigeic species such as *D. octaedra* that were proportionately more abundant near roadside lakes without fishing permits.

Earthworm abundances can be determined both by human-mediated propagule pressure as well as by natural variation in soil conditions. Reich et al. (2005) sampled earthworms and forest soil properties along a deciduous–coniferous forest gradient and found a correlation between soil pH and earthworm abundances, consistent with the long held view that litter chemistry affects earthworm foraging behaviour and biomass (Pearce 1972; Hendriksen 1990). In our study, however, differences in soil pH under deciduous and coniferous canopies were not as determinant of earthworm abundances as was the proximity to the boat launch (Fig. 3). Given this clustering of earthworms near boat launches, the question remains whether these constitute sink populations or whether they have the potential to spread over time. The risk of exotic earthworms gradually spreading through these forests cannot be dismissed, given that we did find earthworms at 200 m from the boat launches, both along the shorelines and upslope from the water’s edge. Also, our survey detected *L. terrestris* around lakes where fishing permits had issued abundant fishing permits up until a few years prior to our 2013 survey. All of this evidence suggests that introduced earthworms are able to establish and survive in mixed-wood forests of Parc du Mont-Tremblant.

**Fig. 5** Final path diagram confirming a significant positive relationship between the abundance of *Lumbricus terrestris* and potential N<sub>2</sub>O production in soils. Path coefficients (β) represent strength and direction of significant correlations. R<sup>2</sup> values are indicated for each endogenous variable



If we assume that the relative abundance of earthworm species in the non-identified pool of juveniles is similar to the relative abundance of identified mature specimens, it would explain why the “NO\_ID” variable in Fig. 4 correlated strongly with *D. octaedra*. The fact that this dominant epigeic species correlated negatively with all other endogeic and anecic species is consistent with other studies (e.g. Eisenhauer et al. 2007). Some have hypothesized (e.g. Pizl 1992; Tiunov et al. 2006) that this may be due to incompatibilities in their respective activities, whereby endogeic and anecic species gradually eliminate the forest floor that is vital to the survival of epigeic species. Future earthworm surveys in Parc du Mont-Tremblant could test this hypothesis by verifying whether the Introduction of *L. terrestris* by anglers is gradually eliminating *D. octaedra* populations.

Our results provide some clues concerning the nature of interactions between earthworms and soil microbes, although with only observational data, interpretations are necessarily speculative. Our RDA biplot indicates a positive correlation between the abundance of *D. octaedra* and microbial biomass in both the forest floor and mineral soil. While some authors have proposed that epigeic species such as *D. octaedra* might actually reduce microbial biomass in forest floors (McLean et al. 2006; Eisenhauer et al. 2011), the evidence for such a generalization is lacking. For example, several papers (e.g. Brown 1995; Bohlen et al. 2004b; Groffman et al. 2015) have pointed out the complex interactions between earthworms of different feeding habits, organic matter quality, and soil properties that result in either an increase or decrease in the biomass of different microbial groups. The positive correlation between *D. octaedra* and total microbial biomass that we observed could be due to the litter mixing and comminuting activities of this species, resulting in a higher soil carrying capacity for microbial biomass (Groffman et al. 2015). It is curious that *D. octaedra* correlated positively with microbial biomass in the mineral soil, rather than anecic or endogeic species as others have found (e.g. Tiunov and Scheu 1999). Rather, our results are consistent with those of Groffman et al. (2015) who found a significantly greater increase of microbial biomass in the mineral soil following the introduction of an epigeic species (*L. rubellus*) compared to an anecic species (*L. terrestris*). The fact that microbial basal respiration

correlated strongly with the second RDA principal component axis suggests that microbial biomass and microbial respiration vary independently from each other. We minimize the importance of the positive correlations between microbial respiration and either *L. rubellus* (epi-endogeic) in the forest floor or *Aporrectodea sp.* (endogeic) in the mineral soil, given that RDA axis 2 explained only 5% of the total variance in the data set.

We found some intriguing evidence linking earthworms to denitrification, although the exact nature of how earthworms interact with N cycling is potentially quite complex. The global impacts of exotic earthworms on forest ecosystems, notably on soil N cycling, has been reviewed by several authors (e.g., Bohlen et al. 2004b; Frelich et al. 2006). Our RDA output suggests that *L. terrestris* favors the production of soil  $\text{NO}_3^-$ , a mobile anion that is prone to be leached to groundwater with excess rainfall. Accordingly, Ewing et al. (2015) found that Northern hardwood forest plots harbouring exotic earthworms had a lower retention of applied  $^{15}\text{N}$ -labelled  $\text{NO}_3^-$  than control plots without earthworms. Whether exotic earthworms increase soil  $\text{NO}_3^-$  leaching remains controversial, however, as Groffman et al. (2015) recently provided data suggesting that earthworms increase soil available C thereby enhancing microbial N immobilization. Yet the production of  $\text{NO}_3^-$  is necessary for denitrification to occur, and this process is thought to control  $\text{N}_2\text{O}$  emissions in anaerobic soil microsites (Wrage et al. 2001). The mechanism by which earthworms may increase denitrification remains, however, unclear. Some authors report that earthworm biological activity (e.g. feeding, excretion and mucus production) and earthworm structures (e.g. burrows and casts) directly stimulate the chemical reduction of soil  $\text{NO}_3^-$  (Horn et al. 2003; Drake and Horn 2006; Costello and Lamberti 2009; Majeed et al. 2013). Conversely, Bertora et al. (2007) argued that earthworms stimulate  $\text{N}_2\text{O}$  production primarily through indirect effects on soil structure. In our study, we could not establish a significant direct correlation ( $P = 0.064$ ) between the abundance of *L. terrestris* and potential denitrification. Faced with a similar situation, Sackett et al. (2012) used exploratory path analysis to reveal causal pathways among measured variables. Accordingly, we found a significant pathway in which *L. terrestris* increases potential  $\text{N}_2\text{O}$  production via its negative effect on soil C:N ratio.



This reflects a positive effect of *L. terrestris* on soil organic matter decomposition, which ultimately results in mineral N release and turnover. Subsequently, the path diagram corroborates the potential production of N<sub>2</sub>O via both the nitrification and denitrification pathways.

## Conclusions

The global environmental impacts of exotic earthworms in North America has prompted some researchers to suggest comprehensive conservation policies that would limit the spread of these alien species (e.g. Hendrix and Bohlen 2002; Callahan et al. 2006). In terms of influencing conservation decisions, studies showing negative impacts of earthworms on forest plant biodiversity (e.g. Hale et al. 2006; Drouin et al. 2016) probably carry more weight than do data on less tangible and more diffuse impacts of earthworms on biogeochemical cycles. Yet, the chronic effects of earthworms on the cycling of reactive N through the biosphere and geosphere may be more pernicious and irremediable than other impacts. The problem is that altered biogeochemical cycles are less liable to detection than is the loss of conspicuous plant species. The two salient points to emerge from our study are (1) recreational fishing is responsible for the introduction of *L. terrestris* in forests ecosystems of Mont-Tremblant National Park, and (2) this exotic earthworm species can lead to faster N cycling with potential increases in soil N<sub>2</sub>O emissions. Future research should strive, therefore, to quantify these fluxes under *in situ* conditions, and scale them up to the landscape scale.

As we have shown, there remains a large forested area in southern Quebec that is earthworm-free and deserving protection through comprehensive policies limiting the spread of exotic earthworms. The *Société des Établissements de Plein Air du Québec* (SÉPAQ) is the governing body whose mandate is to preserve the natural heritage of our forests through the management of 24 national parks occupying an area greater than 37,400 km<sup>2</sup>. In their mission statement, SÉPAQ states that “a national park’s primary purpose is to ensure the conservation and permanent protection of areas representative of the natural regions of Québec and of natural sites with outstanding features, in particular because of their biological diversity,

while providing the public with access to those areas.” Accordingly, SÉPAQ has established a series of conservation measures, such as forbidding domestic animals such as dogs and cats from entering national parks. Paradoxically, SÉPAQ sells live exotic earthworms to anglers at their Visitor Centers, thus contributing substantially to their spread into new habitats. Further work should include plans to inform and educate the public on the issue of exotic earthworms and to assist resource managers in developing future policy that would end their dispersal into protected forest areas.

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