Earthworm interactions with denitrifying bacteria are scale-dependent: Evidence from physiological to riparian ecosystem scales

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Abstract

Earthworms are implicated in denitrification, the microbially mediated reaction that results in gaseous nitrogen (N₂O and N₂) loss from terrestrial ecosystems. At the physiological scale, the anaerobic earthworm gut is a favorable microsite for endemic and transient denitrifiers that produce up to 11 nmol N₂O g⁻¹ earthworm h⁻¹. Besides this direct earthworm–denitrifier interaction, the earthworm’s ability to consume, fragment and mix organic residues with soil will accelerate N mineralization and create suitable conditions for opportunistic soil denitrifiers. At the drilosphere scale, earthworm biostructures (burrows, casts) and earthworm-worked soil create gradients of redox conditions and are enriched in inorganic N and soluble C substrates used by denitrifiers. Therefore, earthworms indirectly stimulate soil N₂O emissions by soil denitrifiers. Although these small-scale effects may imply that ecosystems with large earthworm populations are more likely to lose N through denitrification, there is scant experimental data to confirm this supposition. Evidence from simulated streams and agroecosystems suggests that earthworms can stimulate N₂O emissions at the ecosystem-scale, but environmental factors (temperature and moisture) may overwhelm earthworm-induced denitrification. A critical review of earthworm–denitrifier interactions in riparian buffers, a hotspot for both groups of organisms, indicated that hydrodynamics controls denitrification during flooding periods by profoundly changing soil moisture and substrate concentrations that favor denitrifier activity. Earthworm effects on denitrifiers may be detected during drier periods. Thus, earthworm–denitrifier interactions cannot be extrapolated from the physiological- and drilosphere-level to explain denitrification in riparian ecotones due to seasonal variation in hydrological processes occurring at this scale.

Zusammenfassung

Regenwürmer sind an der Denitrifikation beteiligt, der mikrobiell vermittelten Reaktion, die die Freisetzung von gasförmigem Stickstoff (N₂O und N₂) aus terrestrischen Ökosystemen bewirkt. Auf der physiologischen Ebene ist der anaerobe Regenwurmdarm ein günstiges Habitat für endemicische und vorübergehende Denitrifikanten, die bis zu 11 nmol N₂O g⁻¹ Regenwurm h⁻¹ produzieren. Neben dieser direkten Regenwurm-Denitrifikanten-Interaktion, beschleunigt die Fähigkeit des Regenwurms, organismen Detritus zu konsumieren, zu zerkleinern und mit Erde zu mischen, die Stickstoffmineralisation und schafft günstige Bedingungen für opportunistische, bodenbewohnende Denitrifikanten. In der Drilosphäre erzeugen vom Regenwurm geschaffene Strukturen (Gänge, Ausscheidungen) und vom Regenwurm verarbeiteter Boden Gradienten von Redox-Bedingungen, und sie sind angereichert mit anorganischem N und löslichen C-Substraten, die von Denitrifikanten genutzt

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Denitrification, denitrifiers, and earthworms

Denitrification is the most important biological source of gaseous nitrogen (N) emission from soil, accounting for global N losses of about 7.1 × 1012 mol y⁻¹ (Canfield et al., 2010). The term ‘denitrifier’ refers to any organism that catalyzes at least one step in this complex, multi-step reaction, including species from more than 60 genera of Bacteria as well as Archaea and Eukaryotes (Zumft and Körner 2007; Demanèche et al., 2009). Nitrous oxide (N₂O), an intermediate byproduct of denitrification, is a potent greenhouse gas and the most important ozone-depleting substance in the atmosphere (IPCC 2007; Ravishankara et al., 2009). Soils are the dominant source of N₂O emissions and produce 6–7 Tg N₂O-N y⁻¹ (Syakila and Kroeze 2011).

Earthworms could contribute to soil N₂O emissions, according to meta-analysis that reported greater net N₂O emission from soil with than without earthworms (Lubbers, López González, Hummelink, & Van Groenigen, 2013). This meta-analysis was based on 13 studies about earthworm-induced N₂O emissions, mostly performed under laboratory conditions. These small-scale studies provide strong evidence of a stimulatory effect of earthworms on denitrifying bacteria in the earthworm gut and in earthworm drilosphere (Drake and Horn 2006; Paul et al., 2012), but it is not known if such earthworm–denitrifier interactions can be extrapolated to the ecosystem-level, given the limited experimental data from field-scale studies. In this review, we examined earthworm–denitrifier interactions at the physiological scale (within individual earthworms), drilosphere scale (earthworm-worked soils) and ecosystem scale, with the goal of determining whether or not earthworm–denitrifier interactions could be expected to increase soil N₂O emissions in riparian buffers, a natural biofilter that reduces runoff and attenuates excess nutrients. Riparian buffers are a special ecotone that support larger earthworm populations and greater denitrification than the adjacent terrestrial or riverine ecosystems, although a dynamic relationship exists between earthworms and denitrifiers because of fluctuating water levels in the riparian buffer.

Earthworm–denitrifier interactions at the physiological scale: direct effects

At the physiological scale, earthworm–denitrifier interactions are regarded as direct effects because earthworm gut provides a “heaven” for transient denitrifiers (Horn, Schramm, & Drake, 2003; Drake & Horn, 2006). Populations of transient microorganisms increase when earthworms mix organic residues and soil in their middens prior to consuming them. Upon ingestion of this pre-digested material, facultative denitrifiers are activated within the anoxic earthworm gut which possesses neutral pH, high mineral N and labile C concentrations (“sleeping beauty paradox”) (Brown, Barois, & Lavelle, 2000; Horn et al., 2003). Although the “sleeping beauty paradox” is not perfect, as it is hard to explain how transient microorganisms could significantly improve earthworm N nutrition during their short gut transit time (<24 h), it does illustrate a mutualistic relationship between earthworms and ingested microorganisms (Brown et al., 2000). Denitrifying bacteria are up to 300-fold more numerous in the earthworm gut than in the bulk soil (Karsten and Drake 1995; Ihssen et al., 2003) and biologically active. The earthworm gut can emit 0–11 nmol N₂O g⁻¹ earthworm h⁻¹ with the N₂O accounting for around 50% of the total N₂O + N₂ due to incomplete denitrification (Karsten and Drake 1997; Matthies et al., 1999; Horn et al., 2006). In situ microsensor reveals most N₂O production occurred in the foregut (2.7 µM N₂O) and midgut (5.6 µM N₂O) and declines in the hindgut (0.2 µM) (Horn et al., 2003). The N₂O-emitting bacteria in the earthworm gut are widely found across earthworm species and aquatic macrofauna, suggesting the
Table 1. Earthworm digestion processes that transform organic substrates to assimilable N compounds within the earthworm body (N gains), prior to their turnover from earthworm tissues or transit through the earthworm body (N losses).

<table>
<thead>
<tr>
<th>Process</th>
<th>Explanation</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N gains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consuming substrates in various stages of decay</td>
<td>Plant litter, roots, animal dung, decomposing soil organic matter, amorphous humus, sand particles, clay minerals, etc. Soil: 1000–3500 mg soil g⁻¹ earthworm fresh weight d⁻¹ by endogeics Organic substrates: 1–80 mg organic substrate g⁻¹ earthworm d⁻¹, depending on earthworm species and food quality</td>
<td>Obtain energy and mineral nutrition from organic substrates, depending on substrate availability in the ecological niche they inhabit</td>
<td>Table 2 (Curry &amp; Schmidt 2007)</td>
</tr>
<tr>
<td>Selecting substrates</td>
<td>Preference for organic substrates with high N content and particle size between &lt;1.0 mm</td>
<td>Acquire assimilable N compounds</td>
<td>Curry &amp; Schmidt (2007)</td>
</tr>
<tr>
<td>Varying gut transit time</td>
<td>2–24 h</td>
<td>Modulate the digestion process</td>
<td>Brown et al. (2000)</td>
</tr>
<tr>
<td>Producing extra- and intra-cellular enzymes</td>
<td>Highest enzyme activity in foregut and midgut Epigeic <em>Eisenia fetida:</em> 25 mg tyrosine g⁻¹ earthworm h⁻¹ Anecic <em>Metaphire guillelmi:</em> &gt;135 mg tyrosine g⁻¹ earthworm h⁻¹ Enzymes come from earthworm cells; indigenous microflora (bacteria, archaea, fungi, etc.); ingested clays and soil; and transient microorganisms.</td>
<td>Produce protease and hydrolyse the peptide bonds in proteins, as well as other hydrolitic and degradative enzymes, e.g., chitinase, cellulases, phosphatase</td>
<td>Lattaud, Locati, Mora, Rouland, and Lavelle (1998); Zhang, Li, Shen, Wang, and Sun (2000).</td>
</tr>
<tr>
<td>Creating internal mucus</td>
<td>Secretion rate depends on earthworm ecological groups: endogeics &gt; anecics &gt; epigeics N-rich mucopolysaccharide Large mucus production: 50–800 mg mucus g⁻¹ gut content (dry weight basis)</td>
<td>Lubricate soil–litter mixtures prior to ingesting them; keep the gut lubricated to facilitate food passage; provide a moist anoxic microhabitat with readily assimilable C and N substrates for gut microorganisms; and recycling mucus conserves N in earthworm tissues</td>
<td>Barois (1992); Trigo and Lavelle (1993); Binet, Fayolle, and Pussard (1998); Trigo et al. (1999); Brown et al. (2000).</td>
</tr>
<tr>
<td>Assimilating N</td>
<td>High N assimilation efficiency of 10–30% Assimilation efficiency depends on food quality and probably depends on earthworm ecological group Rapid N replacement that &gt;30% N of earthworm body being renewed in a month</td>
<td>Ensure adequate N for basal metabolism, growth and reproduction</td>
<td>Barois et al. (1987); Binet and Trehen (1992); Bouché and Al-Addan (1997); Whalen and Parmelee (1999).</td>
</tr>
<tr>
<td><strong>N losses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secreting external mucus</td>
<td>20–269 μg N g⁻¹ earthworm d⁻¹ (together with urine) Accounts for 0.3–1.7% loss of earthworm tissue N per day Independent of food quality</td>
<td>Facilitates physiological functions, e.g., defecation, reproduction, movement</td>
<td>Table 3 (Brown et al., 2000; Whalen et al., 2000)</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Process</th>
<th>Explanation</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excreting urine</td>
<td>NH₄⁺ and urea</td>
<td>Excreting nitrogenous byproducts of earthworm metabolism</td>
<td>Curry &amp; Schmidt (2007)</td>
</tr>
<tr>
<td>Defecating casts</td>
<td>A mixture of partially digested, fibrous organic matter and mineral soil particles</td>
<td>Clears intestinal tract of undigestable substances</td>
<td></td>
</tr>
<tr>
<td>Reproduction</td>
<td>A minor N loss in the form of genetic material contained within earthworm cocoons. Cocoons contributes to less than 1% annual biomass</td>
<td>An essential function of creating offspring and allows for persistence of the species</td>
<td>Whalen, Parmelee, and Edwards (1998)</td>
</tr>
<tr>
<td>Mortality</td>
<td>Organic N in earthworm tissues is rapidly decomposed and cycles through microbial biomass N to produce mineral N 55% organic N in earthworm tissues transfers to microbial biomass after 2 d</td>
<td>Transfers N from dead earthworms to the soil environment</td>
<td></td>
</tr>
</tbody>
</table>

The earthworm–denitrifier relationship has co-evolved (Steif et al., 2009; Wüst et al., 2009).

Earthworm feeding habits indirectly affect denitrification and N₂O emissions, due to the fact that earthworms consume substantial amounts of organic substrates on a daily basis to obtain energy and meet their nutritional requirements for N and other minerals (Table 1). These N gains are obtained by selective consumption of organic substrates, varying gut transit time, producing extra- and intra-cellular enzymes and using internal mucus to facilitate the release of amino acids and NH₄⁺ that will be assimilated into earthworm tissues (Tables 1 and 2). Some of the processes whereby earthworms lose N from their bodies such as secreting external mucus, excreting urine, defecating casts and mortality will result in organic N transformation into mineral N forms (Tables 1 and 3). Temperate ecosystems with earthworm fresh biomass of 1–3 t ha⁻¹ can contribute to the direct N mineralization of up to 96 kg mineral N ha⁻¹ y⁻¹, of which 35–76% is attributed to earthworm biomass turnover and the rest from N secretion/excretion (Lee 1983; Christensen 1988; Parmelee and Crossley, 1988; de Ruiter et al., 1993; Marinissen and de Ruiter 1993; Curry et al., 1995; Whalen and Parmelee 2000; Eriksen-Hamel and Whalen 2009). The transfer of mineral N from earthworms to soil, a consequence of their daily excretion of N-rich mucus and urine, defecation of partially decomposed organic substrates in their casts and the rapid decomposition of dead earthworm tissues, increases the soil mineral N concentration and provides a source of nitrate (NO₃⁻) to soil denitrifiers at the drilosphere and ecosystem scales. The conceptual relationships between

Table 2. Nitrogen content of organic substrates and their consumption rate by earthworms.

<table>
<thead>
<tr>
<th>Earthworm species</th>
<th>Life stage</th>
<th>Organic substrate</th>
<th>C:N ratio</th>
<th>Consumption rate a</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbricus terrestris</td>
<td>Not described</td>
<td>Elm leaves</td>
<td>25</td>
<td>80</td>
<td>Needham (1957)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Adult</td>
<td>Ryegrass</td>
<td>44.8</td>
<td>13</td>
<td>Binet and Trehen (1992)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Adult</td>
<td>Alfalfa leaves</td>
<td>13.7</td>
<td>13</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Adult</td>
<td>Red clover leaves</td>
<td>10.9</td>
<td>12</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Adult</td>
<td>Corn leaves</td>
<td>21.1</td>
<td>6</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Adult</td>
<td>Bromegrass leaves</td>
<td>26.2</td>
<td>2</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. rubellus</td>
<td>Adult</td>
<td>Alfalfa leaves</td>
<td>13.7</td>
<td>52</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. rubellus</td>
<td>Adult</td>
<td>Red clover leaves</td>
<td>10.9</td>
<td>36</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. rubellus</td>
<td>Adult</td>
<td>Corn leaves</td>
<td>21.1</td>
<td>18</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>L. rubellus</td>
<td>Adult</td>
<td>Bromegrass leaves</td>
<td>26.2</td>
<td>5</td>
<td>Shipitalo et al. (1988)</td>
</tr>
<tr>
<td>Aporrectodea tuberculata</td>
<td>Juvenile</td>
<td>Ryegrass leaves</td>
<td>32.2</td>
<td>8.5</td>
<td>Whalen and Parmelee (1999)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Juvenile</td>
<td>Ryegrass leaves</td>
<td>32.2</td>
<td>1.4</td>
<td>Whalen and Parmelee (1999)</td>
</tr>
<tr>
<td>A. tuberculata</td>
<td>Juvenile</td>
<td>Soybean leaves</td>
<td>27.8</td>
<td>9.8</td>
<td>Whalen and Parmelee (1999)</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Juvenile</td>
<td>Soybean leaves</td>
<td>27.8</td>
<td>2.6</td>
<td>Whalen and Parmelee (1999)</td>
</tr>
</tbody>
</table>

a mg organic substrate g⁻¹ earthworm fresh weight d⁻¹.
Table 3. Nitrogen losses from earthworms through urine excretion and external mucus secretion.

<table>
<thead>
<tr>
<th>Earthworm</th>
<th>Life stage</th>
<th>Organic substrate</th>
<th>C:N ratio</th>
<th>Daily N loss$^a$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. terrestris</em></td>
<td>Not described</td>
<td>Elm leaves</td>
<td>25</td>
<td>269</td>
<td>Needham (1957)</td>
</tr>
<tr>
<td><em>Allolobophora caliginosa</em></td>
<td>Adult</td>
<td>Elm leaves</td>
<td>25</td>
<td>88</td>
<td>Needham (1957)</td>
</tr>
<tr>
<td><em>L. terrestris</em></td>
<td>Juvenile</td>
<td>Soybean leaves</td>
<td>12</td>
<td>177</td>
<td>Whalen et al. (2000)</td>
</tr>
<tr>
<td><em>L. terrestris</em></td>
<td>Adult</td>
<td>Soybean leaves</td>
<td>12</td>
<td>533</td>
<td>Whalen et al. (2000)</td>
</tr>
<tr>
<td><em>A. tuberculata</em></td>
<td>Adult</td>
<td>Soybean leaves</td>
<td>12</td>
<td>620</td>
<td>Whalen et al. (2000)</td>
</tr>
<tr>
<td><em>L. rubellus</em></td>
<td>Adult</td>
<td>Soybean leaves</td>
<td>12</td>
<td>578</td>
<td>Whalen et al. (2000)</td>
</tr>
<tr>
<td><em>L. terrestris</em></td>
<td>Not described</td>
<td>Not described</td>
<td>–</td>
<td>60–160</td>
<td>Tillinghast (1967)</td>
</tr>
</tbody>
</table>

$^a \mu g N g^{-1}$ earthworm fresh weight d$^{-1}$.

Still, we do not know how the selective consumption of organic substrates influences earthworm–denitrifier interactions. Using $^{15}$N stable isotopes to trace the N flows from organic substrates through the earthworm and into the soil environment would permit us to address questions like (1) does the choice of organic substrates ingested by the earthworm influence denitrifier activity and denitrification in the gut; and (2) whether ecosystems with N-rich organic substrates and large earthworm populations would produce higher soil N$_2$O emissions than would be predicted from the activity of soil denitrifiers alone.

Earthworm–denitrifier interactions in the drilosphere: indirect effects

The drilosphere is the zone where earthworms interact with the soil environment through bodily contact and their biostructures – casts, middens and burrows (Edwards, 2004). Bodily contact is a transient phenomenon (within 1–2 d), occurring when earthworms secrete mucus from the body surface to facilitate their passage through the soil or when they die (Whalen et al., 1999; Whalen, Parmelee, & Subler, 2000). In contrast, biostructures can persist for months or years, remaining as biologically active ‘hotspots’ for microbial activities including denitrification beyond the lifespan of the earthworm that created them. Earthworm biostructures

![Fig. 1. Conceptual model of earthworm-mediated N transformations in riparian buffers. The N balance within an earthworm body (physiological scale) is illustrated within the dashed circle. Substrates released from the earthworm body can contribute to N mineralization, ammonia oxidation and nitrification reactions in aerobic soils. In contrast, the earthworm gut is permanently anaerobic and a hotspot for denitrification. The surface of casts, middens, and burrow linings support aerobic processes in the N cycle, while the core of casts, middens and the subsurface of the burrow lining are microsites for anaerobic processes (drilosphere scale). In riparian buffers, aerobic conditions prevail during dry seasons and would stimulate aerobic N transformations, while flooding events would promote denitrification. The earthworm model was *L. terrestris.*](image-url)
create micsites with anaerobic conditions and higher labile C and NO₃⁻ concentrations than bulk soil, resulting in up to 3 times greater denitrifier activity within fresh casts and burrow linings (Six et al., 2004; Bottinelli et al., 2010). Cast production, which can reach in the hundreds of t ha⁻¹ each year in the primary topsoil layer (10–30 cm depth), contributed to 29% of the N loss through denitrification in a permanent pasture (Lavelle 1988; Elliott et al., 1991; Knight et al., 1992). The connection between earthworm biostructures and denitrification under aerobic and anaerobic conditions is illustrated in Fig. 1. A meta-analysis concluded that 42% greater N₂O emissions come from earthworm-worked soil, on average, than from soil without earthworms (Lubbers et al., 2013). More data is needed to rank earthworm functional groups (e.g., anecics, endogeics and epigeics) for their ability to stimulate or inhibit denitrification, since each group has a characteristic life history, feeding habit, soil-moving ability, and biostructure properties (Rizhia et al., 2007; Speratti and Whalen 2008; Giannopoulos et al., 2010; Majeed, Miambi, Barois, Blanchart, & Brauman, 2013). The mechanisms whereby earthworms impact net N₂O emissions from soil are not known, but could include earthworm actions that:

1. stimulate the activity of denitrifiers producing N₂O or inhibit the denitrifiers that consume N₂O (i.e., completely reduce N₂O to N₂) (Nebert et al., 2011; Depkat-Jakob et al., 2013),

2. change the level of substrates (labile C, NO₃⁻) available to denitrifiers (Nebert et al., 2011), and

3. alter soil aggregation and porosity, thus affecting gas diffusion, soil moisture and redox conditions for denitrifiers (Giannopoulos et al., 2010; Paul et al., 2012).

Denitrification and N₂O emissions are a function of soil redox potential and earthworm-created biostructures are expected to change micro-oxic conditions. Casts and middens (macroagggregates) produce a gradient of oxygen (O₂) availability from oxic surface to anoxic core (Cécillon, Cassagne, Czarnes, Gros, & Brun, 2008; Jouquet et al., 2011). As well, burrows (macropores) can significantly affect air, water and solute movement through the soil profile according to their size and connectivity (Fig. 1). Gas diffusion coefficients of anecic and endogeic earthworm burrow systems are similar (0.66 × 10⁻⁵ to 1.00 × 10⁻⁵ m² s⁻¹), but anecic burrows provide vertical continuity of gas-transmitting pores from subsoil to surface soil (Capowiez, Monestiez, & Belzunces, 2001). However, it is hard to distinguish porosity due to earthworm burrowing from root channels, soil cracks and other macropores. Still, it is possible that earthworm biostructures would support more denitrifiers than other macroaggregates/macropores because they contain labile C and mineral N derived from the earthworm body (e.g., mucus) and from partially decomposed, fragmented organic substrates present in casts and along burrow linings. Another possibility is that earthworm burrows can offer a pathway for N₂O diffusion, particularly vertical burrows by anecic earthworms that have an opening at the soil surface. This would result in N₂O emissions to the atmosphere, rather than the complete reduction of N₂O to N₂ before gaseous N is released from soil. Comparison of N₂O emissions from earthworm biostructures and other soil structures needs to be done in oxic to anoxic soil conditions, as there is no report on this topic to date.

Earthworm–denitrifier interactions in riparian ecosystems: the case of riparian buffers

At the ecosystem scale, riparian ecotones in temperate humid regions are a hotspot for denitrification, producing from 0.4 to 8.2 mg N₂O-N m⁻² d⁻¹ depending on spatial-temporal factors (Hefting, Bobbink, & de Caluwe, 2003; Dhondt, Boeckx, Hofman, & Van Cleemput, 2004; van den Heuvel, Hefting, Tan, Jetten, & Verhoeven, 2009). Spatially, denitrification in riparian buffer is controlled by the intensity of flooding, topography and soil hydraulic conductivity, as well as the supplies of labile C and NO₃⁻ coming from riparian vegetation, runoff and sediment from the adjoining land and upstream sources (Groffman, Gold, & Simmons, 1992; Clément, Pinay, & Marmonier, 2002). Temporal variation in denitrification is due to seasonal flooding events, vegetation growing seasons, and NO₃⁻ leaching (Clément et al., 2002).

Riparian buffers are legislated or proposed as a best management practice in many jurisdictions to intercept runoff and reduce the sediment transport into waterways from agricultural fields, forestry operations, roadsides, commercial and private properties. When implemented at the boundary of agricultural land, riparian buffers are also effective in capturing excess NO₃⁻ and phosphate before these nutrients enter waterways. Riparian buffers are expected to be a favorable habitat for earthworms because they are permanently vegetated with perennial herbaceous plants, shrubs and trees, so the soils are relatively undisturbed, although they are subject to periodic inundation due to flooding of streams and rivers. In agricultural riparian buffers, the spatio-temporal variation of the earthworm population is responsive to water levels, as lower numbers are recorded following extended periods of flooding (Zorn, Van Gestel, & Eijsackers, 2005) and drought season (Parmelee et al., 1990; Whalen & Costa 2003). While flooding events reduce the populations of Lumbricus terrestris and Lumburic rubellus, periods of inundation do not affect Aporrectodea caliginosa or Allolobophora chlorotica (Zorn et al., 2005). Moisture-loving earthworms are more abundant in agricultural riparian buffers than surrounding agroecosystems, i.e., A. chlorotica, L. terrestris, Eiseniella tetraedra, L. rubellus (Reynolds & Reynolds 1992; Bradley, Whalen, Chagnon, Lanoix, & Alves, 2011). These changes in earthworm abundance and community structure could affect earthworm–denitrifier interactions within riparian buffers.

Should we expect earthworm–denitrifier interactions to produce significant N₂O emissions in riparian buffers? Earthworm-worked soil from agricultural riparian buffers
had up to 4 times greater denitrifier activity and basal
denitrification rate than the no-earthworm control in a lab-
орatory incubation study (Bradley et al., 2011). Costello
and Lamberoti (2008) established an artificial stream to sim-
ulate earthworm functions in riparian buffers and found
that earthworm presence increased NH4+ leaching, NO3−
leaching and gave 4 times more denitrification than the
no-earthworm control. This lab simulation suggested that
earthworms would increase the N flux in riparian ecosystems,
but hydrodynamics would control the proportion of NO3−
lost through denitrification or by NO3− leaching. Neverthe-
less, these short-term, controlled studies cannot represent
earthworm–denitrifier interactions in riparian buffers because
(1) earthworm population dynamics and the spatial distribu-
tion of earthworm assemblages control the physiological- and
drilosphere-scale interactions with denitrifiers throughout
the growing season, (2) the NO3− generated by earthworm activi-
ties may go to plant N uptake or NO3− leaching (Lubbers, Brussaard, Otten, & Van Groenigen, 2011), which reduces
the NO3− concentration available to denitrifiers, and (3)
earthworm–denitrifier interactions depend on soil moisture
conditions, such that earthworms increase N2O emission in
oxic soils, but reduce N2O emissions in anoxic soils and under
fluctuating oxic–anoxic moisture conditions (Chen, Whalen,
& Guo, 2014).

Seasonal variation in earthworm-induced N2O emissions
at the field scale was documented in two studies. Borken,
Gründel, and Beese (2000) tested gas fluxes from repacked
forest soil columns and found that the anecic earthworm
L. terrestris increased N2O fluxes of 68% from late spring
to early fall. Lubbers et al. (2013) measured gas fluxes in
intact columns from a managed grassland soil and found
that epigeic L. rubellus addition treatments increased N2O
fluxes from 286 to 394% in the fall season compared to
no-earthworm added treatment, but there was no earthworm
influences on N2O fluxes in the spring season. These stud-
ies suggest earthworm–denitrifier interactions could lead to
a measurable increase in N2O emissions under field condi-
tions, but environmental factors (i.e., the fluctuation in
weather and moisture) can overrule earthworm-induced
N2O emissions. Another possibility is that high denitrification rate attributed by earthworm can be captured by roots and
microorganisms, resulting in less N2O emits. In this case, the
earthworm–denitrifier interactions that can be detected at the
physiological- and drilosphere scales cannot be recognized
at the riparian scales. Lacking data from riparian buffers, we hypothesize earthworm effects on N2O emissions will be
limited because:

(1) In the spring season, plant N-uptake and leaching will
reduce the NO3− concentration, which is the key, direct
substrate for denitrifiers responsible for denitrification
(Zumft, 1997). Moreover, flooding events will reduce
earthworm abundance and simplify the community struc-
ture. Thus, denitrifier activity will be controlled more by
hydrodynamics than earthworms.

(2) In the summer season, flooding events are infrequent
and environmental conditions favor earthworm-mediated
decomposition and N cycling, which contributes directly
to earthworm–denitrifier interactions. However, drier soil
conditions in summer months reduce earthworm activi-
ties (i.e., foraging, burrowing, casting) as well as soil
N2O emissions. Earthworms make a small contribution
to denitrification during this period. The contribution of
earthworms to denitrification is minimal.

(3) In the fall season, the senescence of vegetation releases
organic substrates and evapotranspiration ceases, cre-
ating anoxic conditions in riparian buffers. Earthworm
abundance increases and earthworms resume their role in
decomposition and N cycling activities, but have less
impact on N2O emissions than the plant community,
which is no longer competing for NO3−. Denitrification is
attributed to plant–denitrifier interactions. Although
leaching can lower the NO3− concentration, denitrifiers
have access to NO3− and labile C when fall flooding
events occur, implying a role for hydrodynamics in deni-
trification during this period.

Conclusions

We acknowledge the value of carefully con-
trolled laboratory and field mesocosm studies to evaluate
earthworm–denitrifier interactions. The
earthworm–denitrifier interaction can be detected in
the laboratories. Nevertheless, at the riparian scale, the
interaction is modulated by environmental factors that may
conceal the earthworm–denitrifier relationship in some
cases. Thus, extrapolation from small scales to field scale
must be done cautiously and more research is required at
different scales to fully evaluate the scale-dependency of
earthworm–denitrifier interactions. We suggest the following
topics for future research:

(1) At the physiological scale, 15N tracer studies to evalu-
ate the N mass balance within the earthworm, including
N losses through denitrification, considering how food
quality might affect N2O and N2 production by in vivo
denitrifiers.

(2) At the drilosphere scale, the activity of denitrifiers
associated with earthworm biostructures, the com-
parison of earthworm biostructures and other soil
macroaggregates/macro pores; N2O sources from nitrifica-
tion, mesocosm studies with plants, particularly riparian
vegetation; and

(3) More systematic field-scale investigations of
earthworm–denitrifier interactions in the ecosystem
scale, particularly in riparian buffers. As outlined in the
hypotheses, the seasonal variation in biotic and abiotic
controls on denitrification must be considered. Stable isotope
15N tracing through the earthworm-based food web
could be helpful, as would studies that compare riparian
buffers having large, naturally occurring earthworm populations with those possessing few or no earthworms.

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