Immediate responses of cyst nematode, soil-borne pathogens and soybean yield to one-season crop disturbance after continuous soybean in northeast China

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Abstract

Habitat disturbance affects numerous ecosystem components and processes, but its effect on continuous soybean system is less available. Soybean was seeded following six preceding crops, including grain soybean (*Glycine max* L. Merill.), wheat (*Triticum aestivum* L.), sugar beet (*Beta vulgaris* L.), tobacco (*Nicotiana tabacum* L.), corn (*Zea mays* L.) and hemp (*Cannabis Satia* L.), on a Mollisol farmland that had previously been cropped to continuous soybean for seven years in Northeast China. Soybean after hemp reduced the number of second-stage juveniles of soybean cyst nematode (J2) by 29.8% compared to continuous soybean, while soybean after corn had the lowest J2 number. The number of soil-borne pathogens of *Fusarium*, *Rhizoctonia* and *Pythium* after corn and hemp and root rot disease severity index after all crop disturbance, except sugar beet, were significantly lower than continuous soybean. Soybean yield after hemp disturbance was improved by 10.8%, while sugar beet disturbance had the greatest negative impact on soybean yield. No differences were found among crop disturbance for protein and oil content in soybean seed. Crop disturbance changed the habitat already developed in the continuous soybean system. Adoption of hemp disturbance has the potential to be an alternative approach in managing continuous soybean production system in Northeast China.

Keywords: Crop disturbance; Ecosystem components; Cyst nematode; Soil-borne diseases; Seed yield; Continuous soybean; Preceding crop.

Introduction

Agroecosystem is constantly disturbed by anthropogenic activities and comes in many forms with a variety of ecological effects (Qunn, 2004). A disturbance is any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability or the physical environment (White and Pickett, 1985). Disturbance has demonstrated effects on plant community characteristics, while the functional attributes of ecosystems are also governed to some extent by disturbance (Beets, 1990; Kruess and Tscharntke, 1994; Celika et al., 2011). There are three types of disturbance within agroecosystem, i.e. disturbance of crop habitat, fragmentation of noncrop habitat and the degradation of noncrop habitat (Ruthenberg, 1980; Tilman, 1999; Bommarco, 1998; Qunn, 2004). Habitat disturbances can affect numerous ecosystem components and processes, including pest population dynamics, natural-enemy community structure, pest-natural enemy interactions and noncrop community structure through tillage and applications of fertilizers and pesticides (Marino and Landis, 1996; Matson et al., 1997; Lawton et al., 1998; Ostman et al., 2001). The most highly disturbed crop habitats are annual crops, such as potato, corn and other vegetables, that are tilled once or more every year and often receive high inputs of various agrochemicals (Wissinger, 1997; Landis and Menalled, 1998; Letourneau, 1998). Although there have been many studies of the effects of disturbance on patterns of plant establishment and growth, community structure/turnover in forests, on the responses and adaptations of insects, on weed population in grassland and on nematode communities in cropland (Villenave et al., 2001; Ominski and Entz, 2001), their potential impact on soil-borne diseases, growth and yields in continuous soybean systems has not been documented.

Northeast China, which is known as the "bread basket of China", is the main soybean producer of the nation. In 2009, the total production of soybean in this region was 55.7% of the nation's total (Liu et al., 2011). Most farmers in the region adopted continuous soybean due to its higher economic return and natural agro-ecological conditions suited to soybean production. Continuous soybean cultivation in this region can also be attributed to the fact that soils are too wet or even waterlogged in early spring in some areas which makes it impossible for early seeding of alternative crops such as spring wheat for practicing a normal crop rotation (Liu and Herbert, 2002). Continuous soybean system however, imposes

negative impact on soil conditions which in turn reduces soybean productivity (Liu et al., 1990; Zheng, 1999; Liu and Yu, 2000). Soybean cyst nematode and soil-born pathogens are the main causes of crop yield reduction of continuous soybean (Xu et al., 2000). Thus, soybean producers in this region are seeking practices to maintain natural soil fertility, attain economic sound grain yield and thus improve their farm sustainability.

Crop sequence/rotation in combination with other management practices often considered as the most effective and inexpensive method to manage a number of plant diseases (Holtzer et al., 1996; Krupinsky et al., 2002; Turkington et al., 2003; Krupinsky et al., 2007a).

In the current study, we have assumed the continuous soybean system and conventional tillage as normal cultivation, whereas any interference within this system is defined as a disturbance. Thus, crop disturbance in the present study is defined as seeding a disturbing crop for one season after continuous soybean system and then switching back to continuous soybean again. By this definition, crop disturbance in nature in this study is a synonym to the term of preceding crop in conventional tillage. We hypothesized that crop disturbance would cause changes in the abundance of pathogens/nematode, reduces disease severity and therefore improves soybean yield, which could be adopted as an alternative management practice in continuous soybean system in supporting the agronomic benefits of the system. The specific objective of current study was to examine, under field conditions, the immediate responses of cyst nematode, soil-borne pathogens, disease severity index and soybean seed yield to one-season crop disturbance in a previous continuous soybean system.

Materials and Methods

Field trials were established in the fall of 2006 at Glory Village, Hailun City, Heilongjiang Province (47° 21' N, 126° 49' E) in Northeast China. The farmland had previously been cropped to continuous soybean for seven years. The soil is a typical Chinese Black soils (classified as Eluviated Black Chernozem in the Canadian system of soil classification or Mollisols in the USA taxonomy system). The soil lies on glacial parent material and has well-developed horizons, with 30 cm thick A horizon and have been under cultivation for at least 40 years. The texture of the soil is silty clay loam with 40% clay and containing 24.1 g organic C kg^{-1}. Soil pH is neutral (7.0±0.1) as determined in distilled water (1:5 V/V). The area has a humid continental

climate; cold and arid in winter and hot and moist in summer. Average annual temperature is 1.5 ^{o}C and long-term annual precipitation is approximately 500-600 mm, with 70% of the total occurred from July to September.

In 2007, soybean (*Glycine max* L. Merill. cv. Heinong 35), wheat (*Triticum aestivum* L. cv. Long-mai 26), sugar beet (*Beta vulgaris* L. cv. KWS0143), tobacco (*Nicotiana tabacum* L. cv. LJ911), corn (*Zea mays* L. hybrid Hai-yu 6) and hemp (*Cannabis satia* L. cv. Gruyev) were planted separately at normal viable seeding rate to develop the following treatments: (1) rotation (wheat-corn-soybean), (2) continuous soybean, (3) one-season wheat disturbance and continuous soybean (wheat treatment), (4) one-season sugar beet disturbance and continuous soybean (sugar beet treatment), (5) one-season tobacco disturbance and continuous soybean (tobacco treatment), (6) one-season corn disturbance and continuous soybean (corn treatment) and (7) one-season hemp disturbance and continuous soybean (hemp treatment). The sowing dates were April 6 for spring wheat, April 23 for hemp, April 30 for sugar beet, May 5 for corn, May 8 for soybean and transplanting date for tobacco was July 3.

Fertilizer rates applied at sowing time were: 120 kg N ha^{-1}, 53 kg P$_2$O$_5$ ha^{-1} for sugar beet and corn; 45 kg N ha^{-1}, 59 kg P$_2$O$_5$ ha^{-1} for tobacco; 75 kg N ha^{-1}, 30 kg P$_2$O$_5$ ha^{-1} for hemp; 90 kg N ha^{-1}, 45 kg P$_2$O$_5$ ha^{-1} for wheat and 20.5 kg N ha^{-1}, 53 kg P$_2$O$_5$ ha^{-1} and 15 kg K$_2$O ha^{-1} for soybean. Treatments were organized in a randomized complete block design with three replications. Individual plot size was 5.36 m wide by 8.0 m long and the row spacing for all crops was 0.67 m. Weeds were controlled manually.

In 2008, except the plots of normal rotation in which corn hybrid Haiyu 6 was planted, soybean cultivar Heinong 35 was seeded on 5 May to resume continuous soybean system again to all other plots. The rates of fertilizers applied for corn and soybean were the same as in 2007.

The number of soybean cyst nematode per 100 g dry soil in the top soil (0-20 cm) was counted after harvest in the year of crop disturbance (2007) and the first year of soybean after disturbance (2008). The number of second-stage juveniles of soybean cyst nematode (*Heterodera glycines*, Ichinohe), soybean root rot disease severity index (DSI), as well as main soil-born disease pathogens, such as *Fusarium, Pythium and Rhizoctonia* were examined during the growing season of the first year after disturbance in 2008.

Soybean cyst nematodes were identified and calculated using the Fang's method (1998). The number of second-stage juveniles (J2) of soybean cyst

nematode followed the method described by Liu et al. (1988). The root rot disease severity index (DSI) was calculated by 10 plants from each plot using a 0-5 scale developed by Cardoso and Echandi (1987), where 0=no lesions on hypocotyl, 1= lesions ≤ 2.5 mm long, 2= lesions 2.5-5.0 mm long, 3= lesions ≥ 5.0 mm long, 4= lesions girdling plant and wilting visible on leaves and 5= seedling damped-off or dead. The modified method of Liu et al. (2008) was used to isolate *Fusarium* and the method of Ma and Xin (1988) was used to isolate *Pythium* and *Rhizoctonia*. The number of second-stage juveniles of cyst nematode, DSI and root rot pathogens was determined respectively at soybean growth stages of V_3, R_2 and R_4 described by Fehr and Caviness (1977).

Soybean seed yield in 2008 were determined by harvesting the center rows of each plot followed by cleaning and weighing of samples. All seed yields were adjusted to a moisture content of 130 g kg^{-1}. Yield component including pod number per plant, seed number per plant, seed number per pod and seed size (mg/seed) were determined by randomly selected 10 harvested plants in each plot. Protein and oil contents in the soybean seed were measured by transmission spectrometer (Foss Electric Multispec Division, Brampton, ON, Canada) and were calibrated with conventional method. Analysis of variance (ANOVA) was performed using PROC MIXED procedure (SAS Institute Inc. 1996). All statistical differences were evaluated at probability level of P<0.05.

Results

The number of soybean cyst nematode

The number of soybean cyst nematode in top soil after crop harvested in the disturbed year and the first year after disturbance was shown in Figure 1. Crop disturbance significantly decreased the cyst nematode number compared with continuous cropping soybean in the disturbed year (P<0.05). The top soils from rotation, wheat, sugar beet, tobacco, corn and hemp disturbance plots had 43-65% less number of cyst nematode than continuous soybean. No difference was found in the number of soybean cyst nematode among rotation, wheat, tobacco, corn and hemp treatments, while those in rotation, wheat, corn and hemp treatments were significantly lower than those in continuous soybean and sugar beet treatments (P<0.05).
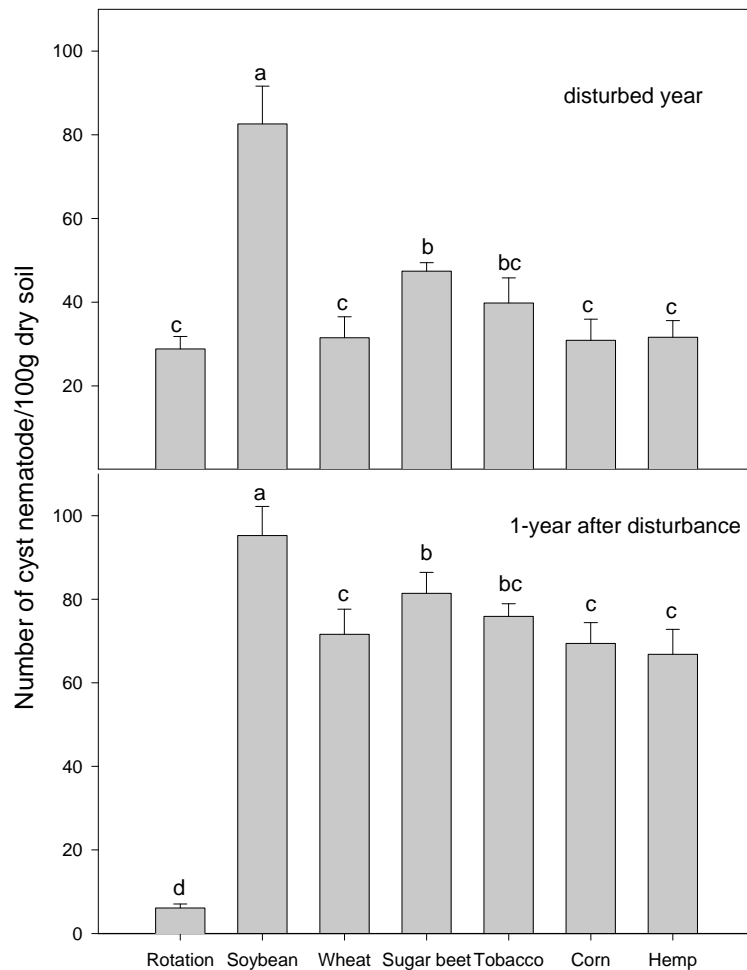
Figure 1. The number of soybean cyst nematode collected at harvest from topsoil in the year of crop disturbance and the first year of soybean after crop disturbance.

Wheat plots in rotation had similar number of cyst nematode as wheat disturbance treatment. However, when soybean was planted in the disturbed plots the following year, the number of soybean cyst nematode in crop disturbance treatments increased consistently compared with the disturbed year except rotation treatment (the crop in the plot was corn) in which the number of cyst nematode declined significantly (Figure 1, P<0.05). Similar to the disturbed year, continuous soybean had the highest number of soybean cyst nematode compared with other treatments (P<0.05). Among

soybean plots after crop disturbance, hemp treatment had the lowest number of cyst nematode, which was 29.8% less than continuous soybean (P<0.05). The number of cyst nematode in plots after soybean was significantly different than that of soybean plots after wheat, sugar beet, tobacco, corn and hemp disturbance (P<0.05, Figure 1). However, no differences were found among wheat, tobacco, corn and hemp treatment. The number of cyst nematode in crop rotation was only 6.1 per 100 g dry soil, which was 6.4% of nematodes counted in the continuous soybean treatment.

Crop disturbance also affected the number of second-stage juvenile (J2) of soybean cyst nematode (Table 1). At V_3, R_2 and R_4 stages, J2 number in continuous soybean was significantly higher than in other treatments (P<0.05). The lowest J2 number during growing period of soybean was observed in corn and wheat treatments. No differences were found at V_3 and R_2 stages among sugar beet, tobacco and hemp treatments, but that of hemp treatment decreased significantly at R_4 stage to the similar level of wheat treatment.

Table 1. The number of second-stage juvenile (J2) of soybean cyst nematode per plant root and soybean root rot disease severity index (DSI) during growing season in the first year of soybean after crop disturbance.

Crop	J2 cyst nematode (individual/plant)			Disease severity index (%)		
	V_3	R_2	R_4	V_3	R_2	R_4
Soybean	93.8[a]	113.7[a]	99.3[a]	46.2[a]	54.1[a]	52.9[a]
Wheat	22.0[c]	34.1[c]	36.8[d]	30.1[b]	33.6[b]	39.7[b]
Sugar beet	58.2[b]	64.7[b]	73.9[b]	49.5[a]	56.1[a]	49.9[a]
Tobacco	55.4[b]	61.2[b]	64.0[c]	28.0[b]	35.9[b]	33.3[bc]
Corn	18.5[c]	30.1[c]	28.5[e]	25.3[b]	36.5[b]	34.5[bc]
Hemp	48.3[b]	52.9[b]	39.7[d]	31.5[b]	29.4[b]	35.7[bc]

Values followed by different letters in the same column are significantly different (P<0.05). The crop planted in rotation treatment was corn, thus no data were collected.

The root rot disease severity index and pathogen number

Although there was no difference between continuous soybean and sugar beet disturbance for disease severity index (DSI) at V_3, R_2 and R_4 stages, DSI after continuous soybean and sugar beet was significantly higher than that of other treatments (P<0.05, Table 1). The number of root rot pathogens during soybean growth varied significantly among various crop disturbances. Continuous soybean had consistently the highest number of *Fusarium* than all other treatments at V_3, R_2 and R_4 stages (P<0.05, Table 2). The number

of all three pathogens after sugar beet and wheat was also significantly lower than continuous soybean (P<0.05), except the number of *Pythium* at V_3 stage after sugar beet and the number of *Rhizoctonia* and *Pythium* at R_2 stage after wheat. Compared with continuous soybean, the number of *Fusarium, Rhizoctonia* and *Pythium* after corn and hemp and the number of *Fusarium* after tobacco were significantly lower at all three stages (P<0.05), whereas no differences were found between tobacco and continuous soybean treatment in regard to *Rhizoctonia* and *Pythium*. Except the number of *Pythium* at V_3 stage after sugar beet and the number of *Rhizoctonia* and *Pythium* at R_2 stage after wheat. The number of all three pathogens after sugar beet and wheat was also significantly lower than continuous soybean (P<0.05).

Table 2. The number of root rots pathogens during the first year of soybean growing after crop disturbance in a continuous soybean system ($\times 10^4$ cfu /g dry soil).

Stage	pathogen	soybean	wheat	sugar beet	tobacco	corn	hemp
	Fusarium	7.38[a]	2.85[b]	2.24[bc]	3.08[b]	2.64[b]	1.96[c]
V_3	*Rhizoctonia*	3.73[a]	1.97[b]	2.11[b]	3.15[a]	1.99[b]	2.20[b]
	Pythium	2.79[a]	2.18[b]	3.07[a]	2.96[a]	2.42[b]	2.12[b]
	Fusarium	8.69[a]	2.27[b]	1.97[b]	2.61[b]	3.20[b]	2.19[b]
R_2	*Rhizoctonia*	2.89[a]	2.86[a]	1.93[b]	2.89[a]	2.24[b]	2.11[b]
	Pythium	0.93[a]	0.85[a]	0.77[b]	0.81[ab]	0.64[c]	0.69[c]
	Fusarium	6.31[a]	3.50[b]	2.89[bc]	2.35[c]	2.91[bc]	2.49[c]
R_4	*Rhizoctonia*	4.09[a]	1.57[c]	2.51[b]	3.73[a]	3.05[b]	3.16[b]
	Pythium	0.91[a]	0.66[c]	0.73[c]	0.85[a]	0.67[c]	0.59[c]

Values followed by different letters in the same row are significantly different (P<0.05).
The crop planted in rotation treatment was corn, thus no data were collected.

Yield and yield components

Soybean produced 10.8% more yield after hemp treatment but those after other crop treatments were not significantly different from that of continuous soybean treatment (Table 3). Hemp disturbance treatment had the highest productive pod number and significantly higher seed number per plant than sugar beet, tobacco and corn disturbance treatments (P<0.05) although it was similar to continuous soybean and wheat disturbance treatment (Table 3). Seed size was not significantly different among continuous soybean treatment and wheat, tobacco, corn and hemp disturbance treatments, while soybean after sugar beet treatment had the smallest seed size.

Only seed protein content after wheat and corn treatments were significantly different, however, they were not different from other treatments (Table 3). No differences were found among crop disturbances for protein + oil contents. Continuous soybean and corn disturbance had the lowest oil content in seed, which were significantly different from that of wheat treatment. Plant height after sugar beet treatment was significantly reduced compared to other treatments (Table 3).

Table 3. Yield components and seed quality in the first year of soybean after crop disturbance in a seven-year continuous soybean field.

Components	Soybean	Wheat	Sugar beet	Tobacco	Corn	Hemp
Plant height (cm)	86.3[a]	87.2[a]	64.1[b]	88.4[a]	90.4[a]	89.9[a]
Pod Number plant[-1]	33.4[b]	32.5[b]	28.4[c]	31.0[bc]	31.4[bc]	36.9[a]
Seed number plant[-1]	93.3[a]	88.0[ab]	75.9[c]	78.3[c]	80.5[c]	93.9[a]
Seed size (mg)	190[a]	188[ab]	182[b]	191[a]	193[a]	187[ab]
Yield per plant (g)	15.1[b]	15.4[b]	13.0[c]	13.8[c]	14.2[bc]	16.8[a]
Seed protein (%)	43.9[ab]	43.4[b]	43.8[ab]	43.7[ab]	44.0[a]	43.8[ab]
Seed oil (%)	18.6[b]	19.0[a]	18.9[ab]	18.8[ab]	18.6[b]	18.7[ab]
Protein + oil (%)	62.7[a]	62.6[a]	62.8[a]	62.5[a]	62.6[a]	62.5[a]
Yield (kg ha^{-1})	3175[b]	2971[b]	2829[b]	3088[b]	3046[b]	3518[a]

Values followed by different letters in the same row are significantly different (P<0.05).

Discussion

Soybean cyst nematode and root rot diseases occurred seriously in continuous soybean (Xu et al., 2000). The present studies found that topsoil collected in the year of hemp disturbance and first year of soybean after hemp disturbance contained lower number of soybean cyst nematode than continuous soybean, while soybean after corn had the lowest number of J2 cyst nematode among the five crop disturbances. The study also demonstrated that the number of soil-borne pathogens of *Fusarium, Rhizoctonia* and *Pythium* after corn and hemp was significantly lower during growing season whereas DSI after crop disturbance except sugar beet, were all lower than continuous soybean. This finding indicates that crop disturbance changed the habitat already formed for the survival of cyst nematode and pathogen after seven-year continuous cultivation of soybean. This may be particularly important and useful as a remediation to continuous soybean system when yield is compromised.

Crop diversification can moderate plant diseases through crop selection and inter ruption of disease cycles, particularly in case of pathogens that are residue-borne (Krupinsky et al., 2002; Turkington et al., 2003). Rotation of

crop species is a valuable tool for reducing plant diseases in cropping systems (Mc Mullen and Lamey, 1999; Hassan et al., 2009). Crop sequence/rotation in combination with other management practices can be one of the most effective and inexpensive methods to manage a number of plant diseases (Holtzer et al., 1996; Krupinsky et al., 2002; Turkington et al., 2003; Krupinsky et al., 2007a). Thus, appropriate crop sequence is an important management practice that may lower the plant disease risk in cropping systems through lengthening the time between susceptible crops so that pathogen populations have time to decline (Krupinsky et al., 2007b).

Changes in the sequence of crops are known to enhance the yield of cereal crops such as wheat. Kirkegaard et al. (2004) reported that wheat can yield 20% more following broad-leaf crops compared with wheat following wheat across broad regions of North America, northern Europe and Australia. Generally, crops seeded on their own residue perform poorly compared with following different crops (Johnston et al., 2005; Krupinsky et al., 2006; Miller et al., 2003). However, the present studies showed that compared to continuous soybean, soybean yield was reduced by 11.0% after sugar beet, by 6.4% after wheat and was increased by 10.8% after hemp. This demonstrates that the preceding crop species before soybean had a remarkable influence on continuous soybean yield. The immediate yield gain by hemp provides a simple and alternative technique to be applied in continuous soybean system if farmers contemplate switching to other crops after continuous soybean and then to continuous soybean. The immediate effect of crop disturbance on continuous soybean yield in this preliminary study is promising, in that it identifies a crop treatment that can be used in such cropping system, which has not been documented elsewhere in the literature.

Hemp plants have been demonstrated to produce potential toxic phenolic chemicals that were leached from the plant foliage (Kaminsky, 1981). It is likely that the primary mechanism for alleviating yield loss of continuous soybean after hemp disturbance might be due to the reduction in soil-borne pathogens, soybean cyst nematode, second-stage juvenile and DSI. However, it is not true in the case of other crops because though these parameters declined, yield was not increased. This finding adds new evidence to the crop rotation in agricultural land for improving crop yield, reducing incidence of pests such as insects, disease and weeds and reducing economic risk (Cook, 2006). The general effects on soil-born pathogens lend support to the conclusion that crop disturbance was the most important factor determining populations of soil-borne pathogens in the following crop growing season.

We surmised that these effects include protection from cyst nematode life cycle in top soil layers, reduction of soil-born pathogens and enhanced microbial activity of the surface soil where plant roots elongate. Thus, we raise the possibility that biochemical changes following hemp interference may actually stimulate soybean growth and thus yield. The potential importance of this phenomenon needs further investigation. The involvement of crop disturbance may provide a useful approach to managing the continuous soybean systems. It is not clear how long the control benefits of crop disturbance might last. As new crops are incorporated into grower's continuous soybean system, more information is necessary to determine the long-term benefits and/or disadvantages of previous crop and crop residues on soybean production.

Future research addressing the persistent races of cyst nematode, root exudates, microbe populations and allelopathic chemicals could identify practices that would further increase soybean yield, a step that would make the practice of crop disturbance more robust. It is our hope that the ideas presented herein will serve as a useful guide for future studies as to the kinds of data needed to elucidate the effects of crop disturbance, including its interplay with biological processes.

Acknowledgement

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