Effectiveness of Reduced Tillage on the Cast Production of *Pheretima (Amynthas) carnosa* and Yields of Chinese Cabbage on Volcanic-ash Soil

Satoshi KANEDA¹, Miyuki NAKAJIMA^{2,3}*, Yasufumi URASHIMA^{2,4} and Toshifumi MURAKAMI²

¹ National Institute for Agro-Environmental Sciences (Tsukuba, Ibaraki 305-8604, Japan)

² NARO Tohoku Agricultural Research Center (Fukushima, Fukushima 960-2156, Japan)

Abstract

Soil aggregation by earthworms is important for sustainable agriculture because soil aggregates are highly permeable and retain water. Casts are a soil aggregate and indicator of earthworm activity. The effects of reduced tillage using a rotary tiller on earthworm cast productivity and yield of Chinese cabbage were investigated in volcanic-ash soil. Five tillage practices were investigated; conventional tillage (CT), no tillage (NT), and three reduced tillage practices: shallow tillage, row tillage, and shallow row tillage respectively. Cast production of *Pheretima (Amynthas) carnosa*, the species which dominated the study site, and yields of Chinese cabbages were measured during three crop seasons. Crop yield under the NT treatment was lower than that under the CT treatment (p<0.05) in fall 2004, while in spring 2005 Chinese cabbage under NT treatment did not head out. Yields under reduced tillage treatments tended to decrease in spring 2005, but were similar to those under CT treatment in the other two cropping seasons. Under CT treatment, the surface cast production was almost zero. Cast production under reduced tillage exceeded that under CT treatment, but was lower than that under NT treatment. Accordingly, reduced tillage can combine the ability to preserve aggregate formation by earthworms while also preventing any decline in Chinese cabbage production yield in the cropping system studied.

Discipline: Soils, fertilizer and plant nutrition **Additional key words:** earthworm, row tillage, shallow tillage, soil aggregate, sustainable agriculture

Introduction

Tillage, pesticide use and chemical fertilizer application are important means of increasing crop production. However, mismanagement of tillage and pesticide and fertilizer applications results in water pollution and soil degradation, hence recent intensive efforts to develop environment-conscious agricultural systems. When environment-conscious agricultural techniques are adopted, any potential or actual yield loss should be covered by facilitating soil fertilization through soil microbe and fauna functions. Although tillage is important for crop management, it may also degrade aggregates and reduce the densities of earthworms, which boost the development of soil aggregates⁵. Earthworms enhance nutrient cycling and soil aggregate formation^{1,3,11}. Furthermore, Martin (1991)¹² showed that aggregates made by earthworms delayed the decomposition of organic matter. Since keeping organic carbon in soil undecomposed is important for soil fertilization, promoting the role of earthworms in soil aggregate formation should also boost improved soil fertilization.

The effect of tillage on earthworm density depends on the tillage machinery used, soil types and earthworm species⁵. Earthworms can be separated into three major groups based on their burrowing and feeding habits: endogeic, epigeic and anecic. Endogeic species live in soil layers, whereas epigeic species are surface dwellers, and anecic species build permanent, deep vertical burrows in the soil and rise to the surface to search for food^{2,10}. Generally, till-

Present address:

³ NARO Tohoku Agricultural Research Center (Morioka, Iwate 020-0198, Japan)

⁴ Agriculture, Forestry and Fisheries Research Council Secretariat, Ministry of Agriculture, Forestry and Fisheries (MAFF) (Chiyoda, Tokyo 100-8950, Japan)

^{*}Corresponding author: e-mail miyukin@affrc.go.jp

Received 4 February 2013; accepted 8 July 2013.

age harms all these earthworm groups, particularly epigeic and anecic species⁵, whereas endogeic species sometimes proliferate if tillage adds organic material to the soil⁵. Ploughing is a conventional tillage method in Western countries, and the effects of tillage on earthworm communities have often been compared with those of non-inversion reduced tillage. During these studies, the adoption of noninversion reduced tillage, e.g. disc harrowing, increased the abundance of anecic and epigeic species compared with plough tillage^{6,8}. Conversely, rotary tillers are conventionally used in Japan. To date, there have been no studies investigating how the tillage area and depth resulting from rotary tillage affects the role of earthworms in soil aggregate formation and earthworm density. Volcanic-ash soils are the dominant soil on Japanese agricultural land¹⁸. In addition to differences in tiller type, the effect of reduced tillage on Japanese agricultural land has not been well studied. Enami et al. (1999)⁷ showed that the negative effects of tillage on earthworm abundance were stronger in volcanic-ash soil than non-volcanic-ash soils, while the benefit of reduced tillage in terms of earthworm abundance and biomass only emerged in non-volcanic-ash soil (e.g. Ernst and Emmerling, 2009⁸). In volcanic-ash soil, the negative effects of tillage on earthworms were investigated by Nakamura (1988)¹⁴ and Nakamura et al. (2003)¹⁵, but the effects of reduced tillage on the role of earthworms in soil aggregate formation and density were not studied. Estimating the effects of reduced tillage on earthworms in volcanic-ash soil is useful for accelerating the functioning and use of earthworms in sustainable agriculture.

No tillage and reduced tillage would induce less destruction of soil aggregate and facilitate soil aggregate formation by earthworms. Promoting the soil aggregate structure would support sustainability, namely, long term productivities on which we can not detect the effects of soil aggregate formation in few years. Conversely, in some cases, environmentally friendly tillage decreased the crop yield in the short term. For carrying out sustainable agriculture, not only long term productivity and also short term productivity are needed. Therefore in this study the effects of reduced tillage using rotary tiller on the role of earthworms in soil aggregate formation as indicator of long term productivity and on the crop yield as short term productivity on a volcanic-ash soil were investigated.

Methods

In the experimental field, the anecic earthworm species *Pheretima* (*Amynthas*) *carnosa* (Goto & Hatai, 1899), which grows to over 20 cm long, dominated, comprising over 95% of the total biomass in the field⁹. Therefore, we focused on this species in the study. Earthworms form soil aggregates by casting and exuding external mucus into the soil, which

acts as a soil-binding agent. Although anecic species excrete casts both onto the soil surface and into the soil, we could not distinguish between newly excreted casts and soil aggregates in the soil. In addition, it was difficult to collect casts deposited in the soil at no-till sites without disturbing the plots. Accordingly, surface cast production alone was often measured and used to indicate activity (e.g. Zeller and Arnone, 1997¹⁹, Sharpley et al., 2011¹⁷). Furthermore, a previous study showed a strong positive relationship between earthworm biomass and the surface cast production of P. (A.) carnosa9. Therefore, in this field experiment, surface cast production was measured to estimate the effects of tillage on earthworm casting activities. Two experiments and one field survey were conducted. A pot experiment was performed to confirm the validity of counting surface casts as an indicator of earthworm activities. In a previous study⁹, we investigated herbicidal effects on this species by measuring surface cast production under no-till sites. In the previous study, soil bulk density was the same between treatments. However, in this study, bulk density changed with tillage treatments and the bulk density of the tilled soil was low compared with no-till. In addition, the bulk density of the tilled soil increased due to the treading stress applied when farm work was done in the plots. If soil density affects earthworm casting behavior, we cannot estimate the role of earthworms in soil aggregate formation by measuring surface cast production between different tillage treatments. Moreover, a field experiment was performed to declare the effects of tillage on soil surface cast production and crop yield. During the field experiment, we could assess the initial effects of tillage on earthworms, because the study site had been managed as a no-till site for more than a decade. These data were obtained by counting the number of injured earthworms on the soil surface at the time of the first tillage. A field survey was performed to obtain data on the vertical distribution of earthworms. In this study, since tillage depth was manipulated between treatments, information on the vertical distribution of earthworms was useful to estimate the extent of earthworm damage caused directly by machinery.

1. Field experiment

(1) Study site

The study was conducted at the Department of Upland Farming, National Agricultural Research Center for Tohoku Region, Fukushima, Japan (37°43'N, 140°23'E; altitude 176 m a.s.l.). The soil was a humus-rich volcanic ash (an Umbric Andosol, according to the FAO classification; 55% sand, 26% silt and 20% clay)¹³. The air temperature and precipitation were monitored near the experimental field. Mean monthly air temperatures and monthly precipitation were calculated from the daily data. Precipitation from April to June in 2005 was less than that in 2004, while in

October precipitation in 2004 exceeded that in 2005. The maximum average temperature over a month differed between the two years: the maximum average was in July 2004 and in August 2005 (Fig. 1).

(2) Treatment

The field experiment was set up in a field measuring 94×30 m. Five tillage practices were investigated; conventional tillage (CT), no tillage (NT), and three reduced tillage practices. For the reduced tillage practices, we manipulated the tillage depth and area to reduce the amount of soil disturbance. The whole surface of CT plots was tilled to a depth of 15 cm with a rotary tiller, while in the NT plots, Chinese cabbage was directly planted. Shallow tillage (ST) was conducted by tilling to a depth of 7 cm. Row tillage (RT) tilled to a depth of 15 cm, but tilled with a width of only 40 cm in an 80-cm width row for planting. In the RT treatment, the area tilled by the rotary tiller was half that in the CT treatment. Shallow row tillage (SRT) was done by tilling to a depth of 7 cm, but tilled with a width of only 40 cm. There was no litter on the surface in the tilled area. Conversely, there was some litter, which was dead weed on the surface in the no-tilled area. The experiments commenced in the fall of 2004 with three replicate plots (4 \times 7 m) per tillage treatment.

(3) Cropping

Chinese cabbage (Brassica rapa L. 'Oukou 65') was cultured in fall 2004, spring 2005 and fall 2005. Table 1 shows the field operation. Because the experimental field had been under no-till management for an extended period, in the first cropping, tillage was done twice. Weeding was done manually. Because the weed growth rate was high in spring, the second cropping involved weeding done twice, while herbicide was applied to every treatment in the second and third cropping. Chinese cabbage was transplanted by hand at rows spaced at 80 cm and a hill space of 40 cm within rows in every cropping. Chemical fertilizer was applied to the rows just before transplanting at a rate corresponding to approximately 18 kg N, 8 kg P, and 15 kg K per 10 a (1,000 m²) in all cropping seasons. A cyclo-di-urea (2oxo-4-methyl-6-ureidohexahydropyrimidine) mixed fertilizer (15% N, 15% P₂O₅, 15% K₂O), which is a slow-release fertilizer was used in 39% of the total fertilizer.

(4) Measurement

To measure the effects of tillage treatments on surface cast production, soil surface casts were collected before the tillage of the first cropping during twelve days and approximately weekly during each cropping period. Fresh casts were collected from a quadrat of 80×80 cm located at the center of each plot, whereupon they were dried at 105° C and weighed.

Damaged earthworms on the surface were counted in the RT and SRT plots after the first tillage, on 26 August, 2004.

Reduced Tillage Impacts on Earthworm Cast Production



Fig. 1. Monthly precipitation (bars) and temperature (solid line) during the experiment

Table 1. Field operation in field experiment

	2004	2005	2005
	2004	2005	2005
	fall	spring	fall
Tillage	26 Aug. 8 Sep.	8 Apr.	1 Sep.
Weeding	26 Aug.	28 Apr. 18 May	11 Oct.
Herbicide application		4 Apr.	30 Aug.
Chinese cabbage seeded	30 Aug.	16 Mar.	23 Aug.
Chinese cabbage transplanted	13 Sep.	19 Apr.	12 Sep.
Chinese cabbage hervested	24 Nov.	21 Jun.	24 Nov.

Fresh heads of Chinese cabbage were weighed 60 days after planting. Fifty-six individuals were planted per plot, and only thirty individuals inside the plot were used to calculate the mean value. Undeveloped individuals, e.g. those whose heads were not formed, were not used to calculate the mean value.

2. Pot experiment

The effects of tillage on soil bulk density and soil surface cast production were measured for validation as a sampling surface cast in the field experiment. Soil core samples were collected in five replications from the NT, CT without treading stress (T), and CT with treading stress (TS) plots. Polycarbonate tubes (10.5 cm in diameter, 12 cm high) were inserted to a depth of 10 cm, and then gently pulled out. The upper opening of the core was covered with a screen mesh made of polyester to prevent the earthworms from escaping, while the bottom of the core was covered with polycarbonate. As food, 1.93 g (105°C dry weight basis) of centipede grass (Eremochloa ophiuroides (Munro) Hack.) was placed on the surface and one worm (average fresh weight 4 g) was inserted into each pot. The core was kept in a climate chamber, the temperature of which was controlled at 22.5°C during the day with 52700 lux (12 h) and at 20°C overnight (12 h). Relative humidity was controlled at 70% during the day, and 75% overnight. Soil surface casts were collected eight and fifteen days after the start of the experiment respectively, and weighed (105°C dry weight basis).

3. Field survey

The vertical distribution of *P*. (*A*.) carnosa was investigated during one year in an adjacent study plot, which had been under no-till conditions. Soil samples were taken from depths of 0-5, 5-25 and 25-40 cm in October 2003, February, June, August, and November 2004. For the second tillage, on 8 September, 2004, sampling was performed at soil depths of 0-7, 7-15 and 15-40 cm. Six soil profiles (25×25 cm) were excavated at all sampling times and earthworms were removed from the soil by hand-sorting. Only the sub-adults and adult density were counted because only they could be identified.

4. Data analysis

The effects of tillage on cast production and soil bulk density in pot experiment were estimated by one-way ANOVA and a Tukey-Kramer's honestly significant difference test, while the effects of tillage and cropping season on cast and Chinese cabbage production were estimated by repeated measures ANOVA. The mean value of thirty Chinese cabbage head samples was used for statistical analysis. To obtain normal distributions for the data before conducting ANOVA, the cast weight and weights of the heads of Chinese cabbage were transformed to log (x + 1), but the



Fig. 2. Effects of tillage treatments on the yield per Chinese cabbage (head weight)

Error bars indicate ±SE. CT: conventional tillage; RT: row tillage; ST: surface tillage; SRT: surface row tillage; NT: no tillage

*: There were no heads of Chinese cabbage in NT in spring 2005. Different characters show statistically significant differences after Bonferroni corrections (α initial=5%). original mean values are presented in Figs. 2 and 3. When significant interaction between treatment and cropping season was detected, the tillage effects were analyzed at each cropping season with Bonferroni correction. Analyses were carried out using SPSS 11.0.1J for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

1. Validation as sampling surface cast in the pot experiment

If the soil density affected earthworm casting behavior, for example high soil density increased earthworm surface casts, this would prevent us from using surface casting activity as an indicator of soil aggregate formation by earth-



Fig. 3. Tillage effects on surface cast production rate in field experiment

Error bars indicate ±SE. CT: conventional tillage; RT: row tillage; ST: surface tillage; SRT: surface row tillage; NT: no tillage.

Different characters show statistically significant differences after Bonferroni corrections (α initial=5%).

Table 2. Effects of tillage on soil bulk density and soil surface cast weight during 15 days in pot experiment (mean)

		Т	TS	NT	р
Soil bulk density	g cm ⁻³	0.729 a	0.816 b	0.925 c	p<0.05
Cast weight	g pot ⁻¹	5.03	4.42	6.16	p=0.243

Data of cast weight show the total soil surface cast weight per pot during the experiment. Different characters show statistically significant differences in values in the Tukey-Kramer test (p<0.05). T: CT without treading stress soil, TS: CT with treading stress soil, NT: NT soil. worms. Although tillage affected soil density, soil density did not affect earthworm casting behavior (Table 2). Buck et al. $(2000)^4$ also showed *Lumbricus terrestris*, which is the same habitat species as this experiment and produced an equivalent amount of cast between two soil densities. With this result we can assume it is valid to measure surface cast production to estimate the effects of tillage on the earthworm function as the soil aggregate formation due to the lack of any link between earthworm casting behavior and soil bulk density.

2. Effect of tillage on Chinese cabbage yield

In the fall of 2004, yields of Chinese cabbage under NT treatment were significantly less than those under reduced and conventional tillage treatments (p<0.05) (Fig. 2). Chinese cabbage did not head in spring 2005 in the NT treatment. Although the yields in ST, RT, and SRT plots tended to decrease compared with that under CT treatment in spring 2005, those under ST, RT, and SRT treatments were almost the same as the yields under CT treatment in the other two cropping periods. In NT treatment, the crop yield fluctuated between cropping, which would be mainly attributable to weed biomass. Even though we did not measure weed biomass, in the first cropping, weed biomass may be high in NT treatment, due to the non-use of herbicide. In the second cropping, despite using herbicide, two times of weeding were needed due to the weed proliferating in spring. Miura et al. (2008)¹³ also showed weed biomass was higher in spring than fall in adjacent experimental plots. In the third cropping, herbicide using, weeding practice and low weed growth rate would help achieve high NT treatment productivity.

Miura et al. (2008)¹³ showed that the yield of Chinese cabbage under no-tillage treatment was lower than that under conventional tillage. Our data were consistent with their study, and showed that reduced tillage helped to avoid a reduction in the Chinese cabbage yield.

3. Effect of tillage practices on earthworm casting activity

There was no difference between treatments in soil surface cast production before tillage commenced. Cast production rates over all treatments before tillage com-

cant differences emerged between NT and the other treatments, while in the second cropping, there were statistically

significant differences between CT and ST tillage treatments, and NT. In the third cropping, cast production rates in the different treatments increased in the following order CT, ST, RT, SRT, and NT, and there were statistically significant differences between treatments.

menced were 17.8 ± 1.01 g m⁻² day⁻¹ (mean \pm S.E.). The

cast production rate in the NT plots was the highest in every

cropping period (Fig. 3). During the first cropping, signifi-

Reduced Tillage Impacts on Earthworm Cast Production

After the first tillage, on 26 August, 2004, injured worms arising from tillage were counted on the soil surface in RT and SRT treatments. The numbers of worms were 3.3 \pm 0.9 and 8.3 \pm 1.8 (mean \pm S.E.) per plot in SRT and RT treatments, respectively (Table 3). The injured individuals died a few days later. Based on this observation, deep tillage induced a profound, direct negative effect on the earthworms. During the second tillage on 8 September, 2004, the vertical distribution proportions of earthworm were 20, 40 and 40% in the 0-7, 7-15, and 15-40-cm soil layers, respectively (Table 4), meaning there were few individual worms in the top 7 cm layer at the time of tillage. In addition, no earthworms were found in the 0-5 cm layer during the year (Table 5). Although slight differences emerged in the depth of the top layer between the two surveys, many individual worms remained under the shallow tillage layer.

However, despite the fact many worms were present in the soil below 5 cm over the year, surface cast production declined significantly, even during the shallow tillage treatments (Fig. 3). The elimination of organic matter from the soil surface, mechanical damage caused to earthworms by machinery, and disturbance of the earthworm habitat are considered major negative factors of tillage on earthworms⁵. Generally speaking, surface organic matter is important for Anecic and Epigeic species^{5,16}. Kaneda et al. (2009)⁹ showed that removing surface litter caused a reduction in the surface cast production of the same species, meaning decreasing surface litter by tillage would induce low surface cast production due to decreasing earthworm casting activity or escaping from tillage plots in this experiment. In addition to mechanical damage to earthworms, the elimination of organic matter from the soil surface and disturbance

Table 3. The number of injured worms arising from tillage in the field experiment (mean ± S.E.)

Treatment	Tillage depth	Number plot ⁻¹		
SRT	7cm	3.3 ± 0.9		
RT	15cm	8.3 ± 1.8		

Earthworms were sampled on 26 Aug., 2004. (n=3) SRT: Shallow row tillage treatment, RT: row tillage treatment.

Table 4. The vertical distribution proportions of earthworms around the field experiment plot (mean ± S.E.)

Soil depth	Number m ⁻²	(%)	
0-7cm	8.0 ± 3.52	20	
7-15cm	16.0 ± 4.16	40	
15-40cm	16.0 ± 5.92	40	

Earthworms were sampled on 8 Sep., 2004. (n=6)

	Sampling date				
	2003	2004			
	21 October	23 February	2 June	4 August	5 November
Total individuals (n)	4	2	9	4	1
Vertical distribution (%)					
0-5 cm	0	0	0	0	0
5-15 cm	25	0	100	0	100
15-25 cm	75	0	0	75	0
25-40 cm	0	100	0	25	0

 Table 5. Vertical distribution of *Pheretima (Amynthas) carnosa* and total numbers of individuals in the field survey

of their habitat induced by tillage apparently decreased cast production in this experiment.

Nakamura (1988)¹⁴ and Nakamura et al. (2003)¹⁵ found that tillage adversely affected earthworm density in volcanic-ash soil. Miura et al. (2008)¹³ showed that surface cast production under conventional tillage was lower than under a no-tillage system. Results in this study were consistent with previous research, and revealed that soil surface cast production in reduced tillage treatments was lower than that in the NT treatment, but exceeded that in the CT treatment. This result is applicable to the same type of anecic species, but for epigeic and endogeic species, further research into the effects of reduced tillage is needed.

Conclusions

This study investigated the reduced tillage effect on earthworm cast production and the yield of Chinese cabbage on volcanic-ash soil. Yields under reduced tillage treatments tended to decrease in spring 2005. At other times, however, yields under reduced tillage treatments were almost the same as those in the CT treatment. Conversely, soil surface cast production under the reduced tillage treatments was lower than that under the NT treatments, but exceeded that under the CT treatment. These results indicate that reduced tillage can help preserve aggregate formation by earthworms and prevent any reduction in yield in Chinese cabbage production in the cropping system studied. Soil aggregate formation is strongly linked to soil fertility. Namely, reduced tillage should keep and facilitate soil fertility without decreasing the crop yield.

Acknowledgements

We wish to thank Mr. Shishido, R., Mr. Kan T. and Mr. Yoshida M. at the National Agricultural Research Center for Tohoku Region, for their help with fieldwork. We also wish to thank Ms. Saito M. at the National Agricultural Research Center for Tohoku Region, for helping sample the casts. We also acknowledge Dr. Miura S. at the National Agricultural Research Center for helpful discussion. S. K. was supported by a grant from the JSPS (Japan Society for the Promotion of Science) Research Fellowships for Young Scientists. This study was partially supported by a Grant-in-Aid for Scientific Research (C) (21880051)

References

- Bossuyt, H. et al. (2005) Protection of soil carbon by microaggregates within earthworm casts. *Soil Biol. Biochem.*, 37, 251-258.
- Bouché, M. B. (1977) Strategies Lombriciennes. *Ecol. Bull.*, 25, 122-132.
- Brown, G. G. (1995) How do earthworms affect microfloral and faunal community diversity? *Plant Soil*, **170**, 209-231.
- Buck, C. et al. (2000) Influence of mulch and soil compaction on earthworm cast properties. *Appl. Soil Ecol.*, 14, 223-229.
- Chan, K. Y. (2001) An overview of some tillage impacts on earthworm population abundance and diversity—implications for functioning in soils. *Soil Till. Res.*, 57, 179-191.
- Edwards, C. A. & Lofty, J. R. (1982) The effect of direct drilling and minimal cultivation on earthworm populations. *J. Appl. Ecol.*, **19**, 723-734.
- Enami, Y. et al. (1999) Use of soil animals as bioindicators of various kinds of soil management in northern Japan. *JARQ*, 33, 85-89.
- 8. Ernst, G. & Emmerling, C. (2009) Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period. *Eur. J. Soil Biol.*, **45**, 245-251.
- Kaneda, S. et al. (2010) Effects of herbicides, glyphosate, on density and casting activity of earthworm, *Pheretima* (*Amynthas*) carnosus. Dojyohiryou gakkaishi (Jpn. J. Soil Sci. Plant Nutr.), 80, 469-476 [In Japanese with English summary].
- Lee, K. E. (1985) *Earthworms: their ecology and relation-ships with soils and land use*. Academic Press, Sydney, pp. 411.
- Marinissen, J. C. Y. & De Ruiter, P. C. (1993) Contribution of earthworms to carbon and nitrogen cycling in agro-ecosystems. *Agric. Ecosys. Environ.*, 47, 59-74.
- Martin, A. (1991) Short- and long-term effects of the endogenic earthworm *Millsonia anomala* (Omodeo) (Megascolecidae Oligochaeta) of tropical savannas on soil

organic matter. Biol. Fertil. Soils, 11, 234-238.

- Miura, F. et al. (2008) Dynamics of soil biota at different depths under two contrasting tillage practices. *Soil Biol. Biochem.*, 40, 406-414.
- 14. Nakamura, Y. (1988) The effect of soil management on the soil faunal make-up of a cropped andosol in central Japan. *Soil Till. Res.*, **12**, 177-186.
- 15. Nakamura, Y. et al. (2003) Earthworm and enchytraeid numbers in soybean-barley fields under till and no-till cropping systems in Japan during nine years. *Mem. Fac. Agr., Ehime Univ.*, **48**, 19-29.
- 16. Rothwell, A. et al. (2011) The impact of cultivation tech-

niques on earthworm population. *In* Soil biology. Biology of earthworms Ser. Vol. 24, ed. Karaca, A., Springer, Berlin, 159-172.

- 17. Sharpley, A. et al. (2011) Land application of manure can influence earthworm activity and soil phosphorus distribution. *Commun. Soil Sci. Plant Anal.*, **42**, 194-207.
- Takata, Y. et al. (2009) Digital soil map of Japanese croplands in 1992. Dojyohiryou gakkaishi (Jpn. J. Soil Sci. Plant Nutr.), 80, 502-505 [In Japanese].
- 19. Zaller, J. G. & Arnone, J. A. (1997) Activity of surface-casting earthworms in a calcareous grassland under elevated atmospheric CO₂. *Oecologia*, **111**, 249-254.