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Floods increase soil microbial activity in paddy soil: a case study in Sakon Nakhon province, Thailand

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Abstract The results showed that the different flooding histories caused the effects on some soil properties. Partially flooded areas had the highest soil electrical conductivity at 0.06 mS/cm, but in the range that did not affect plant growth. All studied areas had low levels of organic matter and fields had available phosphorus of less than 4 mg/kg. There was a low level of exchangable potassium under fully flooded areas but a high level under partially flooded and non-flooded areas. The microbial activity revealed the highest soil respiration under fully flooded areas with 0.16 $mgCO_2$/g soil. The highest bacterial and fungal populations, FDA activity, urease, dehydrogenase, and protease activities were found under fully flooded area conditions. However, soil pH, microbial biomass carbon, microbial biomass nitrogen, and acid-phosphatase activity were not statistically different. No pesticides in carbamate, organochloride, and organophosphate groups were observed. The amounts of mercury, arsenic, lead, and cadmium were lower than the standard set of Thailand. Flooding did not reduce soil quality in the areas of the study; on the other hand, increased microbial activities were beneficial for plant growth. The effects of flooding on soil properties might differ depending on the conditions in each area.

Keywords: Floods, Soil chemical property, Microbial activity, Soil enzyme

Introduction

In the past ten years, the frequency and intensity of extreme weather events increased around the world. Floods are an important problem that are caused from global warming and anthropogenic activities. Regionally, soil is frequently flooded during the growing season for long periods of time. Water-saturated soil has low oxygen and redox potential that causes the disfunction or death of roots. Flooding reduces photosynthesis, respiration, and translocation of nutrients in plants (Yang *et al*., 2019). Frequent flooding reduces agricultural

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productivity and income that is related to food security (Wu *et al*., 2019). In 2011, floods affected 67.9 million people in China and more than 13 million people in Thailand. The reasons for flooding in Thailand are excessive rainfall, land use change, and insufficient drainage and flood protection systems (Singkran, 2017). In the period of the past 32 years, from 1985 to 2016, 69 major flood events have occurred (Singkran, 2017).

Sakon Nakhon is a province that faces flooding frequently. Agricultural areas comprising mostly paddy fields are affected by flooding. Paddy fields around Nong Han Lake, which is the main water reservoir, have seen changes in agricultural practice from intensive to sustainable agriculture with low chemical input. When heavy rain occurs, water flows from urban and industrial areas to Nong Han Lake before draining out into the Mekong River. The flow of water through urban and industrial areas to agricultural areas around Nong Han Lake may affect soil in many aspects. Chemical contamination from flooding of sustainable agricultural areas makes sustainable practice unsuccessful. This study aims to investigate the effects of different flooding conditions on soil properties, focusing on the chemical and biological properties in paddy fields with sustainable agriculture practices in Sakon Nakhon province, Thailand.

Materials and Methods

Study site

The experimental design had its basis in a completely randomized design (CRD) with 3 treatments and 15 replicates. The three treatments were different flooding histories in two consecutive years (2017 and 2018) in Sakon Nakhon province, as fully flooded areas (F1), partially flooded areas (F2) and non-flooded areas (F3). Soil samples were collected from paddy fields in November 2018 after flooding events. Soils were collected at the depth of 0-20 cm. The samples were kept in ice boxes and transferred to the laboratory at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang for chemical and biological analysis.

Soil chemical analysis

The samples were analyzed for soil pH with a 1:1 ratio of soil : water and measured with a pH meter (HANNA Instrument™ HI 8424). Soil electrical conductivity (EC) was measured at a 1:5 ratio of soil : water and measured with a conductivity meter (HANNA Instrument™ HI 8733). Soil organic matter

(SOM) was measured using the wet oxidation method, in which the samples were oxidized with $K_2Cr_2O_7$ under strong acid conditions (H_2SO_4), and the remaining $Cr_2O_7^{2-}$ underwent back titration with a reducing agent (Walkley and Black, 1946). Total nitrogen was measured with the Kjeldahl method, and the samples were digested with concentrated H_2SO_4 followed by distillation into a boric acid solution under alkaline conditions. The available phosphorus was extracted with Bray-II (0.03*N* NH_4F + 0.1*N* HCl) followed by the ascorbic acid method and measured at 882 nm. The extractable potassium was extracted by 1*N* NH_4OAc and analyzed using atomic absorption spectroscopy (Hitachi Z8200). For heavy metal contamination in soil in the form of mercury, arsenic, lead, and cadmium, the samples were digested with concentrated HNO_3 before measurement by the inductively coupled plasma method 3500 B. (3120) (APHA, 2005). Pesticide contaminants in carbamate, organochloride, and organophosphate groups were measured followed the QuEChERS method EN 15662:2008 (British Standard, 2008). The samples were extracted with acetonitrile containing 1% formic acid followed by partitioning with NaCl and $MgSO_4$, and then measured with gas chromatography.

Soil biological analysis

The microbial populations were measured using a culture-based method on asparagine mannitol agar (1 g/l K_2HPO_4, 0.5 g/l KNO_3, 0.2 g/l $MgSO_4.7H_2O$, 0.1 g/l $CaCl_2.2H_2O$, 0.1 g/l NaCl, 0.005 g/l $FeCl_3.6H_2O$, 0.5 g/l asparagin, 1 g/l mannitol and 15 g/l agar, pH 7.4) for bacteria, and potato dextrose agar (200 g/l potato infusion, 20 g/l dextrose and 15 g/l agar) for fungi. The microbial activities were analyzed as microbial respiration, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and fluorescein diacetate (FDA) activity. Soil respiration was measured by the closed chamber method, where CO_2 was trapped with 1*N* NaOH and then total CO_2 was determined by titration with 0.5*N* HCl. Soil microbial biomass carbon and nitrogen were measured by the chloroform fumigation method (Brookes *et al*., 1985; Vance *et al*., 1987). The soils after fumigation were extracted with 0.5*N* K_2SO_4 (1:4 of soil:K_2SO_4). The extractants were subjected to wet oxidation with 0.01*N* $(NH_4)_2Fe(SO_4)_2$ for MBC, and subjected to the Kjeldahl method for MBN. Total microbial activity was measured by hydrolysis of fluorescein diacetate (FDA activity) (Schnürer and Rosswall, 1982). Two grams of moist soils were wetted with 20 ml of 60*mM* phosphate buffer and 0.1 ml of 2 mg/ml fluorescein diacetate. After shaking incubation at 30 ℃ for 60 min, 2:1 of chloroform : methanol was added, and then centrifuged at 6,500 rpm for 10 min. The supernatants were measured at 490 nm.

Soil enzymes

Urease: Five grams of moist soil was wetted with 2.5 ml of 0.08*M* aqueous urea solution and 20 ml borate buffer. After 2 h of incubation at 37 ℃, 30 ml of 1*N* KCl was added and shaken on a mechanical shaker for 30 min. The resulting suspensions were filtered. One milliliter of filtrate was diluted to 10 ml with distilled H_2O and, successively, 5 ml Na salicylate and 2 ml of 0.1% Na dichlorisocyanurate were added. The optical density was determined at 690 nm after 30 min incubation at room temperature (Kandeler and Gerber, 1988).

Dehydrogenase: Twelve grams of moist soil was wetted with 4 ml of 1% triphenyltetrazolium chloride and 2 ml H_2O. After 24 hours of incubation at 37 ℃, 20 ml of 95% ethanol was added. The resulting suspensions were filtered and the filtrates were analyzed by the colorimetric procedure at 485 nm (Casida *et al*., 1964).

Protease: One gram of moist soil was wetted with 5 ml tris buffer solution and 5 ml sodium caseinate solution. After shaking incubation at 50 ℃ for 2 h, 5 ml of trichloroacetic acid solution was added. The resulting suspensions were centrifuged at 10,000 rpm for 10 min. Five milliliters of supernatant were transferred to a new tube and 7.5 ml alkaline reagent was added. After incubation for 15 min at room temperature, 5 ml folin reagent was added. After 1 h of incubation, the resulting suspensions were filtered and measured at 700 nm (Ladd and Butler, 1972).

Acid-phosphatase: One gram of moist soil was wetted with 4 ml modified universal buffer, pH 6.5 and 1 ml of 15*mM* *p*-nitrophenyl phosphate. After incubation at 37 ℃ for 1 h, 0.25 ml toluene and 4 ml of 0.5*M* NaOH were added. After incubation at room temperature for 30 min, the resulting suspensions were filtered and analyzed by the colorimetric procedure at 400 nm (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977).

Statistical data analysis

Data were analysed by the Minitab program version 18. The significance of treatments was set at a P-value of less than or equal to 0.05.

Results

Soil chemical properties

The range of soil pH indicated strong acid (pH 4.94-5.26). Soil EC was lower than 2 mS/cm and showed no toxic effects on plants. Soil organic matter

(SOM) ranged from 0.34-1.07%. Available phosphorus was very low in all fields with a range of 0.96-3.94 mg/kg. Extractable potassium content was low in F1 with 21.62 mg/kg, but high in F2 and F3 with 216.02 and 158.07 mg/kg, respectively (Table 1).

Table 1. Soil chemical properties in the field of study

Field	pH	EC (mS/cm)	OM (%)	Available P (mg/kg)	Extractable K (mg/kg)
F1	4.94±0.35[b1/]	0.01±0.00[c]	0.56±0.26[a]	3.94±1.46[a]	21.62±11.12[c]
F2	5.26±0.70[ab]	0.06±0.04[a]	0.34±0.14[b]	0.96±0.24[c]	216.02±66.92[a]
F3	5.01±0.24[b]	0.03±0.02[b]	1.07±0.57[a]	2.04±0.68[a]	158.07±40.96[a]

[1/]: different letters in each column are significantly different according to Tukey's test

Microbial activities

The microbial population was higher in F1 compared to F2 and F3 (Table 2). The bacteria population in F1 was $1.7x10^5$ CFU/g soil which was 1.7 times higher than F2 and 1.5 times higher than F3. The highest fungi population was also found in F1 with $3.9x10^4$ CFU/g soil which was 8 to 9 times higher than F2 and F3. Microbial activity as measured by soil respiration and FDA activity was the same as the microbial population. Soil respiration and FDA activity were highest in F1. However, flooding history had no effect on soil microbial biomass for both MBC and MBN (Table 3).

Table 2. Microbial population in the field of study

Field	Bacteria (CFU/g soil)	Fungi (CFU/g soil)
F1	166,600±59,171[a1/]	38,533±13,120[a]
F2	96,642±63,209[b]	4,331±2,198[b]
F3	112,920±61,776[b]	4,106±5,676[b]

[1/]: different letters in each column are significantly different according to Tukey's test

Table 3. Microbial activity in the field of study

Field	Respiration (μg CO_2/g)	MBC (ng/g)	MBN (μg/g)	FDA activity (mg/g soil)
F1	155.50±85.80[a1/]	1.34 ±1.04	23.76±22.57	4.76±0.48[a]
F2	57.70±61.64[b]	1.41±2.12	22.79±34.31	0.68±0.51[b]
F3	33.00±42.18[b]	1.77 ±1.58	31.18±26.67	1.92±1.22[c]

[1/]: different letters in each column are significantly different according to Tukey's test

Enzymatic activities

The activity of phosphatase, urease, dehydrogenase and protease was investigated. Phosphatase was not statistically different between the fields which were studied (Table 4). Urease and protease were higher in F1 than other fields with a statistically significant difference. Urease activity in F1 was 1.5 times higher than F2 and 2.3 times higher than F3. Protease activity in F1 was similar to F2 and 3 times higher than F3. Dehydrogenase appeared only in F1.

Table 4. Enzyme activity in soil in the fields studied

Field	Phosphatase (µg/g dwt/hr)	Urease (µg/g dwt/2hr)	Dehydrogenase (µg/g dwt)	Protease (µg/g dwt/2hr)
F1	996 ±643	19.89 ±4.59[a1/]	0.59 ±0.70	3.21 ±1.61[a]
F2	906 ±640	13.23 ±6.01[b]	-	3.08 ±1.30[a]
F3	892 ±483	8.81 ±9.67[b]	-	1.07 ±1.48[b]

[1/]: different letters in each column are significantly different according to Tukey's test

Heavy metal and pesticide contamination

Analysis of heavy metals including mercury, arsenic, lead, and cadmium revealed only arsenic and lead contamination. Arsenic content was 1.16-4.5 mg/kg. Lead content was 9.34-12.2 mg/kg. Analysis of pesticides as carbamate, organochlorine, and organophosphate groups based on the QuEChERS-method (British Standard, 2008) did not find contamination. The limit of detection by this method is 0.01 mg/kg.

Discussion

The fields studied had low soil fertility. The appropriate pH for rice plantation should range from 5.0 to 6.5. The lower soil pH will decrease the growth and the yield of the plants. EC of soil in this study ranged from 0.01-0.06 mS/cm. Soil EC lower than 2 mS/cm has no negative effect on plant growth. Organic matter relates to the structure, porosity, water retention and oxygen content in soil. Organic matter in the fields studies was quite low, while an appropriate SOM should be 1.5-3.5%. Appropriate phosphorus in soil should be 10-25 mg/g, while we observed only 0.96-3.94 mg/kg. Phosphorus induces disease resistance, root formation, and the maturity of plants. All nutrients in soil were lower than recommended levels, except for potassium which appeared in low levels only in F1. Lower levels of potassium in F1 were related to flooding conditions. Potassium can easily be lost via the leaching process (Mendes *et al*., 2016). In soil with low clay particles (Rosolem *et al*., 2010),

organic matter lacked potassium holding capacity (Lehman and Schroth, 2003). The area of study joined in an integrated program that applied mostly organic fertilizer and minimal chemical fertilizer as necessary. Low input of plant nutrients, especially nitrogen, causes low soil fertility. Flood water that passes through many places can bring nutrients and beneficial microorganisms. However, full flooding brought soil with available phosphorus but took the extractable potassium from the field.

Field 1 was located near water resources that caused flooding every year. The passing through of water resources may bring beneficial bacteria and fungi to the field. Microbial activity was the same as the microbial population. MBC and MBN were not statistically different between the different flooding conditions. Microbial biomass refers to the rapid turnover fraction of organic matter in soil (Rice *et al*., 1996). The low soil organic matter resulted in low microbial biomass.

In addition to nutrients and microorganisms, flooding can bring other environmental contaminants such as heavy metals and pesticides. The standard set for heavy metal contamination in soil must not be higher than 30 mg/kg for arsenic and 400 mg/kg for lead (National Environmental Board, 2004). This study did not observe higher heavy metal contamination than the standard set. Furthermore, pesticides in carbamate, organochlorine, and organophosphate groups were not detected. In our opinion, flooding can reduce soil fertility and bring contaminants to agricultural areas; however, flooding may also benefit the soil. The effect of flooding on soil properties might therefore be different depending on the conditions in each area.

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