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# Earthworm ecosystem service and dis-service in an N-enriched agroecosystem: Increase of plant production leads to no effects on yield-scaled N<sub>2</sub>O emissions



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#### ABSTRACT

Earthworms can enhance plant productivity by promoting nitrogen (N) mineralization in N-limited agroecosystems and may also enhance the risk of N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup>-N leaching in N-enriched agroecosystems. However, direct evidence demonstrating the enhancement by earthworms of N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup>-N leaching in the field is scarce, particularly in intensively managed systems. In addition, the interaction of earthworm feeding strategies and organic amendment may profoundly modulate N cycling. We examined these impacts using two earthworm species with distinct ecological strategies (epigeic Eisenia foetida and endogeic Metaphire guillemi) in combination with two manure application methods (surface mulch and incorporation into the soil) in a field experiment. Our results demonstrated that earthworm addition significantly increased the crop yield by 18%-47% and cumulative N<sub>2</sub>O emissions by 19%-25% largely regardless of earthworm species and manure application methods, respectively. However, earthworms did not significantly increase the leachate  $NO_3^--N$  concentration. Earthworm-induced N<sub>2</sub>O emissions were primarily attributed to increased soil N availability  $(NO_3^--N)$  and microbial biomass N) and carbon (C) availability (dissolved organic C). In contrast, a stepwise regression revealed that an earthworm-promoted soil macroaggregation exerted negative effects on N<sub>2</sub>O emissions. Irrespective of earthworm species and manure application methods, earthworms had no stimulatory effects on the yield-scaled N<sub>2</sub>O-N because the promotion of crop productivity counteracted the extent of N<sub>2</sub>O increase. In conclusion, understanding the trade-off between earthworm services and dis-services will contribute to the development of environmentally justified soil management by allowing the full utilization of biological resources.

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#### 1. Introduction

Nitrogen (N) is one of the most limiting nutrient elements for crop production in agroecosystems, and high inputs of chemical N are ubiquitous in intensively cultivated farmland (Elser et al., 2007). However, high N inputs cause a large number of problems associated with high cost, environmental pollution and the deterioration

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of soil ecosystems (Ju et al., 2009), including soil acidification, soil structure degradation, increasing greenhouse gas emissions and  $NO_3^-$ —N leaching to water systems (Chang et al., 2011; Isbell et al., 2013). Reducing or even replacing chemical N fertilization with alternative N sources derived from recycling organic amendments has become a challenge for sustainable agriculture (Jannoura et al., 2014).

Agricultural organic waste such as husbandry manure forms a major source of N for plant growth (Bardgett, 2005). Currently, abundant manure from rapidly expanding animal husbandry is one of the most economic and efficient nutrients available to improve soil fertility, plant production and thus agricultural sustainability (Gattinger et al., 2012). Accumulated evidence has demonstrated

that manure compost can promote soil quality and productivity, e.g. by improving the structure and chemical properties of the soil (Tejada et al., 2008), increasing the plant-available nutrients (Liu et al., 2009), enhancing soil biological activities (Bowles et al., 2014), promoting microbial diversity (Jackson et al., 2012) and soil fauna abundance and diversity (Leroy et al., 2007), and enhancing the crop yield or quality (Palmer et al., 2013). In particular, manure amended to soil provides abundant resources for soil fauna. These resources would reinforce the ability of soil fauna to play critical functional roles in soil ecosystem services, such as organic matter decomposition, nutrient cycling and primary production (Huhta, 2007; Van Groenigen et al., 2014). However, the influences of interaction between organic fertilization and soil fauna on ecosystem services are often neglected (Doan et al., 2013).

Earthworms are considered to be one of the most important soil ecosystem engineers, affecting soil functioning through their burrowing, feeding and casting activities (Edwards, 2004). Continuous and high fertilizer N inputs in the absence of organic amendment often decrease earthworm populations and activities, whereas organic amendments have the opposite effects (Blair et al., 1997; Sharpley et al., 2011). Additionally, it is well known that earthworms with distinct ecological strategies (i.e. habitat preference, food selection, ingestion and assimilation) most likely have different responses to fertilizer inputs (Curry and Schmidt, 2007). Few studies, however, have considered the potential interactions between earthworm ecological strategies and their food resource application methods, especially under field condition (Lubbers et al., 2013a).

Earthworms play a critical role in plant productivity, primarily through the promotion of N mineralization processes (Van Groenigen et al., 2014), but their negative effects on the environment have drawn little attention to date. For example, earthworms may increase nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas with a warming potential that is 310 times greater than that of carbon oxide (IPCC, 2013), and leachate NO<sub>3</sub><sup>-</sup>-N to water systems. Accumulated evidence have demonstrated that Earthworm gut is an ideal environment (anoxic, near-neutral pH, high organic compounds and nitrate) for N<sub>2</sub>O production (Horn et al., 2003), and the habitats within the drilosphere and casts are also hotspots for N<sub>2</sub>O production (Amador and Görres, 2007; Majeed et al., 2013). Previous studies have reported that earthworms could indirectly affect N<sub>2</sub>O emissions by modifying soil biological and physicochemical properties (Giannopoulos et al., 2010; Paul et al., 2012; Lubbers et al., 2013a, b). Additionally, there are controversial findings on the effects of earthworms on  $NO_3^-$ –N leaching. On the one hand, earthworms might increase NO3--N leaching by significantly increasing N mineralization and water infiltration (Wang et al., 2005; Jouquet et al., 2013). On the other hand, earthworms might reduce NO<sub>3</sub><sup>-</sup>-N leaching because their activities could promote the formation of aggregate (e.g. via casts) and thus reduce the leachate through the high protective or holding capacity of aggregates for nutrients and water (Bossuyt et al., 2005; Jouquet et al., 2011). To date, few studies have explored the effects of earthworms on N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup>-N leaching under field conditions where plants, earthworms and manure might strongly interact (Lubbers et al., 2013b). However, knowledge regarding the effects of earthworms on both plant production and N loss will contribute to the understanding of their roles in the trade-off between ecosystem service and dis-service in agroecosystems (Wall et al., 2012).

Recently, polytunnel vegetable cultivation has expanded worldwide, particularly in China, where it covers a total area of 3.3 million hectares (Chang et al., 2011). In efforts to maximize yield to meet the growing food demand, large amounts of N fertilizers have been applied and have reduced or even eliminated the reliance on the inherent biological fertility of soil provided by earthworms. Metaphire guillemi, a native endogeic species, can facilitate soil N mineralization and enhance soil microbial biomass and enzyme activities in the agroecosystems (Tao et al., 2009a, b). In addition, Eisenia foetida is a well-known epigeic species and is the organism most frequently used for processing organic wastes (Ngo et al., 2012). Our field experiment aims primarily to explore the effects of two earthworm species in terms of organic amendment methods (at the soil surface or incorporated into the soil) on the trade-off between earthworm-induced ecosystem services (crop yield) and dis-services (N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup>-N leaching). We hypothesized that (i) earthworms would increase crop yield, N<sub>2</sub>O emissions and leachate NO<sub>3</sub><sup>-</sup>-N by promoting N mineralization and soil N availability, (ii) the net effect of earthworms would depend on the combination of their ecological strategy and the organic amendment method, and (iii) earthworm-induced N<sub>2</sub>O emissions per unit of crop yield would not increase, due to the concomitant increase of crop production in the presence of earthworms.

#### 2. Materials and methods

#### 2.1. Site description and experimental design

A field experiment was established with a tomato-spinach rotation system in 2010 at the Yuhe organic vegetable production base, Suzhou City, Jiangsu Province, China (120°28.732′E, 31°26.798′N). A preliminary survey did not find any native earthworms in this field, most likely due to the intense chemical fertilization. The main soil characteristics were pH 5.96, 6.7% sand, 68.8% silt, 24.5% clay, 21.9 g of organic C kg<sup>-1</sup>, 2.65 g of total N kg<sup>-1</sup> and 168.17 mg kg<sup>-1</sup> of available N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N).

The field experiment, which was conducted in a large polytunnel (Supplementary material Fig. S1). The experiment was organized as a full factorial design, in which manure compost application methods (two levels: applied to soil surface after tillage or incorporated into soil plowing layer during tillage) and the presence of earthworms (three levels: control without earthworms and the introduction of E. foetida (Savigny, 1826) or M. guillemi (Michaelsen, 1895)) were factors. This set-up consisted of six treatments: S (manure compost at the soil surface without earthworms); SE (manure compost at the soil surface with E. foetida); SM (manure compost at the soil surface with M. guillemi); I (manure compost incorporated into the soil without earthworms); IE (manure compost incorporated into the soil with E. foetida); and IM (manure compost incorporated into the soil with M. guillemi). Each treatment was replicated three times and randomly arranged in the field. The replicated plots  $(2.4 \times 1.2 \text{ m})$  were located 0.5 m from each other and separated by a 3-cm-wide concrete frame (60 cm deep belowground and 20 cm aboveground) to prevent the exchange of earthworms, water or nutrients among the plots (Fig. S1B).

Manure compost (hereafter simply termed manure) was obtained from a local cattle farm after composting for one month under aerobic conditions. During the tomato season (from early April to early July), manure (30 t ha<sup>-1</sup>, FW) containing 22.15 g of N kg<sup>-1</sup>, 12.10 g of P kg<sup>-1</sup>, 2.10 g of K kg<sup>-1</sup> and 76% moisture content was applied, whereas during the spinach season (from the end of October to early December), a similar amount of manure containing 21.05 g of N kg<sup>-1</sup>, 11.30 g of P kg<sup>-1</sup>, 3.25 g of K kg<sup>-1</sup> and 80% moisture content was applied. Manure was applied as a single basal fertilizer with no extra chemical fertilizers in any plots due to the initial high nutrient levels in this field (Morra et al., 2010). Earthworm guts were cleaned by the wet-filter paper method for 48 h (Dalby et al., 1996). The earthworms were introduced to the corresponding plots at a density of 60 g m<sup>-2</sup> (100 ± 5 individuals of *E. foetida* adults or 30 ± 2 individuals of *M. guillemi* adults) based on a previous investigation

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