Review

The use of earthworms and biochar to mitigate increase in nitrous oxide production - A minireview

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Nitrous oxide is one of the greenhouse gases that is steadily increasing in the atmosphere at a rate of about 0.2% per year. Since the increasing trend is tied to agricultural processes, it is likely to continue to rise in the future and become important in global warming. Earthworm activities generally result in the high production of N_2O from the worms themselves, their casts, the drilosphere and from other indirect activities. This review seeks to examine nitrous oxide production from soils and mitigate it by the use of biochar and endogeic earthworms that reside deep down the soil profile. Biochar also has the tendency of reducing N_2O produced in the soil. By the combined use of biochar and endogeic earthworms, fertility of soil can be improved as well as the high emission of greenhouse gas (N_2O) reduced in such soil.

Keywords: biochar; nitrous oxide; earthworm

INTRODUCTION

Denitrifier activity is receiving global attention as it is a dominant cause of nitrous oxide emissions from agricultural soils. Emissions from agricultural soil contributes one third of anthropogenic nitrous oxide production globally (Mosier *et al.* 1998). Nitrification, denitrification and nitrifier denitrification are the main microbial activities resulting in N_2O emissions from soil (Wrage *et al.* 2001, Bateman and Baggs, 2005). These processes are affected by combinations of physical, chemical and biological factors (such as soil water content, aeration, mineral nitrogen concentrations and carbon availability) that can only partly be controlled by the farmers (Smith *et al.* 1998). Other agricultural activities that contribute to the emission of nitrous oxide are, wastewater treatments, use of nitrogen-based fertilizer, changing patterns of land use, use of ground water for irrigation, biomass burning and so forth.

Although N_2O is a radiative gas, N_2O contributes to global warming. On a per molecule basis, N_2O has a much greater warming potential than does CO_2 (with a GWP i.e. global warming potential of 298 meaning that a molecule of nitrous oxide can cause 298 times damage as one molecule of carbon dioxide) but its atmospheric concentration is minuscule in the atmosphere (IPCC, 2007 and Blasting, 2008). Also, the atmospheric lifetime of nitrous oxide is estimated to be 120-150 years based on its

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stratospheric losses. The longer the lifetime, the greater the contribution a greenhouse gas will make to global warming (Tuckett, 2009). N_2O in the atmosphere photo degrades to NO, which plays a key role in the production and destruction of ozone and in the oxidation of methane. N_2O undergoes photolysis to yield excited oxygen atoms that attack other N_2O atoms to NO.

The continued increase in atmospheric nitrous oxide contributes to the "greenhouse effect" or global warming. Nitrous oxide like other greenhouse gases such as carbon dioxide and methane, is transparent to short-wave (including visible) radiation but is fairly opaque to long-wave radiation. Thus it allows incoming solar radiation which is mostly short-wave, to penetrate to the earth's surface but traps long-wave, thermal radiation before it can be lost back into space.

The balance can be upset if the concentration of nitrous oxide exceeds normal levels. As nitrous oxide concentration rises in the atmosphere, more long-wave thermal radiation is trapped to the earth, causing the atmosphere to warm up.

Earthworms have been implicated in higher rates of denitrification and nitrous oxide emission, both in vivo and from soils that they inhabit (Burtelow et al. 1998; Costello and Lamberti, 2009, Drake and Horn, 2007; Giannopoulos et al. 2010; Rizhiya et al. 2007). Due to their ability to drive soil ecosystem processes such as soil structure and organic matter dynamics, earthworms are considered to be ecosystem engineers. Maximum densities and biomasses of earthworms are found in forest in the precipitation range of 2000-4000 mm, indicating that tropical annual rainfall values below 2,000 mm are too dry to support earthworm populations (Fragoso and Lavelle, 1995). A general view has been the belief that earthworms are less abundant in tropical rain forest and consequently play less important roles in the soil dynamics (Golley, 1983; Anderson and Swift, 1983).This belief comes from early studies in which very low abundances were found, mainly due to the use of inadequate formalin sampling methods (Madge, 1965; Block and Banage, 1968). Species richness and diversity of Tropical rain forest earthworm communities are not significantly different from those of temperate forests (Fragoso and Lavelle, 1992). However, Fragoso and Lavelle (1992) concluded that at a larger regional scale, tropical countries habour more species than temperate countries.

The objective of this review is to examine nitrous oxide production in soil and ways of managing earthworms and biochar to decrease its emission in the tropical soils.

Nitrous oxide emission from soil

The soil emits some nitrous oxide through microbial processes such as nitrification, denitrification and nitrifier denitrification. During nitrification (i.e. autotrophic nitrification) NH_4^+ or NH_3 is oxidized to NO_3^- via NO_2^-. These reactions are carried out by two groups of microorganisms, the first being the NH_3 oxidizers or the primary nitrifiers where NH_4^+ or NH_3 is oxidized to NO_2^-. The second step is carried out by NO_2^- oxidizers or secondary nitrifiers that oxidize NO_2^- to NO_3^-.

Hydroxylamine (NH_2OH) is the first product in NH_3 oxidation and the reaction is catalyzed by ammonia monooxygenase (Wood, 1986). Electrons are needed for the reduction of oxygen to water and these are derived from the oxidation of NH_2OH to NO_2^- (Hollocher et al. 1981). The next step in NH_3 oxidation is from NH_2OH to NO_2^-. This reaction is catalyzed by hydroxylamine oxidoreductase (McCarthy, 1990). The NO_2^- produced is further oxidized by nitrite oxidoreductase in a one-step reaction to NO_3^-.

N_2O is formed during NH_3 oxidation through chemical decomposition of intermediate such as NH_2OH or NO_2^- itself. This is usually regarded as a form of chemodenitrification (Chalk and Smith, 1983). Incomplete oxidation of NH_2OH can lead to the development of N_2O (Hooper and Terry, 1979). Thus NH_3 oxidation to NO_2^- can be a source of N_2O.

Denitrification is the stepwise reduction of NO_3^- to N_2. The reactions are carried out by denitrifiers across the bacterial taxa including Pseudomonas, Bacillus, Thiobacillus, Propionibacterium and others (Firestone, 1982). These heterotrophic microorganisms are facultative anaerobes that are able to use NO_3^- in place of oxygen as an electron acceptor in respiration to cope with low-oxygen or anaerobic condition. Enzymes catalyzing the reactions are nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Hochstein and Tomlinson, 1988). N_2O is a regular intermediate of denitrification. The portion of the intermediate N_2O that is released is high if the pH of the soil is low, because N_2O reduction is inhibited at low pH (Knowles, 1982). The ratio of N_2O/N_2 also rises if NO_3^- is abundant in soil because NO_3^- is preferred to N_2O as an electon acceptor (Schlegel, 1992). If some oxygen is present, the ratio of N_2O/N_2 also increases. The inhibition of nitrous oxide reductase by oxyen is stronger than the inhibition of other reductases of denitrification (Knowles, 1982). To summarize, N_2O is an intermediate of denitrification which can be released in high quantities in low-oxygen environments with sufficient NO_3^- and metabolizable organic carbon.

Nitrifier denitrification

Nitrifier denitrification is a pathway of nitrification. In nitrifier denitrification, the oxidation of NH_3 to NO_2^- is followed by the reduction of NO_2^- to N_2O and N_2 . This sequence of reactions is carried out by autotrophic NH_3 oxidizers. Thus N_2O is produced as an intermediate in the denitrification pathway.

Earthworm and nitrous oxide emission in soils

Earthworm emit nitrous oxide from the worm themselves, their casts, drilosphere as well as to general changes in soil structure. Earthworm gut provides an ideal habitat for denitrifying organisms to produce N_2O (Karsten and Drake, 1997, Horn *et al*. 2003). Furthermore, the mixing activity in the earthworm gut also leads to increased N_2O emission, denitrification in the fresh earthworm casts (Elliot *et al*, 1990; Knight *et al*. 1992) and in the drilosphere (Parkin and Berry, 1999). Other factors that favor the reduction of nitrate in the gut are the availability of high quality electron donors such as sugars, organic acids and amino acids, the high concentration of ammonium, and water content of approximately 50%, a near neutral pH that greatly enhance anaerobic activities in the gut of the earthworm. The high concentration of organic carbon in the gut might be derived from the ingested plant and soil-derived materials including fungal hyphae and large bacterial cells (Scho¨nholzer *et al*. 2002, Scho¨nholzer *et al.* 1999) that are partially degraded by digestive enzymes (eg. proteases, chitinase, N-acetylglucosaminases, maltases, Tillinghast *et al*. 2001; Tracey, 1951) and the intestinal mucus that is secreted by the earthworm.

Horn et al. (2003) proposed that bacteria from the soil and rhizosphere are ingested by earthworms as part of their diet. The change from the relatively dry and oxygen rich but substrate –poor conditions in the soil to the moist, high osmolarity, anoxic, substrate-rich conditions in the gut leads to activation of ingested bacteria to the onset of the reductive processes that lead to the production of nitrous oxide. The insitu conditions of the earthworm gut activates ingested soil denitrifiers during gut passage and leads to the emission of nitrous oxide and dinitrogen. Horn et al. (2006) noted that the phylogenies of *nosZ* from the gut and soil were similar indicating that denitrifiers in the gut of the earthworm were soil derived and were regular member of the soil denitrifier population. Many of the sequences clustered with known soil derived sequences or were related to nitrous oxide reductases of genera *Bradyrhizobium, Brucella, Dechloromonas, Flavobacterium, Pseudomonas, Ralstonia and Sinorhizobium*. The denitrifier population of the earthworm gut has also been evaluated by cultural methods and the culturable denitrifiers that occur in soil occurred in the earthworm gut (Ihssen *et al*. 2003). Analyses of 16S rRNA genes in earthworm guts and casts indicate that the microbiota in the earthworm gut is largely food derived rather than endemic (Egert *et al.*, 2004).

Based on these data, denitrifiers in the earthworm gut are hypothesized to be ingested soil microorganisms that are more activated by the special microenvironment of the gut (Horn et al. 2003, Ihessen *et al*. 2003). The number of culturable denitrifiers were 256 and 35 fold higher in earthworms (*Lumbricus rubellus* and *Octolasium lacteum*)

than the number of culturable denitrifiers in the soil from which the earthworms were obtained (Kristufek, *et al*. 1992, Lee, 1985, Pedersen and Hendriksen, 1993). Ihessen *et al*. (2003) made a similar observation where increased nitrous oxide emissions measured within the earthworm gut (Horn, *et al*. 2003) coincided with an observed 300-fold increase in culturable bacterial denitrifiers in the gut compared to the bulk soil. In addition, the gastrointestinal tracts of the worms mentioned above harbored substantially higher number of bacteria capable of anaerobic growth than does the soil from which worms were obtained **(**Karsten and Drake, 1995).

The earthworm gut maybe enriched with various aerobic and facultative microbes (Contreras, 1980; Khambata and Bhat 1953; Khambata and Bhat. 1957). The passage of microorganisms through the gut may selectively decrease the numbers of certain microbes such as *Serratia marcesens* and *E. coli* (Day, 1950, Pedersen and Hendriksen, 1993). Earthworms appear to be a mobile hot spot for the production of nitrous oxide in certain terrestrial ecosystems.

The production of nitrous oxide by living earthworms is not derived solely from denitrification. Alternative nitrous oxide producing processes such as nitrification (Davidson, 1991, Firestone, and Davidson**,** 1989, Skiba *et al*. 1993), assimilating reduction of nitrate (Tiedje, 1988) and dissimlating reduction of nitrate (Tiedje, 1988; Conrad, 1995; Stouthamer, 1988) to ammonium could also contribute to nitrous oxide production under in vivo conditions. High numbers of other organisms that are capable of producing nitrous oxide (i.e. nitrate – dissimilating and nitrifying bacteria) are also present in the earthworm gut (Ihssen et al., 2003). Some nitrifiers are able to use nitrite or nitrate as electron acceptors and by using this nitrifier denitrification system can produce nitrous oxide and or nitrogen under oxygen –limited conditions (Freitag *et al*. 1987, Poth and Focht. 1985). Denitrification and reductive dissimilatory reduction of nitrate are most likely the main microbial processes responsible for the production of nitrous oxide by earthworms (Ihssen *et al*. 2003, Karsten and Drake. 1997, Matthies *et al*. 1999).

N_2O production from earthworm casting, burrows and drilosphere

The earthworm castings contain elevated amounts of NH_4^+, NO_3^-, Mg, K and P relative to the bulk soil (Syers *et al*., 1979; Tiwari *et al*., 1989). Denitrification occurs in earthworm casts (Elliot *et al*. 1991; Svensson et al. 1986). Furthermore, in contrast with the bulk soil, fresh earthworm casts exhibit a higher microbial biomass, microbial activity and mineral N content (Amador and Go¨rres 2007, Scheu, 1987).   Earthworm casts have been observed to have

elevated nitrification and denitrification activity (Svensson *et al*., 1986; Elliot *et al*., 1990).

The walls of the burrows of *Lumbricus terrestris* (L) are smooth and cemented together with mucous secretions (Lavelle, 1988). The mucous secretions contain high concentrations of organic N and ammonium (Needham, 1957) and may serve as a substrate for fungi and bacteria (Edwards and Fletcher, 1988). Earthworm burrows contain higher nitrification and denitrification activities due to earthworm excretion of nutrient-rich mucus (Parkin and Berry,1999).

Drilosphere soil (earthworm burrow soil), has a trend of higher denitrification rate in the burrow wall material as compared to nonburrow material. The primary data of Parkin and Berry (1999) indicated elevated populations of nitrifying and denitrifying bacteria associated with the drilosphere soil than the nondrilosphere soil. Denitrification rates were approximately 1000 times lower than nitrification rates indicating that nitrate formed from nitrification has the potential to persist in the burrow. Earthworms excrete ammonia compounds including NH_4^+, urea, allantoin and uric acid (Lee, 1985). In the drilosphere, the earthworm excreta are often pressed into the burrow walls (Lee, 1985). Denitrifying bacteria would find the elevated soil moisture contents, the deposition of earthworms-derived organic carbon such as mucoproteins and the compacted sides of the burrow an advantageous environment for the production of nitrous oxide.

Earthworm priming of denitrifiers in soil

These optimal N_2O-producing conditions are extended into the soil volume that is directly influenced by earthworm activity; casts and burrow wall etc. This is the earthworm priming of the denitrifying community. As a result, N_2O emissions from casts and burrow walls can be up to three times greater than from the bulk soil (Horn *et al*. 2003). Earthworm priming of denitrifiers may ultimately depend upon the earthworm's ecological strategy which determines their access to organic matter. For example epigeic earthworms primarily reside in the soil –litter interface, mixing into the soil and ingesting fresh organic matter while endogenic earthworms inhabit deeper soil layers, feeding predominantly on soil organic matter. Several studies have concluded that earthworm mediated nitrous oxide emission depend largely upon eocological feeding strategy (Giannopoulos *et al*. 2010, Lubbers *et al*. 2011 and Rizhiya *et al*. 2007). These authors also found that earthworms significantly enhanced nitrous oxide emissions after application of crop residue. The effect appeared to be transient and proportionately small in the presence of endogeic species not contributing significantly to cumulative nitrous oxide emissions after 90 days. In contrast, the epigeic species *L. rubellus* exhibited a persistent effect in nitrous oxide emissions significantly contributing to cumulative nitrous oxide emission.). *L. rubellus* is an epigeic worm and feeds more on litter than does the endogeic worm *O. lacteum* that feeds more on soil (Lee, 1985.) in this regard, the gut of *L. rubellus* tends to have higher nitrogen and organic carbon contents than does that of *O. lacteum* (Karsten and Drake, 1995).

Earthworms indirectly involved in nitrous oxide emission

Earthworms affect the production and emission of N_2O and CO_2 indirectly by incorporating plant residue and mixing the soil, by stimulating soil aggregation and by changing soil moisture dynamics and soil diffusivity (Giannopoulus *et al*., 2010; Lubers *et al*., 2011; Rizhiya *et al*., 2007). Nitrous oxide emissions were only significantly enhanced by earthworms when organic fertilizer was added to the soil instead of inorganic fertilizer or no fertilizer at all (Lubbers *et al*., 2013). Some studies about the effect of earthworm on soil greenhouse gas emissions have been conflicting (Tianxiang *et al*., 2008) with some authors noting increased soil N_2O emissions (Borken *et al*., 2000; Karsten and Drake, 1997) and others decreased N_2O emission (Bertora *et al.,* 2007). Other authors have noted that not all earthworms collected from the field emitted nitrous oxide. Possible reasons for these observations are that earthworms increase porosity and thereby gas exchange with the atmosphere, possibly decreasing the amount of N_2O which is further denitrified to N_2 before emission to the atmosphere (Parkin and Berry, 1999). Another reason might be that most denitrifiers possess the capacity to both produce and consume nitrous oxide (Conrad, 1996) and the net release of nitrous oxide during denitrification is regulated by parameters such as pH, the phase of growth and the concentration of nitrate and electron donors (Kester *et al.,* 1997) thus affecting nitrous oxide production in the soil.

Biochar and nitrous oxide emission

Biochar from treewood (Pignatello *et al.,* 2006; Wang *et al*., 2006) and pine needles (Chen *et al,*. 2008) have been shown to sorb significant amounts of diverse polar and non polar aromatic contaminants, humic and fulvic acids. Spokas *et al.,* (2009) noted that biochar additions to soil suppressed N_2O production at all levels with the exception of high nitrogen compost ameneded biochar which increased N_2O production (Spokas and Reicosky, 2009). Possible explanations for biochar suppressing N_2O in soils were – altered soil moisture potential within the soil and biochar system (Tyron, 1948), increased potential for anaerobic or aerobic microsites within the char (Warnock

et al., 2007), increased oxygen diffusion into the soil due to the char particles reducing the bulk density of soil,added nutrients and labile organic matter since the addition of organic material to soils typically increases N_2O especially with low C/N residue. Apart from biochar suppressing N_2O emitted, biochar can enhance long-term soil fertility through several mechanisms. The polycyclic aromatic structure of biochar makes it chemically and biologically stable, allowing it to persist in the environment for centuries (DeLuca *et al.,* 2006). Beside this remarkable chemical structure, biochar has a porous physical structure which leads to very large surface area (Lehmann and Rondon, 2006). This increases the soil cation exchange capacity as well as its capacity to retain dissolved organic matter (Lehmann and Rondon, 2006). Moveover, biochar modifies the community of soil microorganisms as well as their activity, probably because it provides a suitable habitat for them (Pietikainene *et al.,* 2000).

Biochar and earthworms have been shown to directly interact, earthworms ingest biochar particles and reject them in their casts, which is likely to influence biochar distribution in the soil profile (Topoliantz and Ponge, 2003; Topoliantz *et al.*, 2005, Van Zwieten *et al.*, 2009). The interaction between biochar and earthworms have known to be additive and not synergistic. Using tropical endogeic earthworm species, *Pontoscolex corethrurus*, Topoliantz and Ponge, (2003 and 2005) demonstrated the ingestion of biochar particles by earthworm. The earthworm could grind the biochar and mix into the soil and it preferred soil with biochar over soil alone. Biochar may serve to grind organic matter in the gizzard similar to what have been observed for sand (Marhan and Scheu, 2005). Geophagus earthworms may feed on microbes and microbial metabolites (Lavelle, 1988) which are more abundant on biochar surfaces. Bioturbation by mostly anecic earthworms is likely responsible for vertical mixing of biochar within the soil profile (Carcaillet, 2001).

It is often argued that managing soil fauna especially earthworms and applying biochar will be appealing way to increase the fertility of tropical soil. Depending upon the type of ecosystem and the existing population in soil, earthworm population numbers can be adjusted either by direct inoculation of endogeic earthworms into soil or by manipulating the local earthworm populations. Shifts in earthworm communities over the next few decades will significantly affect (and probably enhance) soil greenhouse gases (Lavelle *et al.,* 2001).

By introducing endogeic earthworms into soil, since they feed on soil organic matter and are found deeper in the soil profile they emit little N_2O to the atmosphere. In addition, adding biochar to soil might increase the fertility of the Tropical soil and build up the modern Terra preta. Tropical soils are often poor in organic matter (Tiessen *et al.*, 1994) and tend to have low cation exchange capacities (Glaser, 2007) hence they are low in fertility. The use of earthworms

and biochar may improve on the fertility of such tropical soils. This is because both earthworms and biochar influence soil organic matter dynamics, the release of mineral nutrients and retention. Maintaining and well managing high biomasses of earthworms would be another sustainable way to increase tropical soil fertility (Lavelle *et al.*, 2001).

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