Soil Biology & Biochemistry 75 (2014) 37–44

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Contribution of white grubs (Scarabaeidae: Coleoptera) to N_2O emissions from tropical soils

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ARTICLE INFO

Article history:
Received 28 November 2013
Received in revised form
27 March 2014
Accepted 31 March 2014
Available online 13 April 2014

Keywords:
Tropical soils
N_2O emissions
Gut microbiota
Functional nitrogen genes
Denitrification
Mineral nitrogen

ABSTRACT

Soil biological processes that produce greenhouse gases, such as N_2O, are more intense in tropical soils because of the warm and humid climate; however, the role played by the wide diversity of fauna in these soils in soil N_2O production is still poorly understood. This study attempts to assess the role of scarabaeid grubs (Coleoptera), a major faunal group in tropical soils, in emissions of atmospheric N_2O. It was hypothesized that (i) the guts of these grubs are important sites of N_2O-genesis, since they present environmental conditions (anoxia, high labile C and N mineral contents) that are suitable for N_2O production; and (ii) rates of N_2O emissions will vary according to the density of gut microbial communities that are involved in N_2O emission (i.e. ammonia-oxidizers and denitrifiers). Through laboratory microcosm experiments, *in vitro* emissions of N_2O were determined directly from live grubs of different scarabaeid species (collected from tropical soils of Madagascar and Mexico) and from their surrounding parent soils. Quantitative PCR was used to determine the abundance of the total bacterial community (using the 16S rRNA gene) as well as the ammonia-oxidizing (bacterial AOB and archaeal AOA) and denitrifying (*nirK*, *nirS* and *nosZ*) microbial communities in the grub guts and surrounding soils. The mineral N contents of grub guts and parent soils were also determined using a continuous flow analysis technique. All of the studied grub species emitted significantly higher N_2O than the parent soils and presented a high gut ammonium to nitrate ratio (16:1). Their guts harbor a higher density of total bacterial (4.5-fold) and nitrite reductase (*nirK*) genes (1.5-fold) than the parent soils. However, with the exception of *nirK*, the relative and absolute abundances of all ammonia-oxidizer and denitrifier genes were higher in soils than in the grub gut environment. The average gene abundance of AOA was 10-fold higher than that of its bacterial counterpart (AOB). Emission of N_2O from grubs correlated significantly with the gene abundance of their gut ammonia-oxidizers (AOA and AOB) and denitrifiers (*nirS*), but not with mineral N contents. Based on average biomass values, these scarabaeid grubs are estimated to contribute between 0.2 and 1.8% of total soil N_2O emissions in tropical areas.

1. Introduction

Nitrous oxide (N_2O) is an important climate-forcing greenhouse gas in the Earth's atmosphere, with an atmospheric lifetime of 120 years and a warming potential that is 310 times higher than that of carbon dioxide (CO_2) (EPA, 2010). It has a significant role in the destruction of the ozone layer (Ravishankara et al., 2009). Its concentration in the stratosphere is increasing rapidly at a rate of 0.25% per annum (Haraldsen and Lie, 2012). N_2O emissions, globally estimated to be 17.7 Tg N per year, originate from both natural and anthropogenic sources. Of these, soils under natural and agricultural vegetation account for about 70% of emissions (EPA, 2010). Tropical and subtropical soils are important sources of atmospheric N_2O (Werner et al., 2007) and include tropical rainforests as the

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http://dx.doi.org/10.1016/j.soilbio.2014.03.025
0038-0717/

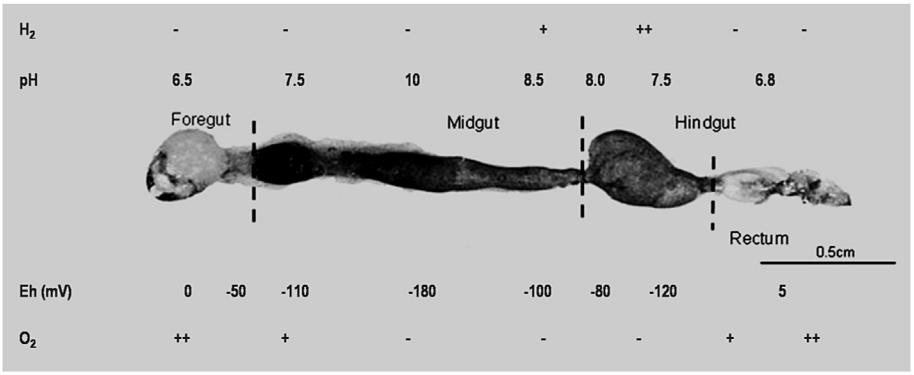

Fig. 1. The gut of a 3rd instar scarabaeid grub, illustrating axial gradients of H_2, pH, redox potential (Eh) and O_2 inside different gut compartments (modified from Lemke et al., 2003; Huang et al., 2010; Photo courtesy of Huang et al., 2010).

highest emitters, contributing as much as 3.5 Tg N per year (Breuer et al., 2000; EPA, 2010).

In the pedosphere, N_2O is generated by different interdependent microbial processes, for example, nitrification (Kowalchuk and Stephen, 2001), denitrification (Firestone et al., 1980; Braker and Conrad, 2011), nitrate ammonification or dissimilatory nitrate reduction to ammonia (DNRA; Bleakley and Tiedje, 1982; Rutting et al., 2011) and nitrifier denitrification (Kool et al., 2011). These processes prevail, either alone or simultaneously, in a given niche depending upon environmental drivers such as soil structure, organic matter quality and availability, pH, temperature, oxygen and moisture distributions (Bremner, 1997; Zhang et al., 2002; Ciarlo et al., 2007). Most of these edaphic drivers of N_2O-genic microbial processes are dependent upon the type of interaction they develop with the other soil biological actors, such as the soil fauna (Lavelle et al., 2006). Tropical (and subtropical) soils are well known to harbor a huge diversity (+20 taxonomic groups per ha; Brown et al., 2001) and density or biomass (5–100 g fresh weight m^{-2}; Lavelle et al., 1997) of soil engineers such as termites, earthworms, scarabaeid grubs, ants, etc. These represent key ecological actors because of the modulation of soil physicochemical and microbiological processes induced by their diverse feeding and foraging activities (Lavelle et al., 1997; Jouquet et al., 2006; Romero-López et al., 2010). However, most studies regarding the involvement of soil macrofauna in N_2O emission have focused mainly on earthworms (Karsten and Drake, 1997; Wüst et al., 2009; Giannopoulos et al., 2010), termites (Brummer et al., 2009; Ngugi and Brune, 2011; Majeed et al., 2012a) and, to a lesser extent, on ants (Jones and Diane, 2006). To date, there has been very little study on the potential role of soil-dwelling coleopterous white grubs (scarabaeid grubs) in N_2O emission dynamics in tropical soils. These grubs represent one of the major edaphic invertebrate groups, with a considerable biomass (5–54 larvae m^{-2} and sometimes up to 600 larvae m^{-2} in severe outbreaks) (Pardo-Locarno et al., 2005; Blanchart et al., 2007; Romero-López et al., 2010). Their gut compartments harbor physicochemical conditions similar to those of the termites (Fig. 1), i.e. steep gradients of pH, H_2, O_2 and redox potential (Eh) concomitant with high gut alkalinity and high levels of microbial diversity (Egert et al., 2003; Lemke et al., 2003; Zhang and Jackson, 2008; Huang et al., 2010). All of these conditions are potentially favorable for the main microbial processes involved in N_2O emissions, such as denitrification and nitrification.

The core objective of this study was therefore to specify the importance of scarabaeid grubs in N_2O emissions and to determine the main possible gut microbial processes at the origin of these emissions. It was postulated that, in tropical and subtropical terrestrial ecosystems, the guts of scarabaeid grubs constitute hotspots of atmospheric N_2O emissions and that these emissions are mediated by *in situ* ammonia-oxidizer and denitrifier microbial communities. To test this hypothesis, *in vitro* N_2O emissions were assessed in a selection of scarabaeid grub species belonging to three most dominant scarabaeid families (Dynastidae, Melolonthidae and Rutelidae) found in tropical and subtropical areas (Pardo-Locarno et al., 2005; Romero-López et al., 2010; Kishimoto-Yamada and Itioka, 2012).

Since physicochemical (e.g. mineral N content) and microbial factors control the functioning of enzymatic N_2O production (Avrahami and Bohannan, 2009; Braker and Conrad, 2011), the main factors that are potentially involved in gut (or soil) N_2O emissions were assessed by quantifying mineral N contents (ammonium and nitrate) and N-related functional genes including ammonia-oxidizers (AOA and AOB) and denitrifiers (*nirK*, *nirS* and *nosZ*).

2. Materials and methods

2.1. Collection of scarabaeid grubs and their parent soils

Grubs were collected from the tropical soils of Antsirabe (Madagascar) and Veracruz (Mexico). Further details regarding the collection sites are provided in Table 1. Coleopteran larvae of five different species (Table 2) belonging to three scarabaeid families of Dynastidae, Melolonthidae and Rutelidae were collected manually by excavating the surface soil layer to a depth of 30 cm. Only third

Table 1
Some characteristics of the collection sites of different scarabaeid grub species studied.

Collection and experimental site	Geographical position	Altitude	Climate	Average annual temperature and precipitation	Soil type
Andranomanelatra, Antsirabe, Madagascar	−19°47′S 47°06′E	1613 m	Cold high-altitude tropical	16 °C, 1300 mm	Ferralsol
La Mancha, Veracruz, Mexico	19°36′N −96°22′W	71 m	Warm sub-humid	26 °C, 1300 mm	Entisol