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CHANGES IN SOIL CHEMICAL CHARACTERS AND ENZYME ACTIVITIES DURING CONTINUOUS MONOCROPPING OF CUCUMBER (*CUCUMIS SATIVUS*)

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Abstract

Soil sickness, a phenomenon of negative plant-soil feedback in continuous monocropping systems, can cause severe yield penalty in agricultural production. Changes in soil chemical and biological characters are thought to account for soil sickness. However, changes in soil properties in continuous monocropping systems and links between these changes and plant growth performance are still not clear. In this study, dynamics of soil chemical characters and enzyme activities were monitored in a continuously monocropped cucumber system, in which cucumber was successively monocropped in pots for nine croppings under greenhouse conditions from 2005 to 2009 in the experimental station of Northeast Agricultural University, Harbin, China. Cucumber showed an obvious stunted growth behavior in the seventh cropping and turned better in the ninth cropping. Soil pH decreased from the first cropping to the seventh cropping and increased in the ninth cropping. Contents of soil available nitrogen, phosphorous and potassium were the highest while activities of soil urease, neutral phosphatase and catalase were the lowest in the seventh cropping. Our results suggested that cucumber in the seventh cropping did not absorb enough soil nutrients, which may lead to the decrease in soil pH and changed soil biological properties. Changes in soil chemical and biological characters may be linked to the soil sickness of cucumber.

Key words: Continuous monocropping, *Cucumis sativus* L., Soil enzyme activity, Soil nutrient, Soil sickness.

Introduction

Plants can change soil biology, chemistry and structure in ways that alter subsequent plant growth and this process is referred as plant-soil feedback (Kulmatiski *et al.*, 2008). In agriculture, a well known phenomenon of negative plant-soil feedback is soil sickness, a reduction in both crop yield and quality caused by continuous monocropping in the same land (Zhou *et al.*, 2012a, 2012b; Gentry *et al.*, 2013; Huang *et al.*, 2013). Soil sickness is one of the major problems in agricultural production, especially for greenhouse crops. For example, cucumber (*Cucumis sativus* L.), a crop of high economic importance in many countries, is vulnerable to soil sickness (Zhou *et al.*, 2011; Huang *et al.*, 2013). Various factors are thought to be accounted for soil sickness, including build-up of soil-borne pathogens, deterioration of soil physico-chemical characters, changes in nutrient availability, and accumulation of allelochemicals (Nie *et al.*, 2009; Zhou & Wu, 2012b; Mahmood *et al*., 2013; Khan *et al.*, 2014). So far, the main causes of soil sickness of cucumber have not been demonstrated conclusively.

Previous studies have mainly focused the differences in plant growth behaviors, soil chemical and soil biological characters between continuous monocropping system and alternative cropping systems (Acosta-Martínez *et al.*, 2007; Zhou *et al.*, 2011). Crop yield, soil enzyme activities and soil nutrient contents were often found to be higher under alternative cropping systems than under monocropping systems (Inal *et al.*, 2007; Zhang *et al.*, 2007; Zhou *et al.*, 2011). Impacts of continuous monocropping of cucumber on soil chemical and biological prosperities have been previously reported (Liang & Chen, 2004; Ma *et al.*, 2004; Fan *et al.*, 2006). However, soils used in these experiments, which were supposed to have the number of croppings as the only influencing factor, were collected from different sites with differing soil management practices, such as fertilization and irrigation regime. These soil management practices are widely known to influence soil chemical and biological characters (Raaijmakers *et al.*, 2009; Thiele-Bruhn *et al.*, 2012; Yuan *et al.*, 2013) and thus may confuse the results. There are few reports about the successive changes of soil characters in long-term continuous monocropping systems in fixed site with uniform soil management practices.

Soil enzymes, which are derived primarily from soil microorganisms, plant roots, plant and animal residues, represent the soil bioactivity, soil fertility, organic matter decomposition and metabolism speed (Lalande *et al.*, 2000; Casucci *et al.*, 2003). Soil enzyme activities can be used as measures of the functionality of the microbiota, and have been indicated as soil characters suitable for use in the evaluation of the degree of alteration of soils in both natural and agro-ecosystems (Gianfreda *et al.*, 2005; Acosta-Martínez *et al.*, 2007).

Main aims of this study were to (1) investigate the changes in soil chemical characters and soil enzyme activities in a long-term monocropped cucumber system; and (2) evaluate whether the changes in soil chemical characters and soil enzyme activities can be linked to the growth performance of cucumber. In a greenhouse experiment, cucumber was continuously monocropped in pots for nine croppings. Cucumber growth conditions, soil pH, soil nutrient contents and enzyme activities were assessed on 30, 40 and 50 d after cucumber planting.

Materials and Methods

Greenhouse experiment: A greenhouse experiment was conducted in plastic pots (30 cm diameter, 25 cm height) from April 2005 to July 2009 in the experimental station of Northeast Agricultural University, Harbin, China (45°41’N, 126°37’E). Two croppings of cucumber (April to July, July to October) were grown each year. Each pot contained 8 kg of soil, which were taken from the upper soil layer (0-15 cm) from an open field that was covered with grasses and was undisturbed for the last 15 years. The soil was a black soil (Mollisol) with sandy loam texture. The basic properties of the field soil are presented in Table 1.

Germinated cucumber (cv. Jinlv-3) seeds were planted in pots on April 25 or July 25 in each cucumber cropping. There was one cucumber plant per pot. New soil from the same open field was taken to fill in new pots on April 25 of each year. Soils from the previous croppings remained in the pots, and cucumber was planted again in these pots on April 25 and July 25 each year. Finally, cucumbers of the first, third, fifth, seventh and ninth croppings were obtained in April 2009 at the same time. The detailed greenhouse experiment set up was presented in the supplementary figure in our previous paper (Zhou *et al.*, 2012b). There were triplicates per cropping with 20 pots in each replicate. All pots were randomly placed in the greenhouse. Fertilization was performed according to local recommendations with decomposed swine manure (15% organic matter, 0.5% nitrogen, 0.5% phosphorous, 0.4% potassium) used as basal fertilization at the rate of 0.75 kg per pot. Urea fertilizer (46% nitrogen content) was used as topdressing at the rate of 25 g per pot 31 days after planting. Irrigation was performed twice weekly with ground water. Weeds were manually removed.

Table 1. Basic properties of the field soil.

pH (1:2.5, w/v)	**EC ($mS\ cm^{-1}$, 1:2.5, w/v)**	**Available nitrogen content ($mg\ kg^{-1}$)**	**Available phosphorous content ($mg\ kg^{-1}$)**	**Available potassium content ($mg\ kg^{-1}$)**
7.78	0.33	89.02	63.36	119.15
OM content ($g\ kg^{-1}$)	Urease activity (NH_3-N mg g^{-1} soil in 24 h)	Neutral phosphatase activity (mg phenol g^{-1} soil in 24 h)	Catalase activity (ml 0.1 N $KMnO_4$ g^{-1} soil in 30 min)	PPO activity (10^2 mg Purpurogallin g^{-1} soil in 3 h)
36.71	2.09	1.05	8.68	10.05

Measurement of plant growth conditions: Cucumber plant height, stem diameter and leaf area were measured on 30, 40 and 50 days after planting. All leaves of one plant were harvested and scanned by the Microtek ScanMaker i800 plus system (WSeen, China) and leaf area was calculated by the LA-S Leaf Area Analysis software (WSeen, China). Root activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) method, and was expressed as ug triphenylformazan (TPF) per g root sample in one hour (Zhang & Qu, 2003).

Soil sampling: Bulk soil samples were collected from five plants in each replicate on 30, 40 and 50 days after cucumber planting. After sieved (2 mm), parts of these fresh samples were used for soil enzyme analysis, whereas the other subsamples were air-dried and used for soil chemical analysis.

Soil chemical analysis: Soil pH was determined in water suspensions at a soil/water ratio of 1:2.5 with a glass electrode. Available nitrogen was determined with the KCl-extraction method. Available phosphorous was extracted with sodium bicarbonate and measured with the molybdenum blue spectrophotometry method. Available potassium was extracted with ammonium acetate and flame photo-metrically measured. Soil organic matter content was measured by the potassium dichromate method (Lu, 1999).

Soil enzyme activities analysis: Soil urease (EC 3.5.1.5) activity was measured as described by Hoffmann and Teicher (1961), and expressed as NH_3-N mg g^{-1} soil in 24 h. Neutral phosphatase enzyme activity was determined by the method of Tabatabai & Bremner (1969), and expressed as mg phenol g^{-1} soil in 24 h. Soil catalase (EC 1.11.1.6) was measured as described by Johnson and Temple (1964), and expressed as 0.1 M $KMnO_4$ ml g^{-1} soil in 30 min. Polyphenol oxidase (PPO) (EC 1.10.3.1) activity was determined as described by Peruccia *et al.* (2000), and expressed as purpurogallin mg g^{-1} soil in 2 h.

Statistical analysis: Soil chemical characters and enzyme activities data were analyzed using two-way analysis of variance (ANOVA) with the number of croppings and growth stage as fixed factors. Mean comparison between croppings was performed based on the Tukey's honestly significant difference (HSD) test at the 0.05 probability level at each cucumber growth stage. The data were tested for normality (Kolmogorov-Smirnov one-sample test) and homogeneity of variances (Levene test) and log-transformed if necessary. The Pearson's correlation coefficients were used to calculate linear correlations between cucumber growth parameters and soil parameters. Tukey's test, ANOVA and correlation analyses were conducted with SAS software (version 8.0).

Results

Cucumber growth performance: ANOVA showed that continuous cropping had significant effects on cucumber plant height, stem height, leaf area and root activity ($p<0.05$, data not shown). Generally, cucumber plant height, stem height, leaf area and root activity maintained at a relatively stable level from the first cropping to the fifth cropping at all sampling dates (Fig. 1). However, cucumber showed retarded growth in the seventh cropping, and then turned better in the ninth cropping. Cucumber plant dry biomass, previously reported by Zhou *et al.* (2012b), showed the similar trend with plant height, stem height, leaf area and root activity.

Soil pH: Compared with the soil before planting of cucumber, soil pH decreased in all cucumber-cultivated soils (Table 1; Fig. 2). ANOVA showed that both continuous cropping and growth stage significantly affected soil pH ($p<0.05$) (Table 2). Soil pH significantly decreased with the number of croppings from the first to the seventh cropping ($p<0.05$); and then significantly increased in the ninth cropping ($p<0.05$). On each sampling day, soil pH in the seventh cropping were the highest among all croppings ($p<0.05$).

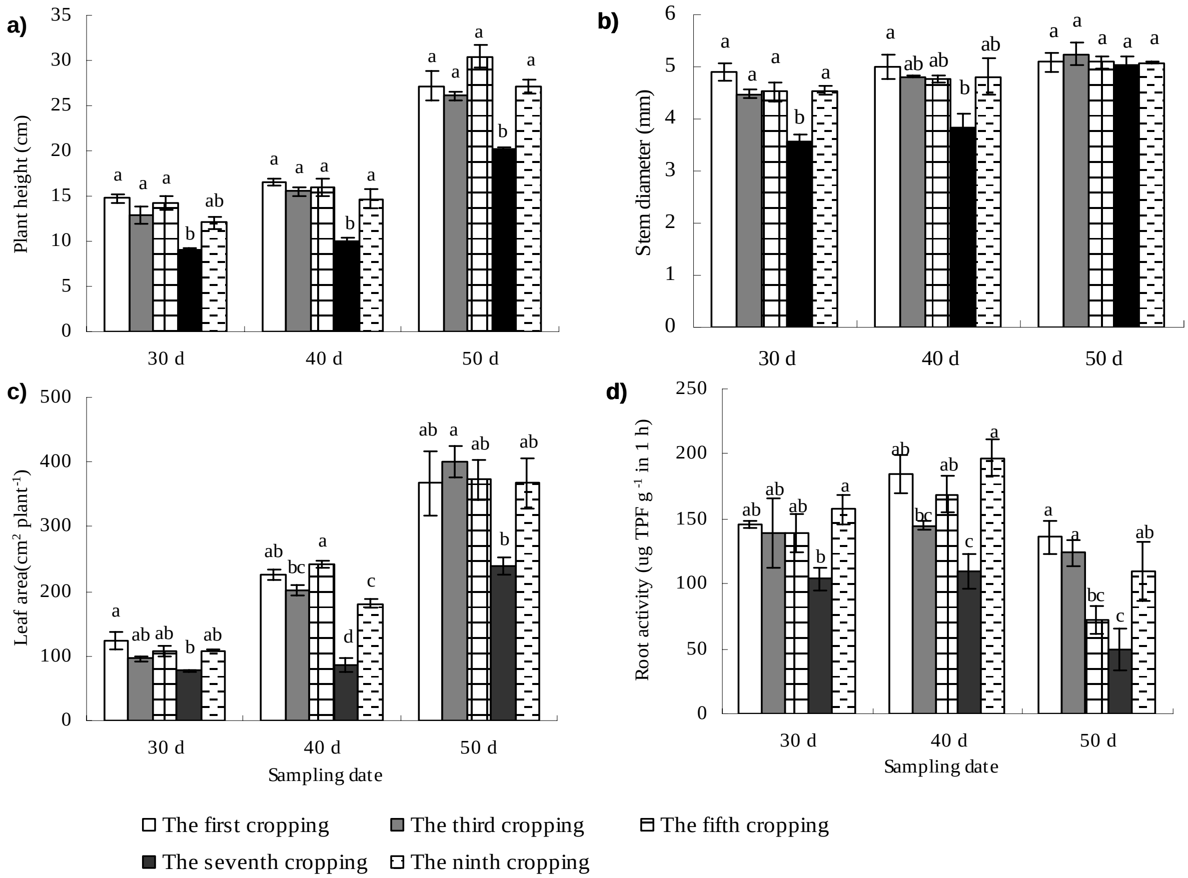

Fig. 1. Dynamics of cucumber plant height (a), stem diameter (b), leaf area (c) and root activity (d) during continuous monocropping of cucumber. Values (mean ± standard error) with different letters on the same sampling day are significantly different (Tukey's HSD test, $p<0.05$).

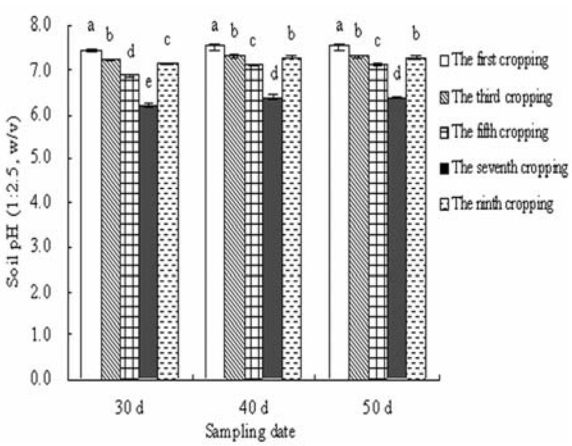

Fig. 2. Dynamics of soil pH during continuous monocropping of cucumber. Values (mean ± standard error) with different letters on the same sampling day are significantly different (Tukey's HSD test, $p<0.05$).

Soil nutrient contents: Soil nitrogen, phosphorous, potassium and organic matter contents in all cucumber-cultivated soils were higher than in the soil sample before cucumber planting (Table 1; Fig. 3). Both continuous cropping and growth stage significantly affected soil nutrient contents ($p<0.05$) (Table 2).

Soil nitrogen and phosphorous contents tended to increase with the number of croppings from the first to the seventh cropping (Fig. 3a, b). On 40 and 50 days after planting, soil potassium content decreased with the number of croppings from the first to the fifth cropping (Fig. 3c). Soil nitrogen, phosphorous and potassium contents in the seventh cropping were significantly higher than in the other croppings ($p<0.05$). Compared with the seventh cropping, soil nitrogen, phosphorous and potassium contents significantly decreased in the ninth cropping ($p<0.05$) (Fig. 3a, b, c). On each sampling day, soil organic matter content did not show a particular trend related to the number of croppings (Fig. 3d). Correlation analyses showed that both soil nitrogen and potassium contents were negatively correlated with plant total biomass ($p<0.05$, r=-0.66 and -0.78, respectively) as shown in Table 3. Soil nitrogen, phosphorous and potassium contents were negatively correlated with soil pH ($p<0.05$, r=-0.80, -0.96 and -0.56, respectively).

Soil enzyme activities: Generally, soil urease, neutral phosphatase, catalase and PPO activities in cucumber-cultivated soils were higher than in the soil sample before cucumber planting (Table 1; Fig. 4). Both continuous cropping and growth stage significantly affected soil enzyme activities ($p<0.05$) (Table 2).

Soil urease, catalase and neutral phosphatase activities in the seventh croppings were lower than in other croppings (Fig. 4a, b, d). Soil catalase activity had the trend to decrease with the number of croppings from the first to the seventh cropping (Fig. 4b). Generally, soil PPO activity did not show a particular trend related to the number of croppings (Fig. 4c). Soil neutral phosphatase activity increased with the number of croppings from the first to the fifth cropping (Fig. 4d). Correlation analyses showed that plant total biomass was significantly and positively correlated with phosphatase activity ($p<0.05$, $r=0.57$), while negatively correlated with PPO activity ($p<0.05$, $r=-0.59$) (Table 3).

Discussion

Liang & Chen (2004) found that cucumber growth performance declined in the fourth cropping, a relatively short term of continuous cropping. However, Ma *et al.* (2004) showed that five croppings had no significant influences on cucumber growth; but relatively long-term continuous cropping, seven and nine croppings, had adverse effects on cucumber growth. In this study, cucumber showed retarded growth in the seventh cropping. These inconsistencies may be attributed to the differences in experiment conditions, such as growth environment (*e.g.* soil texture, temperature and light intensity) and management practices (*e.g.* irrigation and tillage regimes, and the type and amount of fertilizer used). The accumulation of autotoxic substances was thought to be accounted for soil sickness, and cucumber root exudates and plant residues had been shown to have autotoxicity potential (Yu *et al.*, 2000; Zhou & Wu, 2012a; Zhou *et al.*, 2012a). The quantity and composition of root exudates, rates of accumulation and decomposition of plant residues would be different under different crop growth conditions and soil management practices (Marschner *et al.*, 2005), which would lead to different accumulations of autotoxic substances in the soil. Therefore, when cucumber growth performance turned worse may depend on the management practices performed in the continuous cropping system.

One of the notable findings in this study was that, compared with the seventh cropping, the cucumber growth conditions turned better in the ninth cropping, which was also observed in continuously monocropped soybean system (Li *et al.*, 2010). Fusarium disease incidence (data not shown) and the *Fusarium* community size (Zhou & Wu, 2012b) in the ninth cropping were lower than in the seventh cropping. Evidence is now accumulating that soil suppressiveness can be induced by continuous monocropping, which have been described for many plant-pathogen systems, including take-all disease of wheat (*Triticum aestivum*) caused by *Gaeumannomyces graminis* var. *tritici*, Fusarium wilt disease of several plant species, and Rhizoctonia damping-off disease of sugar beet (*Beta vulgaris* L.) (Mendes *et al.*, 2011; Berendsen *et al.*, 2012). Therefore, it is possible that the formation of disease-suppressive soils may account for the improved growth performance in the ninth cropping. Future work should focus on the identification of the causal agent of soil suppressiveness, and the dynamics of these possible biocontrol agents involved.

The deterioration of soil physico-chemical characters is thought to be one of the main causes of soil sickness (Nie *et al.*, 2009; Gentry *et al.*, 2013). The greenhouse cultivation is featured with high input of nitrogen fertilizer or physiological acid fertilizers, which may cause soil acidification and salinization with adverse effects on plant growth (Liang & Chen, 2004; Ju *et al.*, 2007). In this study, soil pH decreased, while soil nitrogen and phosphorous contents increased from the first to the seventh cropping, which concur with previous studies (Liang & Chen, 2004; Fan *et al.*, 2006). Soil nitrogen and potassium contents were negatively correlated with soil pH, indicating that changes in soil pH may be due to the changes in soil nutrient contents in this continuous cropping system.

Soil nutrient contents were the highest and cucumber biomass were the lowest in the seventh cropping, while soil nutrient contents decreased and cucumber biomass increased in the ninth cropping. The same amount of fertilizer was applied in each season, we speculated that changes in soil nutrient contents were due to the changes in cucumber growth condition: when cucumber grew well the ninth cropping, more soil nutrients were absorbed and thus soil pH decreased; and *vice verse* in the seventh cropping. Previous studies demonstrated that soil pH and nutrient contents regulate soil biological properties (Raaijmakers *et al.*, 2009; Thiele-Bruhn *et al.*, 2012). Thus, changes in soil nutrients and pH may further induce changes in soil biological properties, such as the soil enzyme activities as observed in this study.

Soil enzyme activity is critically important for soil quality and can provide indications of changes in metabolic capacity and nutrient cycling due to management practices (Caldwell, 2005). Soil biological characters would change under continuous monocropping conditions (Yao *et al.*, 2006), and, soil enzyme activities usually decreased with the number of cropping (Yu *et al.*, 2011; Zhou *et al.*, 2011). Soil urease, phosphatase, catalase were thought to be involved in the transformation of nitrogen, phosphorous and organic matter, respectively (Tabatabai & Bremner, 1969). Generally, soil enzyme activity and soil nutrient content are thought to be positively correlated (Guan, 1987; Lalande *et al.*, 2000). However, in this study, soil urease and phosphatase activities were negatively correlated with soil nitrogen and phosphorous contents. These indicated that continuous cropping practice had negative effects on soil nutrient cycling.

It is generally accepted that the accumulation of soil-borne pathogens are responsible for soil sickness, a special kind of negative plant-soil feedback (Zhou *et al.*, 2011; Huang *et al.*, 2013). It was demonstrated in our previous study that soil microbial community structures and sizes were changed in the continuously monocropped cucumber system, and soil fungi and *Fusarium* community abundance were linked to the soil sickness of cucumber (Zhou & Wu, 2012b). The instant study showed that soil enzyme activities were also changed. Therefore, not only the community structure but also their related functions were changed. In plant-soil feedback, the performance of plants can be greatly influenced by interactions with the abiotic and biotic soil environment (Wardle *et al.*, 2004) and the changes in soil microbial functions can affect plant growth (Bever *et al.*, 2010). Soil urease, phosphatase and catalase activities were the lowest in the seventh cropping, in which cropping cucumber had the worst growth performance. Thus, our results suggest that, besides the accumulation of soil-borne pathogens (Zhou & Wu, 2012b), changes in soil biological functions, especially the nutrient cycling, may also be linked to the soil sickness of cucumber.

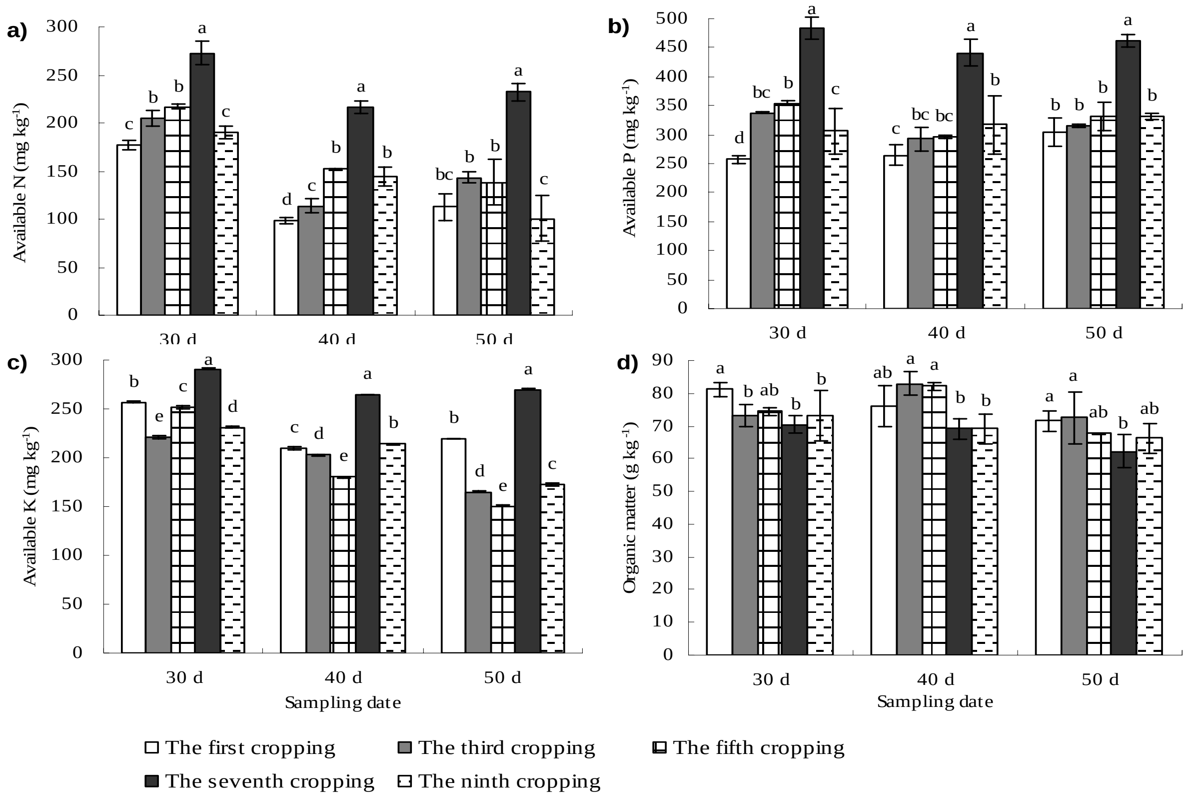

Fig. 3. Dynamics of soil nitrogen (a), phosphorous (b), potassium (c) and organic matter (d) contents during continuous monocropping of cucumber. Values (mean±standard error) with different letters on the same sampling day are significantly different (Tukey's HSD test, $p<0.05$).

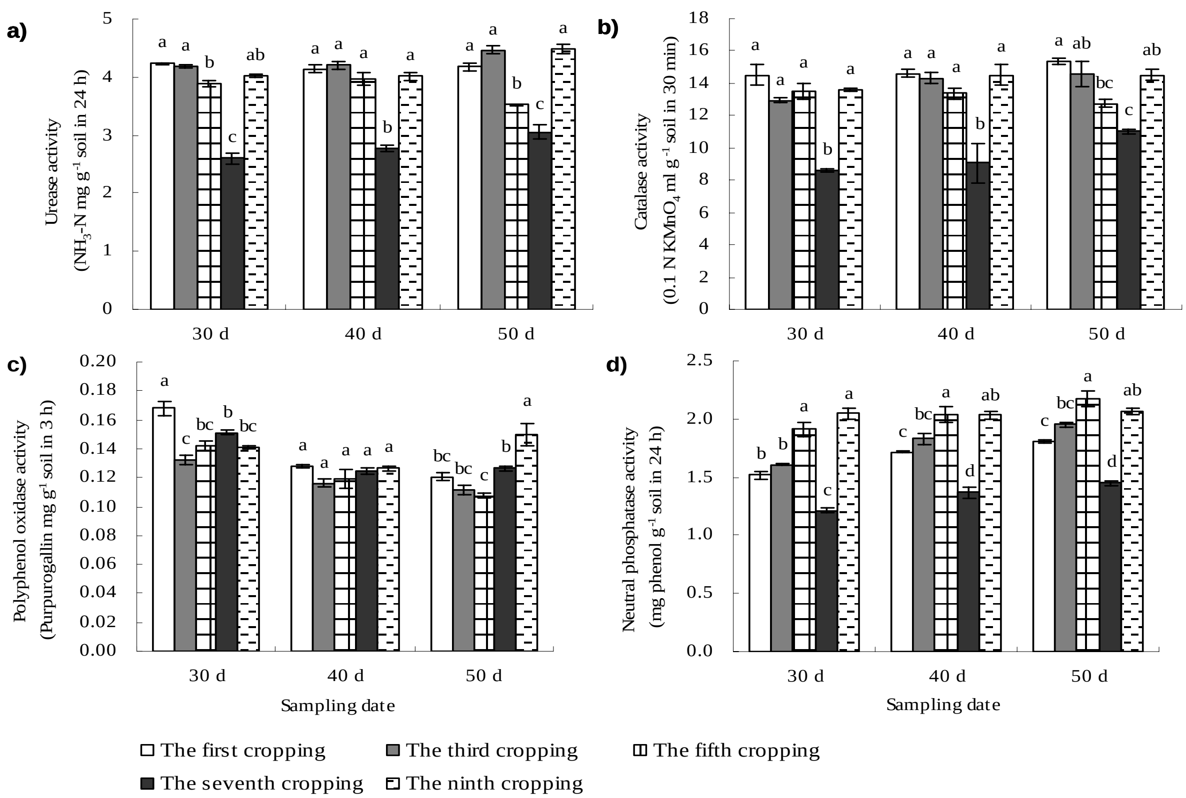

Fig. 4. Dynamics of soil urease (a), catalase (b), polyphenol oxidase (c) and neutral phosphatase (d) activities during continuous monocropping of cucumber. Values (mean ± standard error) with different letters on the same sampling day are significantly different (Tukey's HSD test, $p<0.05$).

Table 2. Summary of two-way ANOVA on soil chemical characters and enzyme activities in the continuous monocropping system.

Source	Cropping number			Growth stage		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Soil pH	4	1045.82	<0.0001	2	50.38	<0.0001
Nitrogen content	4	128.73	<0.0001	2	173.25	<0.0001
Phosphorous content	4	94.47	<0.0001	2	7.01	0.0032
Potassium content	4	11553.8	<0.0001	2	13226.9	<0.0001
OM content	4	8.14	0.0001	2	13.90	<0.0001
Urease activity	4	235.69	<0.0001	2	7.57	0.0022
Catalase activity	4	51.26	<0.0001	2	5.01	0.0132
PPO activity	4	18.98	<0.0001	2	77.14	<0.0001
Neutral phosphatase activity	4	163.23	<0.0001	2	42.64	<0.0001

Table 3. Correlation of soil chemical characters and enzyme activities with each other and with cucumber total biomass in the continuous monocropping system.

	Soil pH	Nitrogen content	Phosphorous content	Potassium content	OM content	Urease activity	Phosphatase activity	Catalase activity	PPO activity	Total biomass
Soil pH										
Nitrogen content	-0.80									
Phosphorous content	-0.96	0.77								
Potassium content	-0.56	0.78	0.59							
OM content	0.52	ns	-0.62	ns						
Urease activity	0.92	-0.74	-0.88	-0.63	ns					
Phosphatase activity	0.56	-0.66	-0.62	-0.83	ns	0.64				
Catalase activity	0.94	-0.80	-0.90	-0.60	ns	0.95	0.69			
PPO activity	ns	ns	ns	0.55	ns	ns	ns	ns		
Total biomass	ns	-0.66	ns	-0.78	ns	ns	0.57	ns	-0.59	

Values presented are significant at $p = 0.05$ and ns = Not significant at $p = 0.05$

Conclusion

All, our results indicate that cucumber shows obvious growth obstacle in the seventh cropping, and the growth condition turned better in the ninth cropping. Soil nitrogen, phosphorous and potassium contents were the highest and activities of urease, catalase and neutral phosphatase were the lowest in the seventh cropping. The decrease in soil pH may be due to the heavy input of fertilizers. Changes in soil chemical and biological characters may be linked to the soil sickness of cucumber.

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