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Full Length Article

Duck Trampling in Rice–Duck Farming Alters Rice Growth and Soil CH_4 Emissions

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Abstract

Rice–duck farming utilizes duck activity to mitigate methane (CH_4) emissions from paddy fields. Ducks trample the root zone of rice (*Oryza sativa* L.) and produce vibrations in the soil as they move through the paddy field. This study aimed to determine the response of the CH_4 flux, rice growth and microbial community under vibration. Vibration was simulated using a device composed of a motor, rod and wire and was applied in pots at intensities of 9 sec d^{-1} (V9), 39 sec d^{-1} (V39), 90 sec d^{-1} (V90) and 0 (CK). Compared with CK, the CH_4 flux at 21 d decreased in V9 and V90, whereas it was unchanged in V39. At 14 d, the CH_4 flux in V9, V39 and V90 was 65%, 70% and 35% lower than in CK, respectively. Height and elongation rate of rice reduced in V39 and V90. The dry shoot biomass and the dry root biomass decreased in V90. Compared with CK, the length and surface area of roots in V39 and V90 significantly decreased. The average diameter of roots increased 21% in V39. Root activity increased by 30% and 32% in V9 and V39, respectively. The average well color development increased from 24 h to 120 h in V39. The Shannon, Simpson and evenness indexes increased in all treatments at 120 h. The vibration can decrease the soil CH_4 flux, possibly related to the change of rice development and the microbial community.

Keywords: Morphological traits; CH_4 flux; Microbial community; Vibration

Introduction

As the second most prevalent greenhouse gas influencing global warming, methane (CH_4) in the atmosphere is greatly affected by natural sources and human activities (Weller *et al.*, 2016). Paddy fields have been identified as an important atmospheric CH_4 source from current anthropogenic activities and global CH_4 emissions from paddy fields are estimated to be 44.9 ± 1.0 Tg each year (Ito and Inatomi, 2012; Zhang *et al.*, 2012). Different managements in paddy fields have led to the fluctuation of CH_4 emissions and many strategies have been used to mitigate CH_4 emissions from paddy fields (Sanchis *et al.*, 2012). In South China, an agricultural technology called rice–duck farming (RDF) has gained increasing attention and practice as it effectively mitigates CH_4 emissions in paddy fields (Xu *et al.*, 2017). In RDF, the ducks move constantly in the spaces among rice plants in paddy field and their activity can lower CH_4 emissions by increasing the dissolved oxygen in water and by improving the soil redox potential (Zhan *et al.*, 2011). In addition, the ducks also stimulate growth of rice plants through diverse behaviors such as touching stems, rubbing leaves and trampling the root zone (Zhao *et al.*, 2015). Such stimulation has significantly influenced the anatomical structure of rice culm internodes (Zhang *et al.*, 2013). As 60%–90% of the CH_4 from fields is released via the aerenchyma of rice and final emissions are regulated by the CH_4 production and oxidation in the root zone (Aulakh *et al.*, 2000; Huang *et al.*, 2005), the stimulation of rice plants could affect the CH_4 emissions by changing the morphological traits and physiological processes of the plant.

As the ducks move around in the paddy fields, the ducks constantly trample the soil and generate vibrations, which can propagate to the entire rice plant. However, until now, little information has been provided about the responses of CH_4 emissions under external vibration stimulation. Therefore, this study aimed to determine whether CH_4 flux, rice growth and microbial community are changed under vibration, which will improve our understanding of the role of biological activities in CH_4 emission processes.

Materials and Methods

Plant Material and Pot Experiment Conditions

Rice seeds (*Oryza sativa* L. cv. Huanghuazhan) were sterilized with 0.01% $KMnO_4$ for 30 min. After removing

the residual $KMnO_4$, the sterilized seeds were soaked in aerated purified water for 24 h in darkness before germination on wet filter paper in an incubator under 16 h (day) and 8 h (night) at 27°C for 6 d. The seedlings were transplanted into pots and grown in a chamber under a 16 h day (27°C) and 8 h night (25°C) cycle. The soil was an Aquic Dystrudepts (Soil Survey Staff, 2014) and was collected from the surface (0–10 cm) in a paddy field at Guangzhou, China (23°14'N, 113°37'E). Each pot (115 × 160 mm: diameter × height) was filled with the 5-mm sieved air-dried soil (800 g) with a water level of 2–3 cm. The soil has a pH of 6.2, 30.06 g kg^{-1} organic matter, 0.12 g kg^{-1} alkali-soluble N, 0.05 g kg^{-1} available P, 0.05 g kg^{-1} available K, 1.45 g kg^{-1} total N, 0.52 g kg^{-1} total P and 20.12 g kg^{-1} total K. The pots were irrigated with purified water for 2 d before the application of N ($CO(NH_2)_2$, 0.162 g kg^{-1}), P ($Ca(H_2PO4)_2 \cdot H_2O$, 0.121 g kg^{-1}) and K (KCl, 0.120 g kg^{-1}). After 21 d, the seedlings that had attained a height of 18–19 cm were transplanted into pots. One seedling was planted in a plastic pot as a replicate and the vibration started after one week.

Vibration Parameter Set-up and Equipment

The vibration (*V*, vibration duration (s) per square from trampling) was quantified based on data from field observation and previous studies (Table 1) (Huang *et al.*, 2005; Zhang *et al.*, 2009a; Zhao *et al.*, 2015) using Eq. 1:

$$V = \frac{D \times T \times P}{A}, \quad \text{Eq. 1}$$

Where *D* is duck quantity [Individual quantity (ind) of 150 to 525 per 10000 m^2), *T* is active time (active time of one individual moving around in 10000 m^2 fields, from 28800 sec ind^{-1} to 86400 sec ind^{-1} (8 to 24 h ind^{-1})], *P* is trampling times [one individual trampled two times in each movement (*P* = 2)] and *A* is disturbance area (10000 m^2). Values of *D* = 345 ind and *T* = 57600 sec ind^{-1} were used as 345 individuals per hectare is common practice in fields, and the active time of one individual is 57600 s (16 h) each day. The minimum, common and maximum vibration duration was calculated as 8.64, 39.74 and 90.72 sec d^{-1} pot^{-1} based on the surface area of 0.01 m^2 per pot.

The Measurement and Set-up of Vibration Intensity

A mechanism composed of mini cylinder-shaped vibrating motor (M408, Shenzhen), glass rod and thin wire was designed to apply vibrations (Fig. 1A). The motor was fixed on the glass rod using scotch tape. The glass rod was fixed onto the top of inner wall by thin wire. In one cup, three glass rods adhered with three vibrating motors were distributed at a 120° angle. Vibration treatments were set as follows: vibration of 9 sec d^{-1} pot^{-1} (V9), vibration of 39 sec d^{-1} pot^{-1} (V39) and vibration of 90 sec d^{-1} pot^{-1} (V90). Vibration was performed by starting each motor for 3 sec, 13 sec and 30 sec with a timer. No vibration was used in the control (CK) pots. In each cup, the motors were started one by one to avoid mutual interference. All treatments and CK were laid out in a randomized design with five replications and the vibration lasted for 21 d.

Vibration intensity was measured on three hybrid ducks (290–300 g) in RDF using a vibration meter (AS63A, China) in a box (60 cm × 50 cm × 35 cm) with paddy soil and water (2 cm) (Fig. 1B), providing values of 0.78 ± 0.11 and 0.68 ± 0.09 mm s^{-1} at measurement times of 08 h00 and 20 h00, respectively. The experimental vibration intensity of each motor in pots with soil (800 g) and water (2–3 cm) was set to 0.77 ± 0.05 mm s^{-1} using the adjustable electric voltage. The vibration intensity was similar between experimental set-up and the measured intensity at 08 h00 and 20 h00 ($P > 0.05$).

Determination of Growth Traits

The rice plant height was determined using a ruler after 21 d. The elongation rate was calculated from equation of $(H_f - H_i)/t$, where *Hf* is final height (cm), *Hi* is initial height (cm) and *t* is 21 d. The roots were carefully washed 3–5 times with purified water to remove adhered soil. The shoots and roots were dried at 105°C for 2 h and 80°C for 24 h in an oven (Jinping 101S-3A, China). The root/shoot ratio was calculated based on the dry weight determined using an electronic balance (METTLER, Switzerland) after 21 d. Morphological traits of roots were measured with a root analysis instrument (WinRhizo-STD4800, Canada) (Zhang *et al.*, 2009b). Root activity (μg g^{-1} h^{-1}) was determined using the triphenyl tetrazolium chloride method after 21 d (Liu *et al.*, 2013).

Determination of CH_4 Flux of Rice

The CH_4 fluxes were measured by the closed chamber method (Mishra *et al.*, 1997). Gas samples were collected from 10 h00 to 12 h00 at 0, 7, 14 and 21 d based on a previously published method (Bharati *et al.*, 2001). Individual pots were carefully placed in a bucket (diameter 22 cm) and covered with a fabricated Perspex chamber (16 cm diameter × 75 cm height). The bucket was filled with water to form an airtight space. The gas samples were homogenized using an electric fan installed in the chamber. The temperature was monitored using a wired thermometer with a sensor in the chamber. Air tightness of the device was detected before usage. Gas was sampled using a syringe penetrating through a tiny hole in the chamber at 0, 8, 16, and 24 min. Gas samples were stored in pre-evacuated glass vials for the determination within 48 h. The CH_4 was determined using a gas chromatograph (Thermo Trace 1300, USA). The CH_4 flux was calculated using Eq. 2. (Ali *et al.*, 2015):

$$F = \frac{M}{V} \cdot \frac{dc}{dt} \cdot H \cdot \frac{273}{273+T}, \quad \text{Eq. 2}$$

Where F is the CH_4 flux (mg CH_4 m^{-2} h^{-1}), M is molar mass (16 g mol^{-1}), V is molar volume (22.4 L mol^{-1}), dc/dt is the increase rate (μmol mol^{-1} h^{-1}), H is chamber height (m) and T is air temperature (°C).

Microbial Community Measurement

The catabolic diversity of the soil microbial community was determined with Biolog Eco-microplates (Biolog Inc., USA). Rhizosphere soil (5 g) was extracted with sterilized water (50 ml) to prepare a suspension, which was shaken at 100 rpm for 30 min in a sterilized flask. The plates were incubated at 25°C for 168 h with a dilution (10^{-3}) of 150 μL. The optical absorbance at 590 and 750 nm was measured every 24 h using a Biolog Microstation (USA). Equations 3–7 were used to calculate the average well color development (AWCD) and the Shannon (H), Simpson (D) and evenness (E) biodiversity indexes (Garland and Mills, 1991; Schutter and Dick, 2001):

$$AWCD_{(590nm-750\,nm)} = \frac{\sum_{i=1}^{31} C_i}{31} \quad \text{Eq. 3}$$

$$P_i = \frac{C_i}{\sum_{i=1}^{31} C_i}, \quad \text{Eq. 4}$$

$$H = -\sum (P_i \times \ln P_i), \quad \text{Eq. 5}$$

$$D = 1 - \sum P_i^2, \quad \text{Eq. 6}$$

$$E = H / \ln S, \quad \text{Eq. 7}$$

Where C_i is the optical absorbance at 590 nm minus the optical absorbance at 750 nm after correction with the control well, P_i is the ratio of C_i to the sum of microbial activities of all substrates and S is the number of substrates used by the microbial community and represents species richness.

Statistical Analysis

Data were analyzed using SPSS 19.0 (USA). One-way ANOVA and multiple comparisons (Duncan method) were applied to analyze the differences among treatments and the control ($P < 0.05$ or $P < 0.01$).

Results

Growth Traits of Rice under Vibration in Soil

Plant height in V39 and V90 decreased by 13% and 17% ($P < 0.05$) relative to CK (Fig. 2A). The elongation rate in V39 and V90 was 22% and 28% lower than in CK ($P < 0.01$) (Fig. 2B). The shoot biomass was decreased in V90 by 16% compared with CK ($P < 0.05$) (Fig. 2C). The root biomass in V90 was 20% and 18% lower than in V9 and CK ($P < 0.05$) (Fig. 2D). Total biomass in V90 was reduced by 16% compared with CK ($P < 0.05$) (Fig. 2E). Compared with CK, root lengths in V39 and V90 decreased by 37% and 29% and surface area was by 28% and 27% ($P < 0.01$) (Table 2). Root volumes were similar between the treatments and CK. The average diameter in V39 was 21% higher than in CK. Root activity in V9 and V39 was 30% and 32% higher than in CK, whereas a decrease was found in V90 ($P < 0.01$). The root/shoot ratio in treatments was unchanged under vibrations ($P > 0.05$) (Fig. 2F).

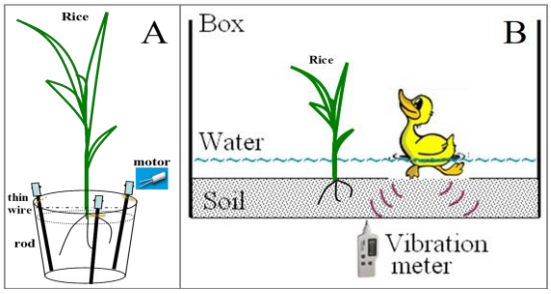

Fig. 1: Vibration mechanism (**A**) composed of vibrating motor, glass rod and thin wire; the glass rod was fixed onto the top of inner wall by thin wire. The motor was fixed on the glass rod using scotch tape. Three glass rods were distributed at a 120° angle; Vibration intensity (**B**) was measured on ducks using a vibration meter in a box with paddy soil and water

Microbial Community under Vibration in Soil

The AWCD value in treatments and CK increased rapidly before 48 h and slowed after 96 h (Fig. 3). The AWCD at 48 h in V9 and V39 was higher than in CK and V90 ($P < 0.05$). The AWCD at 96 h and AWCD at 120 h in V39 and V90 were both higher than in CK ($P < 0.05$). The AWCD at 168 h in V90 increased 9% compared with CK ($P < 0.05$). The H, D and E indexes at 120 h in treatments were all higher than in CK ($P < 0.05$) (Table 3).

The CH_4 Flux under Vibrations

At 7 d, the CH_4 flux in V9 and V90 decreased compared with CK ($P < 0.05$) (Fig. 4). At 14 d, the CH_4 flux in V9, V39 and V90 was 65%, 70% and 35% lower than in CK ($P < 0.01$). The CH_4 flux in V90 was higher than in V9 and V39 ($P < 0.05$). At 21 d, the CH_4 flux in V9 and V90 was lower than in CK ($P < 0.05$). The CH_4 flux in V9 was 34% lower than in V39 ($P < 0.05$). No significant change in CH_4 flux was found between V39 and CK ($P > 0.05$).

Discussion

Vibration in soil is converted into the mechanical stimuli to shoots by propagation through the roots. The V39 and V90 treatments inhibited stem elongation.

Table 1: Simulated vibration parameter set-up of duck trampling

Treatments	Experimental vibration (seconds d^{-1} pot^{-1})	Calculated vibration duration *V* (seconds m^{-2})	Individual quantity *D* (ind)	Active time *T* (seconds ind^{-1})	Trampling times *P*	Fields area *A* (m^2)
V9	9	864 [a]	150	28800	2	10000
V90	90	9072 [b]	525	86400	2	10000
V39	39	3974 [c]	345	57600	2	10000

[a] Theoretical minimum value; [b] Theoretical maximum value; [c] Common value

Table 2: Traits of morphology and roots activity of rice under vibrations for 21 d

Root traits	Vibration duration (seconds d^{-1})			
	0	V9	V39	V90
Length (cm)	296.07 ± 19.9a	237.63 ± 23.9ab	186.12 ± 9.19b	209.02 ± 5.07b
Surface area (cm^2)	97.26 ± 6.45a	84.08 ± 7.22ab	70.53 ± 4.00b	70.62 ± 2.70b
Volume (cm^3)	2.56 ± 0.23a	2.40 ± 0.26a	2.15 ± 0.22a	1.91 ± 0.14a
Average diameter (mm)	1.05 ± 0.05b	1.14 ± 0.06ab	1.27 ± 0.06a	1.08 ± 0.04b
Root activity ($\mu g\ g^{-1}\ h^{-1}$)	86.42 ± 2.32b	112.08 ± 5.55a	114.29 ± 4.50a	69.49 ± 2.39c

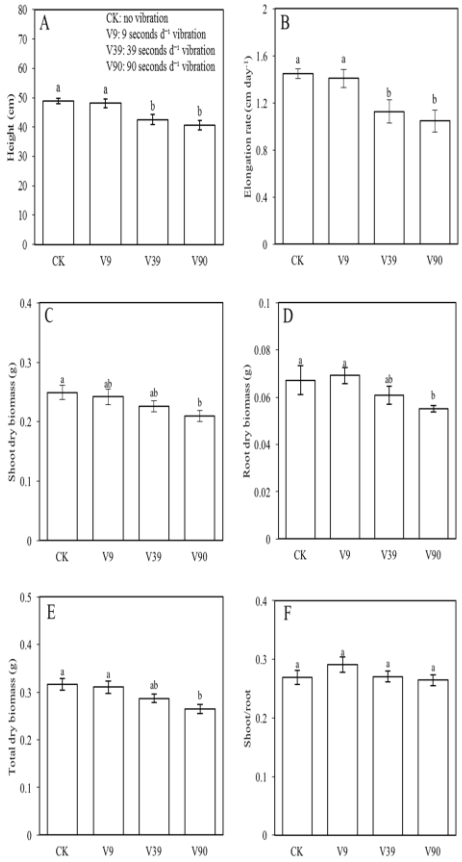

Fig. 2: The height (**A**), elongation rate (**B**), shoot dry biomass (**C**), root dry biomass (**D**), total dry biomass (**E**) and root/shoot (**F**) of rice under vibrations for 21 d

Similarly, *Hordeum vulgare* L., *Cucumis sativus* L. and *Phaseolus vulgaris* L. showed retarded stem elongation under mechanical stimulation (Jaffe, 1973). The V90 treatment hindered biomass accumulation, which may have been related to the micro-scale spatial displacement near roots in soil. The biomass accumulation of *Plantago major* L. was negatively affected by mechanical stimuli (Anten *et al.*, 2010). External mechanical stimulus on the plant leads to the biomass reallocation between the shoot and the root (Coutand, 2010).

No changed in the root/shoot ratio in treatments were observed. For V9 and V39, neither the root biomass nor shoot biomass were affected compared with CK. For V90, the decrease of root biomass and shoot biomass were similar

Table 3: Microbial diversity indices in rhizosphere soil under vibrations for 21 d

Biodiversity index at 120 h [a]	Vibration duration (seconds d^{-1})			
	0	V9	V39	V90
Shannon	3.2825 ± 0.0072b	3.3310 ± 0.0076a	3.3243 ± 0.0085a	3.3230 ± 0.0048a
Simpson	0.9595 ± 0.0005b	0.9622 ± 0.0005a	0.9620 ± 0.0004a	0.9621 ± 0.0003a
Evenness	0.9559 ± 0.0021b	0.9700 ± 0.0022a	0.9681 ± 0.0025a	0.9677 ± 0.0014a

[a] Different letters indicate significant differences among the treatments and CK at $p < 0.05$

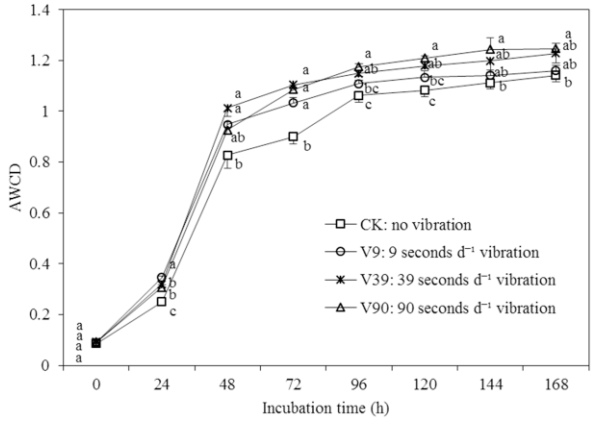

Fig. 3: The average well color development (AWCD) of rhizosphere soil microbial community of rice in pots under vibration for 21 d

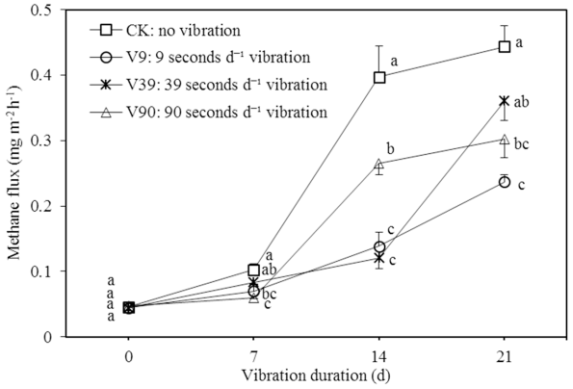

Fig. 4: The methane flux of rice in pots under vibrations at 0, 7, 14 and 21 d

relative to CK.

The microbial communities in the treatments showed an intense metabolic ability. Vibration may activate more nutrients in rhizosphere with a tiny shift of soil particles. Trampling of soils changes the activity and distribution of microbes in microhabitats (Pietola *et al.*, 2005). In the current study, the vibration enhanced the microbial diversity and the individuals became distributed more evenly. Compaction increases the soil bulk density, providing a more suitable environment for microorganism reproduction (Pengthamkeerati *et al.*, 2011). In V9, the AWCD at 120 h was similar to that in CK, whereas the *H*, *D* and *E* indices at 120 h increased, indicating that the vibration affected community structure more than it affected microbial activity.

There are three pathways of CH_4 emission from soils: diffusion, ebullition and plant-mediated transport. Of these, diffusion is often negligible in clay soils in paddy fields (Neue, 1993). In the current study, vibration affected ebullition and plant-mediated transport in rice grown in the pots. The root activity was enhanced in V9 relative to CK. The oxidation of CH_4 in the rhizosphere is regulated by radial oxygen loss as a function of root activity (Bodegom *et al.*, 2001). Higher root activity after vibration is also helpful to decrease the CH_4 transport through the rice plant. Root and shoot biomass decreased in V90 and low plant biomass may be a contributing factor to the reduction of CH_4 emissions (Wang *et al.*, 1997). The CH_4 flux in V39 decreased at 14 d. The decrease of length and surface area of root in V39 led to the decrease of contact area between the root and the soil, which may reduce oxygen release in soil. Vibration may have enhanced the ebullition, resulting in increased CH_4 emissions via bubbles from the pots, which slowly diminished the CH_4 pool throughout the experimental period. Ebullition may be an important pathway under vibration and the proportion of CH_4 released in bubbles should be determined in future research to understand the potential for reduction of final CH_4 emissions.

Conclusion

In short, high-intensity vibration had a negative effect on stem elongation, root development and biomass accumulation of rice; however, vibration increased the microbial biodiversity. The rice growth and CH_4 emissions were closely related to the external vibration stimulation.

Acknowledgments

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