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Earthworm responses to cropping rotation with oilseed rape in no-tillage rice fields and the effects of earthworm casts on human-essential amino acid content in rice grains

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ABSTRACT

The rice-oilseed rape rotation is a major rice-based cropping system in the Yangtze River basin in China, and no-tillage technology has been successfully adopted in this system. The main objectives of this study were to determine: (1) the response of earthworm density to rotation with oilseed rape in no-tillage rice fields; and (2) the effects of earthworm casts produced during the oilseed rape-growing season on the content of human-essential amino acids in resulting rice grains. An on-farm study was conducted from 2015 to 2017 to compare earthworm density in no-tillage fields where rice was rotated with oilseed rape and with a fallow period. A micro-plot experiment was also carried out to compare the grain content of human-essential amino acids in rice with and without the addition of earthworm casts (produced during the oilseed rape-growing season) under three nitrogen fertilizer rates. We observed that medians of earthworm number per m^2 were 68–154% higher in the fields rotated with oilseed rape than in the fallow fields. The contents of human-essential amino acids detected in rice grains were generally increased by the addition of earthworm casts, and there were significant increases (8–13%) for phenylalanine, threonine and valine. Our study suggests that earthworms can be increased by rotation with oilseed rape in no-tillage rice fields, and that their casts can increase the contents of human-essential amino acids in rice grains.

1. Introduction

Earthworms are important components of the soil biota in terms of soil formation and maintenance of soil structure and fertility, and they serve as a keystone group that reflects the abundance of other life forms in the soil (Edwards, 2004). Soil moisture and temperature are the primary factors determining earthworm survival and growth (Wever et al., 2001). In general, earthworms prefer higher soil moisture and lower soil temperature (Clapperton, 1999; Tian et al., 2000). Earthworm activity is also influenced by several agricultural practices, e.g. soil tillage and crop rotation (Hubbard et al., 1999; Mele and Carter, 1999; Pelosi et al., 2009). The absence or reduction of soil tillage is favorable to earthworm populations by providing them with an undisturbed biotope, and with crop residue that serves as a food source for earthworms.

Oilseed rape is an excellent rotation crop for cereals because it helps disrupt the cycle of soil-borne pathogens and maintain good quality soil (Kirkegaard et al., 1997; Huang et al. 2017). Rice-oilseed rape rotation has become a major rice-based cropping system in the Yangtze River basin in China, and no-tillage technology has been successfully adopted in this system (Huang et al., 2011). Compared with leaving fields fallow, oilseed rape cultivation changes soil properties. First, bed cultivation is usually used for cultivating oilseed rape to avoid high soil moisture levels. Second, ground cover provided by oilseed rape plants helps decrease soil temperature. Third, oilseed rape plants can produce a large amount of litterfall, which enriches soil organic matter. These changes in soil properties indicate how rotation of oilseed rape with rice may affect earthworm density in no-tillage rice fields, but no studies have been conducted to confirm and clarify the effects on earthworms.

In a rice-oilseed rape rotation, earthworms are likely to migrate away from the fields during the early stage of rice growth when rice paddies are flooded since they are aerobes (Reynolds, 1994). Therefore, the potential changes in earthworm density and activity induced by

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rotation with oilseed rape may have no direct effects on the growth of subsequent rice crops. However, nutrient-rich earthworm casts produced during the oilseed rape-growing season will remain in the fields, where they can affect the subsequent rice crop. Huang et al. (2016) observed that addition of earthworm casts collected from oilseed rape fields could improve nitrogen (N) uptake, physiological N-use efficiency, and grain yield in rice.

For a long time, high yield has been the first priority of rice researchers due to the high demand for rice production to keep pace with population growth and economic development (Peng et al., 2008). Also, hidden hunger has been prevalent in many Asian countries where rice is the major staple food (Muthayya et al., 2013). Provision of more nutritious foods is in high demand in the market place as people's health becomes a priority for the food industry and for consumers. Enhancing human-essential amino acids in grains is an important objective for improving the nutritional value in rice, generally consumed more for its energy (carbohydrate) content than for its protein or other nutrient components (Wenefrida et al., 2009). Because earthworm casts can affect N utilization in rice as mentioned above, and N is an important component of all amino acids, we hypothesized that earthworm casts may also affect human-essential amino acids in rice grain.

The main objectives of the present study were to determine (1) the response of earthworm density to a rice-oilseed rape rotation in no-tillage rice fields; and (2) the effect of earthworm casts produced during the oilseed rape-growing season on the content of human-essential amino acids in rice grains.

2. Materials and methods

2.1. On-farm research

To determine the response of earthworm density to cropping rotation with oilseed rape in no-tillage rice fields, an on-farm experiment was conducted in Nanxian (29°21′ N, 112°25′ E), Hunan Province, China from 2015 to 2017. The site has a moist subtropical monsoon climate with an annual average temperature of 16.6 °C, an annual average rainfall of 1238 mm, and an annual average sunshine duration of 1776 h. Soil in the fields was a purple calcareous clay (Fluvisol, FAO taxonomy). Two cropping systems (rice-oilseed rape, and rice-fallow) have been implemented in these fields under no-tillage management since 2006. Fixed beds (1.8 m width) and furrows (30 cm width and 20 cm depth) were established in the fields with rice-oilseed rape rotation, while flat surface was used in the fields with rice-fallow rotation.

In each year, for both cropping seasons, Huanghuazhan and other cultivars were planted during the rice-growing season from May to October. Rice crops were established by direct seeding (broadcasting) with a seed rate of 52.5 kg ha^{-1}. Weeds were controlled by applying herbicide (paraquat) at 5–7 days before sowing, and then bispyribac-sodium at the 4–5 leaf stage of rice. The fertilizers applied (per hectare) were: 75 kg urea (46% N) at the 2–3 leaf stage, 525 kg compound fertilizer (19% N, 7% P_2O_5, and 14% K_2O) at the 5–6 leaf stage, and 187.5 kg compound fertilizer at the panicle initiation stage. Alternate wetting and drying irrigation, i.e. the periodic drying and re-flooding of the rice field (Richards and Sander, 2014), was implemented during the whole rice-growing season. Insects and diseases were controlled using chemicals at the tillering, booting, and heading stages. Rice was harvested using a machine, and with the stubble was left on the field. The herbicide paraquat was applied again after harvesting the rice, and a hybrid oilseed rape cultivar Xiangzayou 6 (2006–2010) or Fengyou 273 (since 2011) was planted at 5–7 days after applying the herbicide. Oilseed rape seeds (3 kg ha^{-1}) were mixed with urea (45 kg ha^{-1}) and then broad-seeded on the fields. Top-dressing was done by applying 112.5 kg urea ha^{-1} at the 3–4 leaf stage and 375 kg compound fertilizer ha^{-1} at the 5–6 leaf stage. The herbicide ethametsulfuron was applied at the 4–5 leaf stage of oilseed rape. Oilseed rape was harvested by machine with the stubble left on the field.

Five fields (replicates) were randomly selected in 2015 and fixed in the next two years for each cropping system to determine earthworm density. The fields were separated by a bund of about 0.4 m width or by a farm road of about 2.5 m width. The size of each field was about 1200 m^2. In each year, ten sampling points (pseudo-replicates) were chosen along the diagonal of the selected fields for each cropping system after harvesting oilseed rape to determine earthworm density. In 2017, soil moisture and temperature were measured in a fallow and an oilseed rape field, and litterfall mass was determined in the oilseed rape field (details are presented in Supplementary material). The earthworm density was determined by hand-sorting from soil cores (60 cm long × 40 cm wide × 20 cm deep). The dominant earthworm species in the fields is *Pheretima guillelmi* (Huang et al., 2006).

2.2. Micro-plot experiment

To determine the effect of earthworm casts produced during the oilseed rape-growing season on the content of human-essential amino acids in rice grains, a micro-plot experiment was conducted in a rice field at the research farm of Hunan Agricultural University (28°11′ N, 113°04′ E) in Changsha, Hunan Province, China, in the single rice-growing season of 2016. The soil was a tidal clay (Fluvisol, FAO taxonomy) with the following properties: pH = 5.75, organic matter = 34.2 g kg^{-1}, available N = 81.6 mg kg^{-1}, available P = 34.4 mg kg^{-1}, and available K = 56.71 mg kg^{-1}. The micro-plots were constructed by inserting bottomless PVC boxes (40 cm long × 40 cm wide × 30 cm deep) into the soil to a depth of 20 cm with a collar of 10 cm aboveground.

The hybrid rice cultivar Liangyoupeijiu was grown in micro-plots under a factorial combination of two earthworm cast levels (0 and 34 kg m^{-2}) and three N fertilizer rates (9, 12 and 15 g N m^{-2}). The earthworm cast levels and N fertilizer rates were chosen according to Huang et al. (2016) and the local recommended practices in the study region. The treatments were arranged in a completely randomized block design with three replicates. Liangyoupeijiu has been widely commercialized, being planted over wide climatic areas in southern China and southeastern Asia, from 12 °N to 35 °N, e.g., in Vietnam and the Philippines. This representative hybrid cultivar has a cumulative planting area of about 5.95 million hectares (China Rice Data Center, http://www.ricedata.cn/variety/varis/600132.htm).

The earthworm casts collected from the surface of the no-tillage rice-oilseed rape rotation fields located in Nanxian during the oilseed rape cropping season had the following properties: pH = 7.89, organic matter = 61.4 g kg^{-1}, available N = 128 mg kg^{-1}, available P = 44.2 mg kg^{-1}, and available K = 254 mg kg^{-1}. The pH was determined using a digital pH meter (Model 868, Thermo Orion, MA, USA), organic matter was measured using the potassium dichromate method, available N was obtained with the diffusion method, available P was determined with the Olsen method, and available K was measured using a flame photometer (FP640, Shanghai Precision & Scientific Instrument Inc., Shanghai, China). The N fertilizer was ^{15}N-labeled urea (5.18% isotopic abundance, provided by Shanghai Institute of Chemical Industry, China).

Pre-geminated seeds were sown on a seedbed on 10 May. Seedlings were transplanted on 5 June. Transplanting was done with four hills per micro-plot and one seedling per hill. Earthworm casts were applied and mixed thoroughly up to about 20 cm soil depth at 1 day before transplanting. N fertilizer was split-applied with 50% as basal fertilizer (1 day before transplanting), 30% at early tillering (7 days after transplanting), and 20% at panicle initiation. Superphosphate (4.8 g P_2O_5 m^{-2}) was applied as basal fertilizer. Potassium chloride (8.4 g K_2O m^{-2}) was split equally at basal fertilization and panicle initiation. A floodwater depth of about 5 cm was maintained in the micro-plots until 7 days before maturity. Insects, pathogens, and weeds were controlled using approved pesticides to avoid yield loss.

At maturity, rice grains were sampled for each micro-plot and oven-dried at 70 °C to a constant weight. The dried grains were hulled,