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Emissions of nitrous oxide from casts of tropical earthworms belonging to different ecological categories

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While earthworms' feeding behavior has been shown to be a key driver of greenhouse gas like nitrous oxide (N_2O) emissions for temperate species, this assessment remains to be demonstrated for tropical ones. This study constitutes a comparative assessment of the impact of different feeding strategies of tropical earthworms on their cast emissions of N_2O and the characterization of microbial communities associated with these emission dynamics. N_2O flux was determined *in vitro* from casts of 2 epigeic (*Dichogaster annae* and *Eisenia andrei*) and 4 endogeic/epiendogeic (*Metaphire houlleti*, *Octolasion tyrtaeum*, *Pontoscolex corethrurus* and *Amynthas corticis*) earthworm species in the presence or absence of acetylene. Casts of epigeic earthworms, fed on fresh coffee pulp, emitted about 392-fold higher amounts of N_2O than those of endogeic ones, fed on a tropical soil. Acetylene exposure significantly increased the cast emission rates indicating that (i) apart from N_2O, dinitrogen (N_2) is also emitted by earthworm casts, particularly by the epigeic guild (45%) and (ii) denitrification is the major microbial process responsible for these N-gas emissions from casts. Total bacteria were 10-fold higher in epigeic casts than endogeic but the relative abundance of nitrifier (AOB) and denitrifiers (nirK and nirS) was many times higher in endogeic casts than epigeic ones. The relative gene density of nosZ to (nirK + nirS) was 4-fold higher in epigeic casts than endogeic ones. Moreover, bacterial amoA gene abundance was higher in casts of all species than for their archaeal counterparts. Cast N_2O emission was positively correlated with nitrifier as well as denitrifier gene abundance, while it was positively correlated with epigeic nitrate and negatively with endogeic nitrate contents. Ammonium contents did not show any relationship with cast emissions. Mean ammonium and nitrate contents were many times higher in casts than food substrates except nitrate in endogeic casts which was 4-fold less than parent food substrate.

Introduction

Nitrous oxide (N_2O) is a major greenhouse gas in Earth's atmosphere, exerting a significant pressure on the phenomena of global warming and ozone destruction (IPCC 2007; Ravishankara et al. 2009). More than two-thirds of total N_2O emissions originate from soils (IPCC 2007; EPA 2010) where it is produced by various microbe-mediated interdependent processes such as nitrification, denitrification, nitrate ammonification and/or nitrifier denitrification (Baggs 2011; Rutting et al. 2011). Tropical and subtropical soils are principal emission sources of atmospheric N_2O (Denman et al. 2007; Werner et al. 2007) with highest emission rates reported from tropical rainforests (Breuer et al. 2000; EPA 2010). However, these tropical soils harbor as well a large diversity of macrofauna particularly of earthworms (Fragoso and Lavelle 1992; Edwards and Bohlen 1996; Lavelle et al. 1997) which considerably modulate these N_2O-genic microbial processes either by their diversified feeding and foraging activities or by creating different biogenic structures in a given soil ecosystem (Lavelle et al. 1997; Jouquet et al. 2006; Brümmer et al. 2009).

Based on their feeding ecology, earthworms are generally grouped into three ecological categories *i.e.* (i) epigeic worms living and feeding on surface litter layers, (ii) endogeic worms living in top soil layers and thriving on bulk soil containing soil organic matter, fine roots, fungal hyphae, *etc.*, and (iii) anecic worms that are deep soil dwellers but feeding on decaying surface litter dragged by them into their burrows (Lavelle 1981). Generally anecic worms are dominant in temperate ecosystems while tropical soils, particularly tropical rainforests are predominately populated by litter-feeding

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epigeic and soil-feeding endogeic earthworm categories (Fragoso and Lavelle 1992; Edwards and Bohlen 1996; Hauser et al. 2012).

As much as 60% of N_2O emissions from certain temperate soils are claimed to be derived from earthworm activities (Drake and Horn 2007). Indeed earthworm engineering often brings beneficial, constructive modifications in soil profiles (Edwards and Bohlen 1996; Lavelle et al. 1997; Oyedele et al. 2006), often accompanied by a worm-induced priming effect on organic matter decomposition and nutrient transformations in their substrate materials and hence influences subsequently soil-atmosphere gas exchange processes (Nebert et al. 2011; Bernard et al. 2012; Bityutskii et al. 2012). Surface casting, for instance, is one of the major aspects of earthworm activities in tropical soils with a production rate varying from few tens to several hundreds of Mg dry soil per ha per year (Lal and De Vleeschauwer 1982; Ganeshamurthy et al. 1998; Lavelle et al. 1998; Hauser et al. 2012) and sometimes could cause replacement of even the entire 0–20 cm layer of topsoil (Ganeshamurthy et al. 1998). The physicochemical and biological properties of these earthworm casts are different from the non-ingested food material (Aranda et al. 1999; Blanchart et al. 1999). They exhibit relatively higher micro and macro nutrient pools (Ganeshamurthy et al. 1998; Bityutskii et al. 2012; Teng et al. 2012) and functionally more active microbial communities, particularly of N_2O-genic nitrifiers, denitrifiers and dissimilatory nitrate-reducers (Furlong et al. 2002; Ihssen et al. 2003; Drake and Horn 2007; Depkat-Jakob et al. 2010) and these microbes are essentially stimulated by the indigenous gut micro-conditions of earthworms (Karsten and Drake 1997; Matthies et al. 1999; Drake and Horn 2007). Denitrification activities and N_2O production have been evidenced from casts of various temperate earthworms with varying degrees of intensity among different feeding categories.

However, Wüst et al. (2009) reported an independency of feeding guilds for N_2O emissions in some introduced European endogeic and epigeic earthworm species. While Nebert et al. (2011) evidenced a greater cumulative N_2O emission by epigeic worms than endogeic ones and contended that feeding behavior may influence the earthworms N_2O emissions as it determines the access to soil organic matter for microbial processes, potentially responsible for N_2O production. Moreover, some recent studies on N_2O flux from earthworms of different ecological categories have revealed a differential stimulation of some specific functional domains of ingested microorganisms (*e.g.* of denitrifiers) during the gut passage (Sheehan et al. 2006; Chapuis-Lardy et al. 2010; Depkat-Jakob et al. 2010; Giannopoulos et al. 2011). Giannopoulous et al. (2010) also indicated a similar reflection of feeding strategies of temperate earthworms on their emissions of N_2O. Indeed, an ample amount of literature including aforementioned studies is available to date on the dynamics of nitrifying and denitrifying microbial communities and emissions of N_2O from guts and casts of temperate earthworm species. Recently few studies have characterized the N_2O emissions from tropical earthworms (Wüst et al. 2009; Chapuis-Lardy et al. 2010; Depkat-Jakob et al. 2012). However, to date there is not even a single study conducted on a comparative assessment of direct N_2O emissions from earthworm casts of different feeding guilds in a tropical ecosystem. In the tropics, therefore, more knowledge is required for the better comprehension of the impact of different earthworm feeding guilds, and their interactions with substrate material, on their cast chemical and biological dynamics (Teng et al. 2012), potentially linked to their atmospheric N_2O emissions.

Keeping in view the above mentioned idea of differential impact of earthworm feeding strategies on transient stimulation of microbial processes inside the guts and subsequent N_2O emissions, we hypothesized that in earthworm-populated tropical forest soils, castings produced by earthworms feeding on organic matter rich litter substrate (epigeic) should essentially maintain conditions highly favorable to denitrifying microbial processes and hence produce more N_2O than the casts of worms subsisting on nitrogen poor food soil (endogeic). To test this hypothesis and to ascertain the potential impact of feeding behavior on *in situ* cast N_2O flux, we determined *in vitro* emissions of N_2O, in the presence or absence of acetylene, from freshly produced casts of different earthworm species belonging to two dominant feeding guilds in the tropics *i.e.* endogeics and epigeics.

Moreover, as N_2O-genic microbial processes can be influenced by certain biotic variables such as microbial density and diversity of nitrifiers and denitrifiers (Braker and Conrad 2011; Petersen et al. 2012) and abiotic variables like available carbon (C) and nitrogen (N) contents (Avrahami and Bohannan 2009; Clark et al. 2012), we quantified the gene abundances of functional key enzymes of the N cycle (*i.e.* amoA, nirK, nirS and nosZ) and mineral N compounds (*i.e.* ammonium (NH_4^+) and nitrate (NO_3^-)) in the earthworm casts and their substrates and quantified the possible relationships among them, and between these factors and the corresponding N_2O emission rates.

Materials and methods

Collection of earthworms and their substrates

Earthworms of three endogeic (*Metaphire houlleti*, *Octolasion tyrtaeum*, *Pontoscolex corethrurus*) and one epiendogeic (*Amynthas corticis*) species were collected from the soils of Francisco Javier Clavijero garden, a 7.5 ha botanical garden established in a preserved cloud-forest patch. It is situated in the city of Xalapa (Mexico) at an altitude of 1330 m (19°30′N; −96°56′W), close to the *Instituto de Ecología* (Xalapa). Dominant vegetation, at the collection site, composed of trees species (*Clethra mexicana*, *Quercus xalapensis*, *Turpinia insignis*, *Cinnamomum effusum*) and shrub species (*Palicourea padifolia*, *Eugenia xalapensis*, *Coffea arabica*). Earthworms of two epigeic (*Dichogaster annae*, *Eisenia andrei*) species were collected in the vicinity of the botanical garden from the vermicompost beds of the *Instituto de Ecología*, where the main feedstock was coffee pulp. Though *A. corticis* has often been perceived in previous studies (Fragoso 2001; Garcia and Fragoso 2003) as an epiendogeic earthworm species, we consider and discuss hereafter the casts of *A. corticis* as of an endogeic species because of its similar behavior to other endogeic species *e.g.* coexistence in same soil profile as endogeic species and further similar physicochemical and microbial cast features.

The climatic conditions of the area are humid subtropical with a high rainfall and frequent mist. Mean annual temperature and precipitation are 18 °C and 1490 mm, respectively (Garcia and Fragoso 2003). The soil type is an Andisol with clay-loamy texture. Samples of fresh 2 mm sieved air-dried soil and fresh coffee pulp were taken from the upper 0–15 cm layers from the same relevant collection sites for laboratory rearing of worms and incubation processes.

Mesocosm experimentation

In order to obtain earthworm casts, healthy equal-sized hand-sorted earthworm individuals were released in 5 L plastic boxes containing food substrates (soil or coffee pulp). With the aim of ensuring better adaptability to the rearing conditions inside the mesocosms, earthworms were reared for few days in the laboratory under controlled temperature (25 ± 2 °C) and relative humidity (70%) with prevailing 12 h light:dark cycles. Casts produced overnight were removed each day early in the morning until the final harvesting of fresh casts for experimentation.