



## Research article

# Effects of 18 yr of repeated manure and mineral fertilizer applications on soil properties, crop yield and their plot scale spatial distribution

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## Abstract

A field experiment was conducted to study the effect of repeated manure and mineral fertilizer applications on soil properties and crop yield and their spatial distribution at the plot scale. This experiment was initiated in 1996 with three treatments: 1) chemical fertilizer (CF), 2) chemical fertilizer + manure (CF + M) and 3) manure (M). After continuous application for 18 yr, soil and plants were sampled using 10 m × 10 m sampling grids. The results showed that application of M alone or combined CF + M increased soil total C, total N, organic matter, pH, electrical conductivity (EC) and available P compared with CF. However, M application resulted in significantly higher levels of soil pH and soil EC than for the other treatments. The highest seed yield was observed in M followed by CF + M and the lowest was observed in CF. The contour map indicated there was a medium-to-high spatial distribution for the soil properties and crop yield within and between the treatments. Soil total N content was visually correlated with soil total C, organic matter, soil pH and EC. The contour map of seed yield generally had a high visual correlation with all plant and soil parameters. High spatial distribution of soil properties and crop yield between the treatments clearly showed the effect of organic and mineral fertilizers; however spatial distribution within the treatment might have been due to the uneven distribution of applied fertilizers and soil management practices.

## Introduction

One of the most important intensification factors of crop production is plant nutrition and fertilization which has a significant impact on yield and quality of harvested products (Černý et al., 2010). Good soil fertility management ensures adequate nutrient availability to plants and increases crop yields (Shisanya et al., 2009; Vanlauwe et al., 2015). Applying an adequate amount of fertilizer is an important cultivation

practice for the yield and quality of crops and the application of inorganic fertilizers is a widely practiced method for increasing crop yields (Atafar et al. 2010; Liang et al. 2012). However, long-term application of ammonium nitrogen (N) fertilizer and urea can cause several soil problems, such as lowering soil organic carbon (SOC) contents, acidifying soil and poor soil physical properties (Celik et al. 2010; Liu et al. 2010). Application of organic fertilizers may partially improve these problems (Chang et al., 2014). The addition of organic materials such as crop residues, animal manures and green manures to soils has a direct effect on the soil organic matter content,

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can improve soil fertility and soil physical characteristics, augment microbial activity and can ameliorate metal toxicity, by complexation (Escobar and Hue, 2008). Application of different organic fertilizers in combination with chemical N fertilizer to agricultural lands is a popular practice in crop production (Chang et al., 2014). Long-term fertilizer experiments are valuable for monitoring changes in soil properties, determining yield trends, assessing soil quality and system sustainability (Zhang et al., 2015). Trends in soil fertility changes have been reported from samples obtained in the short-term (Oo et al., 2013a, Oo et al., 2013b; Oo et al., 2015) or long-term (Hati et al., 2006; Masto et al., 2006). In contrast, information is still limited on the spatial distribution in soil properties and on crop yield at the plot scale due to long-term fertilizer experiments involving manure and mineral fertilizer treatments.

Understanding the spatial distribution of soil properties and crop yield at the field scale is essential in refining agricultural management practices while minimizing environmental damage. Variation in soil properties and its related crop yield within the field is a result of long-term and short-term soil management practices and studies have investigated the long-term effects of manures and fertilizers by comparing treatment mean values for soil properties and crop yields (Hati et al., 2006; Masto et al., 2006). However, it is essential to understand the effects of different organic and mineral fertilizer applications on the spatial distribution of the physio-chemical properties of the soil within and between the treatments, as well as any effects on crop yields at plot scale. The hypothesis applied in the current study was that evaluating the visual maps of spatial distribution of soil properties and the related crop yield due to repeated application of manure and mineral fertilizers at the plot scale will be a useful tool to compare the effect of different fertilization regimes on the soil properties and crop yield. Therefore, the present research was undertaken to assess: 1) the effect of different fertilization regimes on the soil properties and crop yield and 2) to understand the impact of long-term repeated applications of organic and mineral fertilizers on the spatial distribution of soil properties and crop yield at the plot scale.

## Materials and Methods

### Description of study area

This experiment was conducted in an upland field of the Field Museum Fuchu, Field Science Center, Tokyo University of Agriculture and Technology (TUAT), Fuchu, Japan (latitude 35°40'N, longitude 139°37' E, at 59 m above sea level). The average annual precipitation in 2014 was 1,560 mm and the highest and lowest air temperatures were 36.7°C and -6.2°C, respectively. The type of soil was described as Andosol and red-yellow soil in the Japanese soil classification (UNESCO, 1974) with an approximate texture composition of 50% sand, 27% silt and 23% clay. The field had been under rye grass-soybean-winter wheat-dent corn rotation for 18 yr. Rye grass and winter wheat are grown from November to June and soybean and dent corn are grown from July to October.

### Experimental layout

The study area was about 1.5 ha and the boundary of the area is shown in Fig. 1. This experiment was initiated in 1996 with three treatments: 1) chemical fertilizer (CF), 2) chemical fertilizer + manure (CF + M) and 3) manure (M). The field was divided into eight plots consisting of three chemical fertilizer plots, four manure plots and one chemical fertilizer + manure plot (Fig. 1). Each plot size was 20 m × 80 m. The chemical composition of the applied cattle manure was 0.68% in nitrogen (N), 0.15% in P<sub>2</sub>O<sub>5</sub> and 0.7% in K<sub>2</sub>O. The manure and mineral fertilizer application rates for each crop are shown in Table 1. The amount of P<sub>2</sub>O<sub>5</sub> in the manure treatments was balanced to that in the chemical application only for the soybean crop by adding 110 kg/ha of P<sub>2</sub>O<sub>5</sub> as triple superphosphate. Data were collected on the soybean crop to compare the effect of different fertilizers on crop growth and yield from July to October 2014. Two varieties of soybean (*Glycine max*), namely Enrei (grid rows 1–4) and Tsukui (grid rows 58) were sown on 15 July 2014 at a spacing of 5 cm × 25 cm. The crop was harvested on 20 October 2014.

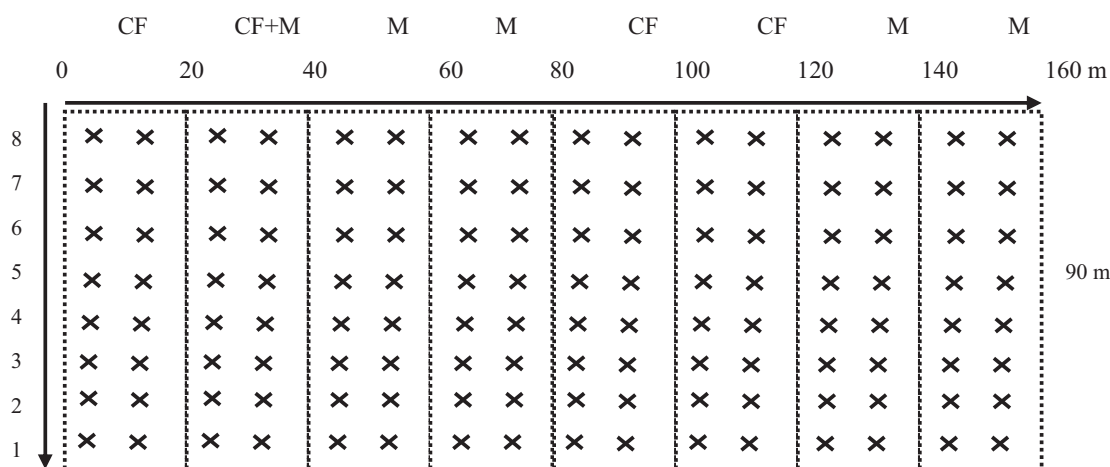


Fig. 1 Layout of sampling area and sampling grids, with 8 × 16 = 128 (X = 10 m × 10 m)

**Table 1** Rate of chemical and organic fertilizer applied to each crop

Crop	Treatment	Manure (kg/ha)	Chemical fertilizer		
			N (kg/ha)	P <sub>2</sub> O <sub>5</sub> (kg/ha)	K <sub>2</sub> O (kg/ha)
Rye grass	CF	0	140	140	140
	CF + M	10000	70	70	70
	M	20000	0	0	0
Soybean	CF	0	30	140	120
	CF + M	10000	15	70	70
	M	20000	0	110	0
Winter wheat	CF	0	120	120	120
	CF + M	9000	60	60	60
	M	18000	0	0	0
Dent corn	CF	0	200	200	200
	CF + M	15000	100	100	100
	M	30000	0	0	0

CF = chemical fertilizer; CF + M = chemical fertilizer + cattle manure; M = cattle manure. Chemical composition of cattle manure = 0.68% nitrogen (N), 0.15% P<sub>2</sub>O<sub>5</sub> and 0.7% K<sub>2</sub>O; NPK compound fertilizer 14:14:14 contained fused magnesium phosphate; NK compound fertilizer 16:0:16 contained potassium sulfate.

### Sample collection and analysis

Soil samples were collected before planting the soybean crop in June 2014 using 10 m × 10 m sampling grid cells. Surface soil samples were collected from the top 15 cm of each grid cell and 128 soil samples were collected (Fig. 1). Each sample was made by mixing five spots randomly collected from one grid cell. The total N and total C contents were analyzed using an NC analyzer (Sumigraph NC-80; Sumika Chemical Analysis Service Co.; Japan). The available P content was analyzed using the Bray-1 method (Olsen and Sommers, 1982). The organic matter content was determined using methods described by Nelson and Sommers (1996). Electrical conductivity (EC) of the soil water was measured in the supernatant suspension of a 1:5 soil:water mixture using an EC meter (OM-51; Horiba; Japan). The soil pH was measured in the supernatant suspension of a 1:2.5 soil:water mixture using a portable pH meter equipped with a combined electrode (glass:Ag/AgCl; Horiba; Japan).

Ten sample plants were collected from each grid cell for seed yield and yield components parameters. All plant growth data shown in this experiment were the average of the two soybean varieties.

### Statistical analysis

Statistical analysis was performed using the Sigma Plot 11 statistical software program. Descriptive statistics were taken for the physical and chemical properties of the soil and crop parameters. Box plots and contour plots were created for spatial distribution analysis of the soil properties and crop parameters due to different management practices. The treatment mean comparison was tested at the 5% level of probability using the least significant difference (LSD) test by Fischer. Spearman's rank order correlation analysis was done for all measured soil and plant parameters.

## Results and Discussion

### Effect of repeated fertilization on physicochemical properties of soil

The descriptive statistics and box plot analysis for soil parameters are presented in Table 2 and Fig. 2, respectively. The soil total C content was significantly lower in CF than in the CF + M and M treatments. The high soil total C content under the M and CF + M treatments could have been the result of the direct addition of organic matter through cattle manure as well as the increase in biomass production stimulated by the addition of manure. Adekayode and Ogunkoya (2011) also reported the addition of organic manure increased the soil organic C content.

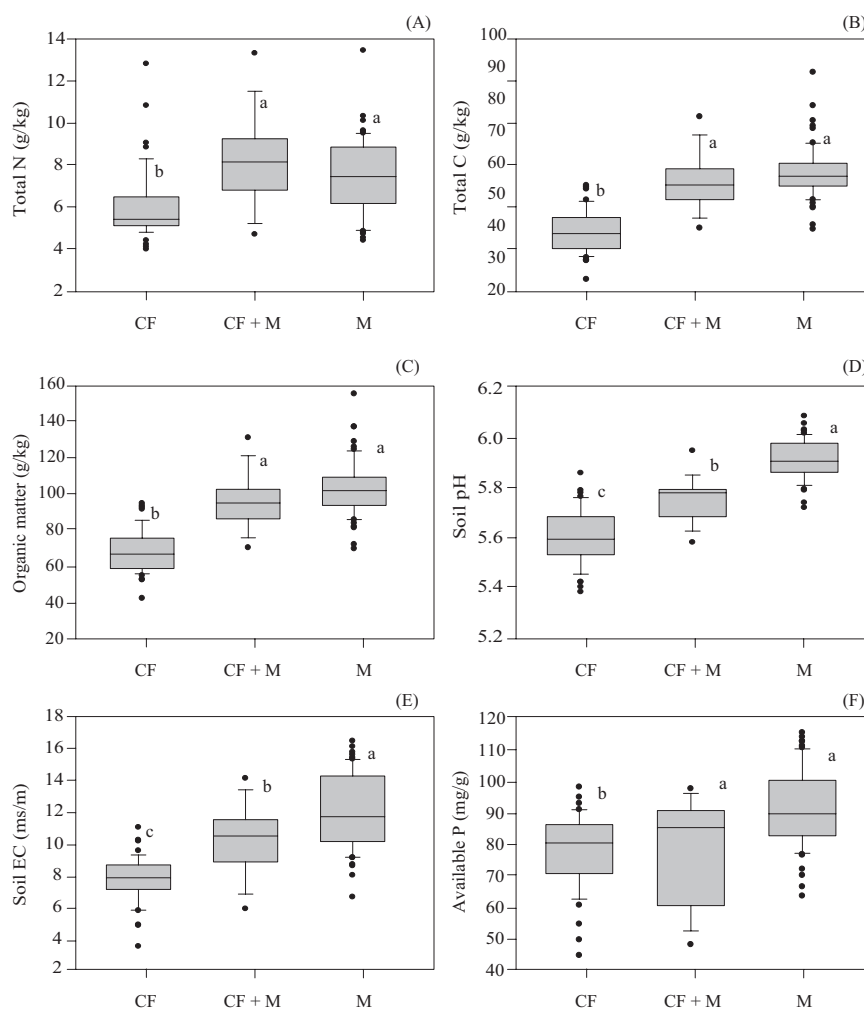
The manured plots had significantly higher total N content than the CF treatment. This result was in agreement with Aziz et al. (2010) and Thamaraiselvi et al. (2012). They reported that increases in the soil total N content were due to farmyard manure application. Based on the soil total nitrogen ratings of Hazeltonan and Murphy (2007), farmyard manure application raised the soil total N to the highest rate. The combination of M and CF produced the highest value of total N than from either M or CF alone. Chang et al. (2014) also observed that the soil total N contents of a combined application of chemical N with different organic fertilizers were significantly higher than for the of control and chemical N treatments.

The quality of a soil is determined by the soil organic matter content since it governs other soil parameters such as physical, chemical and biological properties. The soil organic matter content was significantly higher in the manure treatment due to the direct addition of organic manure to the soil. Relative to fertilizers, manure increased the soil organic matter content which was consistent with other evidence (Dick, 1992) that manure contained significant amounts of organic matter. Hao et al. (2003) also reported that long term applications of solid manure can result in very large increases in the soil organic matter.

**Table 2** Descriptive statistics of soil parameters determined in grids of treatment plots

	Treatment	Number	Mean	SD	Median	Skewness	Kurtosis	Minimum	Maximum
Total C (g/kg)	CF	<i>n</i> = 48	38.5	6.6	37.7	0.25	-0.3	23.3	52.9
	CF+M	<i>n</i> = 16	53.8	8.7	53.0	0.74	1.0	39.5	74.5
	M	<i>n</i> = 60	56.8	8.2	55.8	1.17	3.4	39.1	88.5
Total N (g/kg)	CF	<i>n</i> = 48	6.1	1.7	5.5	2.22	6.2	4.0	12.9
	CF+M	<i>n</i> = 16	8.1	2.1	8.2	0.83	1.6	4.7	13.4
	M	<i>n</i> = 60	7.5	1.8	7.5	0.44	0.7	4.45	13.5
Organic matter (g/kg)	CF	<i>n</i> = 48	66.0	11.8	64.5	0.46	-0.1	40.2	92.3
	CF+M	<i>n</i> = 16	92.4	14.5	92.6	0.67	1.4	68.1	128.4
	M	<i>n</i> = 60	99.9	14.7	99.4	0.95	2.5	67.5	152.5
Soil pH	CF	<i>n</i> = 48	5.6	0.1	5.6	0.10	-0.4	5.4	5.8
	CF+M	<i>n</i> = 16	5.7	0.1	5.8	0.16	1.3	5.6	5.9
	M	<i>n</i> = 60	5.9	0.1	5.9	-0.06	-0.4	5.7	6.07
Soil EC (ms/m)	CF	<i>n</i> = 48	7.8	1.4	7.9	-0.55	1.4	3.6	11.0
	CF+M	<i>n</i> = 16	10.2	2.1	10.5	-0.19	0.3	5.9	14.1
	M	<i>n</i> = 60	12.0	2.3	11.7	0.06	-0.8	6.7	16.4
Available P (mg/kg)	CF	<i>n</i> = 48	78.2	11.6	80.7	-0.90	0.7	44.9	98.5
	CF+M	<i>n</i> = 16	78.4	16.5	85.6	-0.65	-1.2	48.4	98.0
	M	<i>n</i> = 60	91.3	12.2	90.0	0.17	-0.4	63.8	115.8

CF = chemical fertilizer; CF + M = chemical fertilizer + cattle manure; M = cattle manure; EC = electrical conductivity.



**Fig. 2** Box plots of soil properties among treatments, where boxes within plots with same lowercase letter are not significantly different ( $p > 0.05$ ); CF = chemical fertilizer; CF+M = chemical fertilizer + cattle manure; M = cattle manure

Soil pH is an important chemical property governing the availability of nutrients in the soil nutrient pool. The soil pH value of the manure treatment was the highest followed by the CF + M treatment and the lowest pH value was noted in the soil of the CF treatment. These results were in agreement with Benke et al. (2009) who stated that the application of cattle manure moved the soil pH towards neutrality in acidic soils, thus improving nutrient availability. The pH decrease in the CF treatment might be attributed to the efficient assimilation of urea by soil microorganisms leading to the production of acidic metabolites such as organic acids (He and Suzuki, 2004) and to the process of nitrification (Hu et al., 2010).

Electrical conductivity can serve as a measure of soluble nutrients for both cations and anions. Neve et al. (2000) indicated that the mineralization of soil organic matter serves as a measure of soluble nutrients. In the current study, the soil EC value in the CF treatment was significantly lower than for the other treatments, with the M treatment having the highest value. According to Eigenberg et al. (2002), the higher EC value in the M treatment indicates a higher N content in the soil since they stated that the N content of soils may be monitored using EC measurements and specifically mentioned a significant positive relationship. Azeez and Van Averbek (2012) found that the electrical conductivity of the soil significantly increased with the application of poultry, cattle and goat manures.

There was no significant difference in available soil P content between the CF and CF + M plots. The available soil P content was significantly higher in the manure plot than in the other treated plots. This result conformed with the findings of Thamaraiselvi et al. (2012) who reported increases in soil total N and available P due to farmyard manure application. Similarly, Aziz et al. (2010) reported maximum P contents after a maize harvest for the farmyard manure treatment whereas the minimum P content was recorded for the treatments with the application of inorganic NPK fertilizer. In the current experiment, the increase in soil available P associated with the manure application might have been due to the direct addition of P through decomposition of the manure and the addition of a balanced amount of P fertilizer for soybean crop. The improvement in the soil available P with manure addition could be attributed to the addition of P from the manure and

retardation of soil P fixation by organic anions formed during manure decomposition (Tadesse et al., 2013). Although the current study did not analyze some soil physical properties, the manure-treated soil might also have had improved soil physical properties such as soil bulk density, porosity, and aggregate stability compared with CF treatment. A review conducted by Edmeades (2003) reported that manured soils had lower bulk density and higher porosity, hydraulic conductivity and aggregate stability, a higher organic matter content and higher numbers of microfauna relative to fertilized soils, and were more enriched in P, K, Ca and Mg in the top soil and in nitrate N, Ca and Mg in subsoils.

The total C content of the manure treatment and the total N content of the CF treatment had skewness values higher than 1, indicating that there were some extremely high values in these treatments (Table 2 and Fig. 2). Other soil variables for all treatments were slightly skewed (skewness < 1). Negative skewness (ranging from -0.19 to -0.90) was observed in the soil pH of the M treatment and in the soil EC and available P of the CF and CF + M treatments.

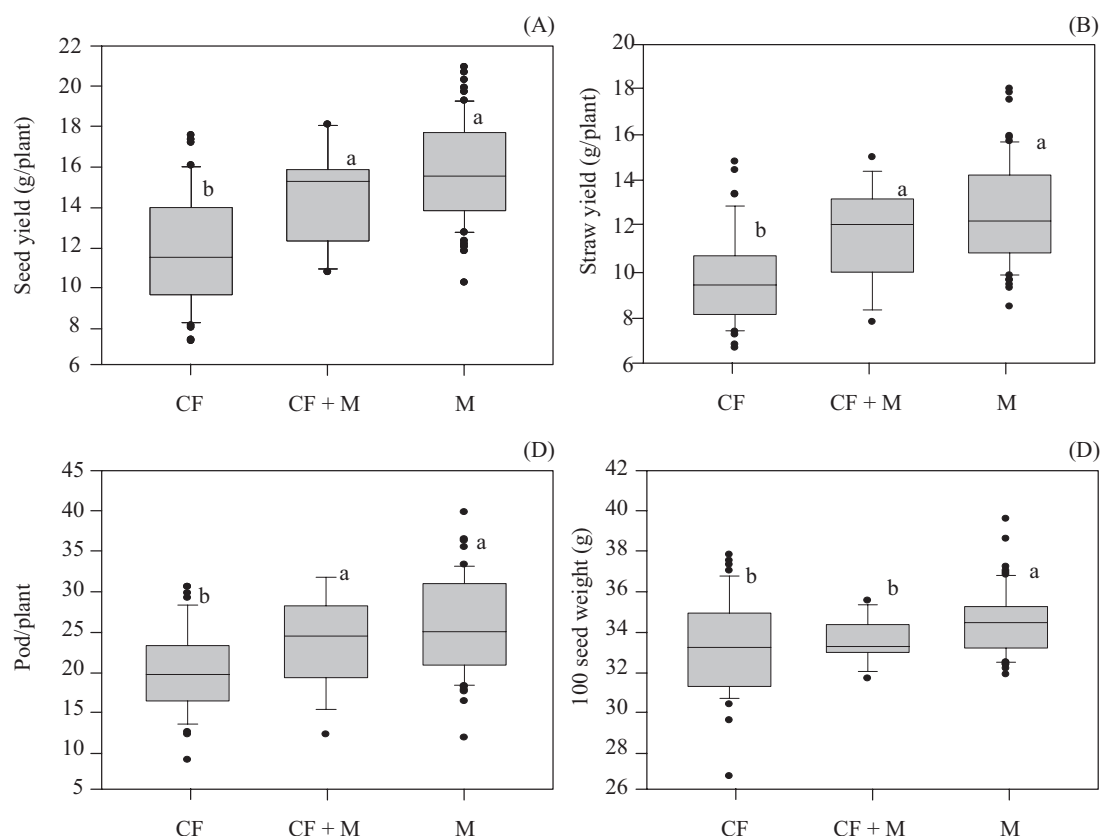
#### *Effect of repeated fertilization on plant growth parameters*

The descriptive statistics and box plot analysis for plant growth parameters are presented in Table 3 and Fig. 3, respectively. The mean value for pods per plant was significantly higher in the CF + M and M treatments than for CF alone. The highest value for 100 seed weight was observed in the M treatment followed by CF + M and the lowest was observed in the CF treatment. In this experiment, a high seed yield was achieved in the treatments containing manure. The higher seed yield and straw biomass in the CF + M and M treatments might be associated with higher pods per plant and 100 seed weight values. The integration of organic and inorganic fertilizers led to the higher yield due to a more balanced nutrient supply and enhanced potential N availability (Shisanya et al., 2009). Brar et al. (2015) also observed that better crop yields of maize and wheat with balanced applications of organic manure and inorganic fertilizers might be attributed to improvements in the soil physical properties along with sufficient supply of nutrients from farmyard manure and inorganic fertilizers.

**Table 3** Descriptive statistics of yield and yield components determined in the grids of treatment plots

	Treatment	Number	Mean	SD	Median	Skewness	Kurtosis	Minimum	Maximum
Seed yield (g/plant)	CF	<i>n</i> = 48	12.0	2.8	11.6	0.3	-1.0	7.4	17.7
	CF+M	<i>n</i> = 16	14.7	2.4	15.4	-0.3	-1.0	10.8	18.2
	M	<i>n</i> = 60	15.9	2.5	15.7	0.2	-0.7	10.3	21.1
Straw yield (g/plant)	CF	<i>n</i> = 48	9.8	2.0	9.5	0.9	0.2	6.8	14.9
	CF+M	<i>n</i> = 16	11.7	2.1	12.1	-0.4	-0.6	7.9	15.0
	M	<i>n</i> = 60	12.7	2.2	12.3	0.4	-0.4	8.6	18.0
Pods/plant	CF	<i>n</i> = 48	20.8	5.2	20.4	0.3	-0.3	9.6	31.4
	CF+M	<i>n</i> = 16	24.8	5.6	25.2	-0.5	-0.2	12.8	32.6
	M	<i>n</i> = 60	26.5	6.1	25.8	0.2	-0.7	12.4	40.8
100 seed weight (g)	CF	<i>n</i> = 48	33.5	2.4	68.2	-0.1	-0.2	27.0	38.1
	CF+M	<i>n</i> = 16	33.8	1.1	33.5	0.3	-0.3	31.9	35.8
	M	<i>n</i> = 60	34.8	1.6	34.7	0.8	0.9	32.1	39.9

CF = chemical fertilizer; CF + M = chemical fertilizer + cattle manure; M = cattle manure.



**Fig. 3** Box plots of growth and yield of soybean among treatments, where boxes within plots with same lowercase letter are not significantly different ( $p > 0.05$ ); CF = chemical fertilizer; CF+M = chemical fertilizer + cattle manure; M = cattle manure

However, in the current study, the highest seed yield was observed in the M treatment. The higher total C, soil pH and organic matter content as a result of the application of manure over the 18 yr period might have also been responsible for the higher yield in the treatment receiving cattle manure. The result was in agreement with Christensen et al. (1994) who observed that manure inputs produced a higher yield ( $p < 0.05$ ) than for a mineral fertilizer treatment for legumes in Denmark. Are et al. (2017) also reported that organic amendments increased the pooled grain yields of maize by 93.1–261.4% compared to the unamended control treatments. In the current study, the increases in pods per plant, 100 seed weight, seed yield and straw yield in response to application of M were probably due to the enhanced

availability of nutrients and the improved soil physical properties. Positive skewness (ranging from 0.07 to 0.87) was observed in the CF and M treatments for seed yield, straw yield, pods per plant and 100 seed weight of the CF + M and M treatments. Negative skewness (ranging from -0.07 to -0.51) was observed in all parameters of the CF + M treatment except for 100 seed weight.

#### *Relationship between soil properties and plant growth parameters*

The results from Spearman's rank order correlation analysis among the soil and plant parameters is shown in Table 4. Total N had a significant and positive correlation with total C and soil organic

**Table 4** Spearman's rank order correlation analysis among plant and soil parameters

	Straw yield	Pods/ plant	100 seed weight	Total C	Total N	Organic material	Soil pH	Soil EC	Ava. P
Seed yield	0.91**	0.82**	0.45**	0.47**	0.46**	0.48**	0.53**	0.39**	0.08 <sup>ns</sup>
Straw yield		0.84**	0.42**	0.50**	0.43**	0.51**	0.56**	0.38**	0.02 <sup>ns</sup>
Pods/plant			0.35**	0.41**	0.41**	0.42**	0.45**	0.26**	-0.02 <sup>ns</sup>
100 seed weight				0.16 <sup>ns</sup>	0.08 <sup>ns</sup>	0.16 <sup>ns</sup>	0.22*	0.12 <sup>ns</sup>	0.12 <sup>ns</sup>
Total C					0.57**	0.88**	0.66**	0.46**	0.24**
Total N						0.47**	0.33**	0.26**	0.01 <sup>ns</sup>
OM							0.70**	0.46**	0.27**
Soil pH								0.56**	0.31**
Soil EC									0.23**

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns = not significant at 0.05 level.



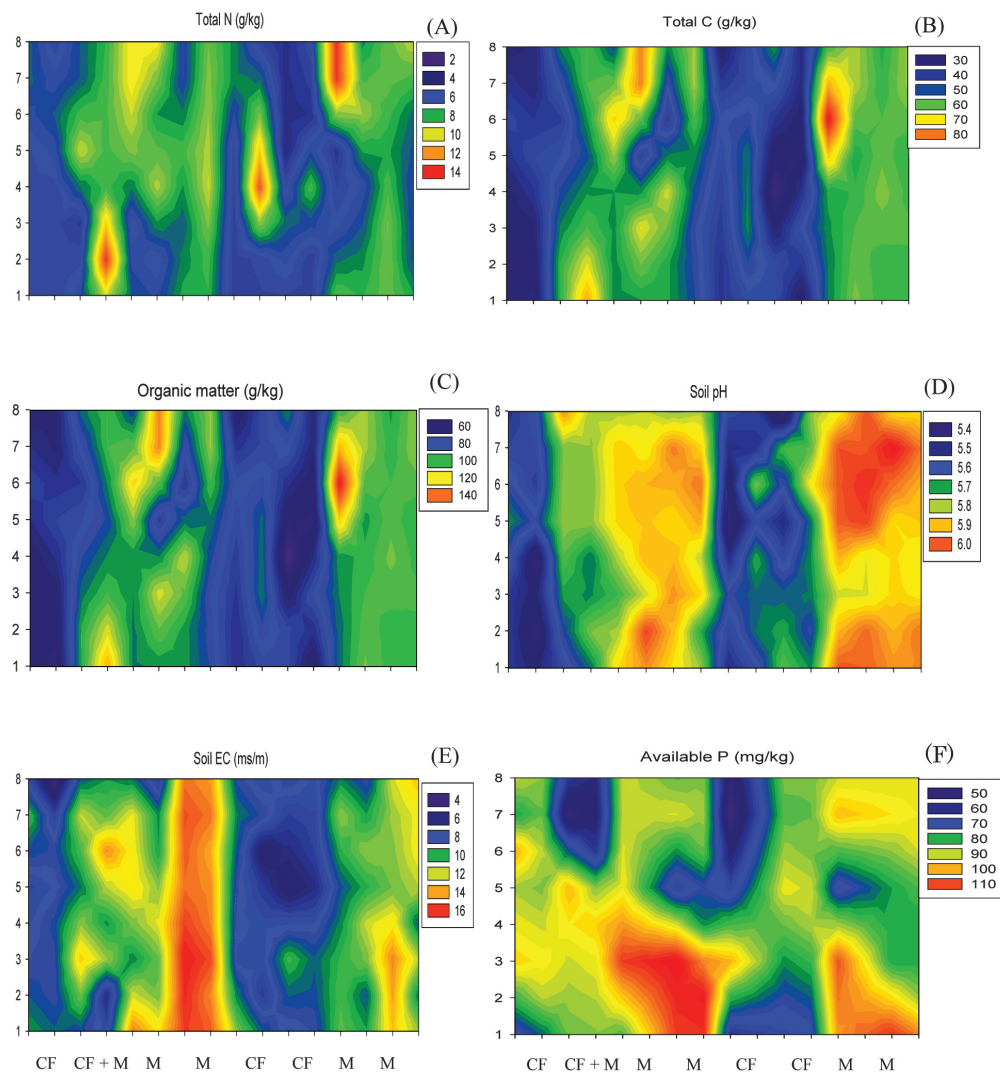
matter. The results indicated that total N increased with increased soil total C and soil organic matter as most of the soil nitrogen was estimated based on the organic matter present in the soil. Soil pH and soil EC had significant and positive correlations with total N, total C, available P and the soil organic matter content. Available P was significantly correlated with total C and the organic matter content.

Seed yield and its related parameters (except 100 seed weight) had significant positive correlations with total N, organic matter content, soil pH and soil EC. Oo et al. (2013a) also reported that seed yield was positively and significantly correlated with the total N content of the soil. Only soil pH had a significant positive correlation with 100 seed weight. Available P had no correlation with yield and its related parameters. Seed yield was significantly and positively correlated with pods per plant and 100 seed weight in this experiment.

#### *Spatial distribution in soil properties and plant growth parameters among the treatments*

The application of manure and mineral fertilizer produced clearly

visible changes in map appearances (Fig. 4 and 5). High spatial distribution patterns for all soil properties and plant growth parameters were observed within and between the treatments in this experiment. The soil total N content had a visual correlation with soil total C, organic matter content, soil pH and soil EC in this experiment. Spearman's rank order correlation analysis also confirmed that total N was positively and significantly correlated with total C, organic matter, soil pH and EC content (Table 3). However, the soil total N content did not visually correlate with the available P content in the soil in this experiment which was also supported by the correlation analysis. The visual comparison using contour distribution maps and box plots indicated that the plots with the CF treatment had lower values of soil parameters than the M alone or CF + M treatments (Fig. 2 and Fig. 4). There was less spatial distribution of measured soil parameters within the CF treatment, while greater variations in soil parameters were observed in the M and CF + M treatments. Higher spatial variations in the M treatment than for the CF treatment highlighted the need to spread the manure as evenly as possible to avoid part of the field getting excessive nutrients and another part not getting enough.



**Fig. 4** Contour plots at plot scale by treatment for: (A) soil total N; (B) total C; (C) organic matter; (D) soil pH; (E), soil electrical conductivity (EC); (F) available P

The contour distribution maps showed that seed yield had a high visual correlation with yield component parameters (Fig. 5). Spearman's rank order correlation analysis also confirmed this result due to the significant correlation between seed yield and yield component parameters (Table 3). The map of seed yield had a visual correlation with maps of total N, total C, organic matter, soil pH and EC in this experiment but no strong visual correlation was detected between seed yield and the map of the available P content in soil. The straw yield, pods per plant and 100 seed weight generally had visual correlations with all soil parameters. In this experiment, visual distribution mapping clearly showed significant differences between the treatments. High spatial distribution of the soil properties and crop yield between the treatments clearly showed the effect of different fertilizer applications. However, high spatial variation within the treatment might have been due to the uneven distribution and overlapped application of fertilizer using the broadcast fertilizer spreader. Moreover, management practices such as soil preparation using rotary tilling might also be one of the reasons for spatial variation in the measured parameters within the treatment.

The results of the present study indicated that the long-term application of manure alone or the integrated application of manure and mineral fertilizers improved the soil total N and total C contents.

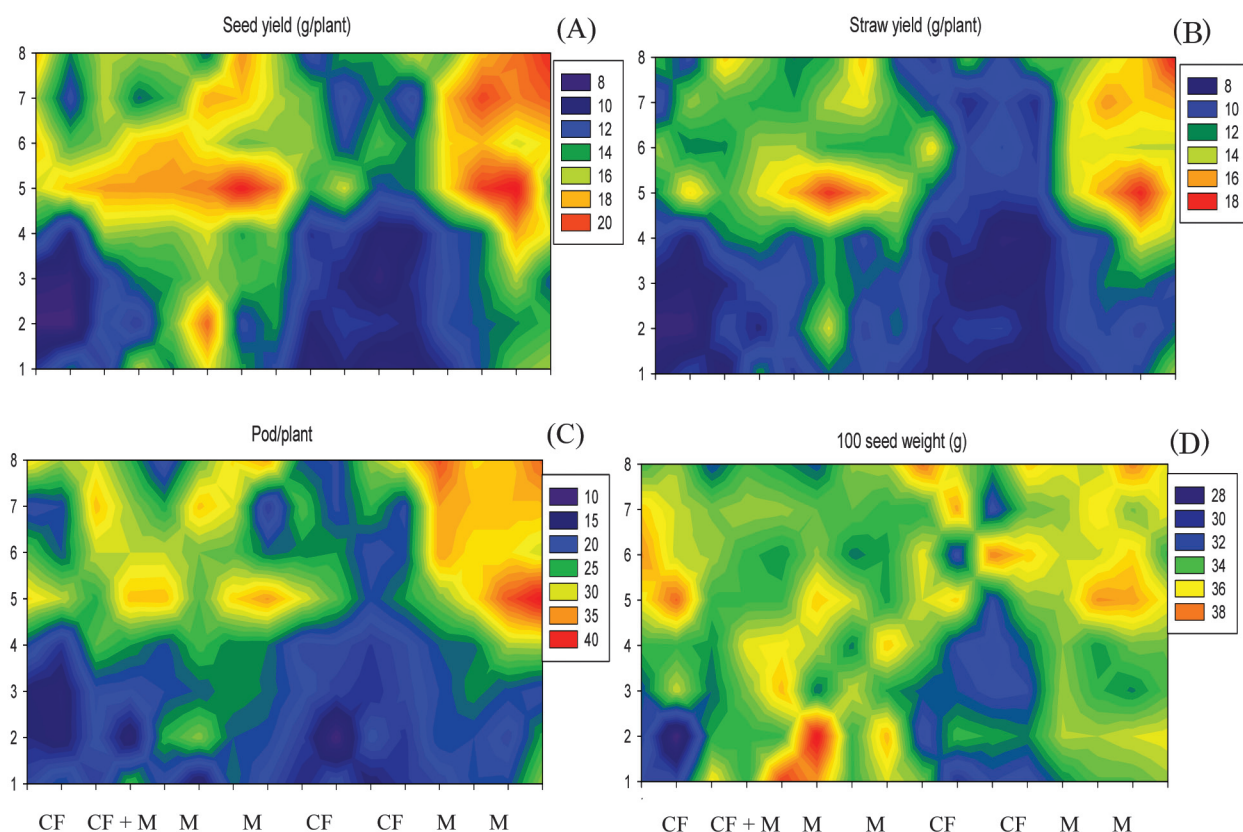
Repeated manure application also increased the soil pH, organic matter and soil EC which favor good growth and development of the crop. Moreover, visual observation using the distribution maps might be useful in understanding the spatial distribution of the soil properties and crop yield within and between treatments rather than comparing the treatment means. According to the visual distribution maps, all soil and plant parameters generally had visual correlation in this study. From the results of the current experiment, it could be concluded that repeated application of manure alone or combined applications of manure and chemical fertilizer resulted in improvements in most soil physiochemical properties and nutrient balances that led to increased and sustained crop production at the plot scale.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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**Fig. 5** Contour plots at plot scale for: (A) seed yield; (B) straw yield; (C) pods per plant; (D) 100 seed weight, where grid rows 1–4 = variety Enrei, grid rows 5–8 = variety Tsukui



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