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Earthworm bioturbation stabilizes carbon in non-flooded paddy soil at the risk of increasing methane emissions under wet soil conditions

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ABSTRACT

Studies on earthworms in rice-based ecosystems tend to focus on some pest species, while the potential of these important soil engineers for beneficially affecting carbon storage and cycling is widely ignored. We carried out a microcosm experiment to quantify the impact of the tropical earthworm *Pheretima* sp. on the C turnover in paddy soils under different conditions of water saturation and N fertilization. The soil was sampled at the lowland farm of the International Rice Research Institute (Philippines). In the absence of earthworms, soil respiration showed a distinct hump-shaped maximum at intermediate levels of water saturation (4-fold higher than in hand-dry soil) and increased 1.5-fold with increasing amounts of N fertilization. Amounts of CH_4 emitted, in contrast, were small at low to moderate soil humidity and became very high under conditions of water saturation (80-fold higher than hand-dry soil). No response to nitrogen addition was observed. Earthworms suppressed both the respiration maximum at intermediate saturation levels (by a factor of 1.4) and the stimulating impact of N fertilization (1.7-fold at maximum fertilizer level). On the other hand, earthworms strongly increased CH_4 release under conditions of high water saturation (3-fold). No consistent response of the soil microflora (bacterial abundance, soil enzymes) to earthworm activity could be established. Our findings suggest that the stabilization of soil organic C via earthworm bioturbation is confined to the range of soil humidity that allows high activity of *Pheretima* sp. Under conditions of intensive agriculture, the stabilizing effect of the worms may even be augmented by the fact that they offset the positive effect of N fertilization on microbial respiration. Earthworms may thus play a vital role in reducing the CO_2 flush from paddy soils after the conversion to non-flooded crops such as aerobic rice or maize. Acceleration of methane emission in very humid soils nevertheless points to a certain risk that is associated with increasing earthworm abundance in production systems that are still exposed to temporary flooding during the wet season.

1. Introduction

Water provision and management are key factors for ensuring global security and sustainability in food production (Mueller et al., 2012). Thus, the enormous water consumption by rice production and its impact on climate change by massive emissions of climate-relevant trace gases (GHG) calls for management practices that save water and also reduce GHG emissions (van Groenigen et al., 2013; Yang et al., 2014). However, a shift from conventional flooded rice cropping to water-saving non-flooded crop rotations bears several risks, since it could possibly lead to soil nutrient mining, aggravating trends of declining soil organic matter contents and adversely affecting the GHG balance (Haefele et al., 2013). Considering the enormous contribution of edaphic organisms to agricultural sustainability (e.g. Brussaard et al., 2007), it seems reasonable to assume that facilitating the establishment of an efficient soil community could be a powerful tool for counteracting adverse effects associated with the conversion to non-flooded conditions. In particular earthworm colonization of paddy soils may hold an enormous potential for increasing soil fertility, plant productivity, stress resistance and soil health (cf. Lavelle, 1988; Lemtiri et al., 2014). While only some earthworms are able to colonize submerged rice fields (Edwards, 2004), reduced flooding will dramatically increase the colonization potential of most species (Emmerling, 1995; Ausden et al., 2001; Zorn et al., 2005).

We conducted a microcosm experiment to quantify the impact of the tropical earthworm *Pheretima* sp. (Megascolecidae) on trace gas release from paddy soils sampled in the vicinity of Los Baños

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(Philippines) under different environmental conditions (water saturation, N fertilization). The effects of earthworms in rice production systems are controversially discussed, since they may include both ecological services and disservices (Blouin et al., 2006; Noguera et al., 2012). For example, the non-native semi-aquatic species *Eukerria saltensis* was found to disrupt seedling establishment, destabilizing the surface layer and accelerating the loss of nutrients (Stevens, 2003). Similarly, earthworms accidentally washed into rice fields have been reported to damage the terrace wall (Joshi et al., 2000) or to cause significant yield losses (Barrion and Litsinger, 1997). On the other hand, native earthworms have been shown to stimulate the growth of rice plants (Noguera et al., 2010). According to Choosai et al. (2010) the species *Drawida beddardi* naturally occurring in the rainfed paddy fields of Northeast Thailand has the potential to increase rice yields.

In general, edaphic animals indirectly affect ecological processes such as GHG emissions by shaping the microbial community via incorporating and redistributing organic and inorganic material (Wolters, 2000; Brussaard et al., 2007). Though the many effects of soil biota on the stabilization of soil organic matter and the modulation of GHG emissions are very well documented for temperate regions, the effect of earthworms on GHG emissions in tropical paddy soils is poorly understood. We hypothesized that:

1. The endogeic earthworm *Pheretima* sp. has the potential for diminishing GHG emissions by reducing CO_2 release.
2. The potential of earthworms for reducing CO_2 release is highest in moderately moistened soils. On the other hand, earthworms can stimulate methane release in water saturated soils.
3. Earthworms diminish the stimulating effect of high amounts of N-fertilizer application on gaseous C release.

2. Methods

2.1. Experimental design

We used soil sampled by cutting intact soil blocks (20 × 20 cm) with spates from the uppermost 10 cm of a paddy rice field at the International Rice Research Institute (IRRI) lowland farm (Los Baños, Laguna, Philippines) classified as Andaqueptic Haplaquoll (USDA). Earthworms (*Pheretima* sp., Megascolecidae) were collected by hand at the field site on the IRRI farm. According to accompanying field investigations, this species was the most frequent and abundant in this region, which dominated the bunds of the experimental fields at IRRI. Therefore we expected *Pheretima* sp. to have an enormous potential for colonizing non-flooded paddy fields and decided to use this species for our experiment.

A full factorial gradient design with seven levels of soil water content, seven levels of nitrogen application and two levels of earthworm inoculation was established using 98 laboratory microcosms. Treatments were randomized across two consecutive blocks of 49 microcosms. The soil water content spanned the range observed under field conditions for irrigated rice and non-irrigated aerobic rice/maize cultivation at the IRRI field station and under which earthworms had persisted in preceding pilot experiments. Soil water saturation levels ranged from 'hand-dry' to flooded (10.4, 20.8, 31.2, 41.6, 51.9, 62.3, 72.7 ml H_2O 100 g^{-1} soil dry weight). Fertilizer (urea) levels ranged from zero to a maximum nutrient amendment in quantities corresponding to a field application of 120 kg N ha^{-1} (0, 20, 40, 60, 80, 100, 120 kg N ha^{-1}). Each combination of soil water saturation level and fertilizer level was duplicated and one out of two microcosms in the pair was subject to the earthworm treatment.

2.2. Microcosm preparation and gas sample analysis

The soil material was mixed and sieved (2 mm mesh) before placing it into the microcosms to remove stones and roots as well as to achieve homogenous texture. Soil pH varied around the average value of 6.4. Earthworms were stored in plastic boxes filled with soil from the sampling area and organic food supply until the experiment started.

The microcosm system is identical to the one described by Wolters (1989) as modified by Vetter et al. (2004). Each microcosm was constructed from a Perspex tube (height: 24 cm, diameter: 4.6 cm), covered by a specially designed cap enabling aeration, water supply and sampling of head space volume. Exactly 158 g dry weight of sieved soil was placed into each microcosm tube, resulting in a column height of 11.5 cm. Afterwards water (containing dissolved urea for the respective fertilizer treatments) was applied. The microcosms were then incubated for 48 h under constant laboratory conditions at 25 °C in an automated climate chamber (maximum temperature variance: +/−0.5 °C). After this period of time, the microcosms subject to earthworm treatment received a single individual of *Pheretima* sp. and were left undisturbed for another five days before measurements started to allow acclimatization of the worms and stabilization of the microbial community. Then, soil CO_2 and CH_4 evolution were measured daily for five consecutive days. Gas samples from the microcosms were taken with 50 ml gas tight polypropylene syringes. Limitation to a total period of ten days was necessary, since preliminary experiments had shown that the release of trace gases from microcosms inoculated with earthworms becomes very variable and divergent starting from day 12 to 15. This can most probably be explained by factors associated with the limited amount of soil within the microcosms (e.g. re-ingestion of soil by the worms, decreased availability of C to bacteria). Due to infrastructural limitations, the experiment had to be split into two blocks, which were installed and operated identically, but measured consecutively.

Soil CO_2 and CH_4 production was quantified by means of a gas chromatograph (Shimadzu GC-14), with measurements being calibrated against standard reference gases each day prior to the control measurement. Microcosms were aerated continuously and moistened every second day to keep the soil water content at prescribed levels. Each individual measurement was preceded by an accumulation period of 15 min. In total 490 measurements were made. The resulting values of soil respiration and CH_4 emission were calculated as a difference in gas concentrations in observation and control sampling with the standardization to g C g^{-1} soil dwt. h^{-1}.

2.3. Bacterial abundance and extracellular enzyme activity

After termination of the experiment, bacterial abundance within each microcosm was determined as described by Buesing and Marxsen (2005) and Marxsen et al. (2010). In brief, a volume of 0.4 ml of all soil samples were fixed with 4% paraformaldehyde solution, washed with PBS buffer and stored at −20 °C in a 1:1 mixture of ethanol and PBS until further processing. After defrosting, each sample was washed and diluted with pure PBS before receiving ultrasonic treatment to detach bacteria from soil particles. Appropriate volumes of the solutions achieved in this way were then filtered onto white polycarbonate filters (pore size 0.2 μm, GTTP, Sartorius, Göttingen). Pieces of the filters were stained with SYBR Green I (Molecular Probes, Eugene, OR, USA) and cells were counted using a Zeiss Axiophot 2 epifluorescence microscope equipped with a 100 W high-pressure bulb and the filterset HQFITCsel (exciter HQ480/40, beamsplitter Q505LP, emitter 527/30; AHF Analysentechnik, Tübingen, Germany).