RESEARCH ARTICLE

Contribution of bacterivorous nematodes to soil resistance and resilience under copper or heat stress

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ARTICLE INFO

Article history:

Received December 10, 2019

Revised May 11, 2020

Accepted May 27, 2020

Keywords:

Stress

Soil resistant

Soil resilience

Bacterivores

Functional stability

ABSTRACT

The functional performance of soil ecosystems following disturbance determines ecosystem stability, and although contributions of bacterivorous nematodes to soil ecosystems are recognized, their roles in functional stability have received little attention. The objective of this study was to evaluate the roles of bacterivorous nematodes in functional stability following stress. In a factorial laboratory experiment, soil microcosms were prepared with two levels of nematode abundance, either an enriched abundance of bacterivores (Nema soil) or background abundance of nematodes (CK soil), and three levels of stress, copper, heat, or an unstressed control. The resistance and resilience of nematode abundance, as well as soil microbial function by determining decomposition of plant residues and microbial substrate utilization pattern using a BIOLOG microplate, were followed post stress. The relative changes of two dominant bacterivores, *Acrobeloides* and *Protorhabditis*, responded differently to stresses. The resistance and resilience of *Protorhabditis* were greater than that of *Acrobeloides* to copper stress during the whole incubation period, while both bacterivores only showed higher resilience under heat stress at the end of incubation. The enrichment of bacterivores had no significant effects on the soil microbial resistance but significantly increased its resilience to copper stress. Under heat stress, the positive effect of bacterivores on soil resilience was only evident from 28 days to the end of incubation. The differences in the responses of soil function to stress with or without bacterivores suggested that soil nematodes could be conducive to ecosystem stability, highlighting the soil fauna should be taken into account in soil sustainable management.

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1 Introduction

Soil is one of the most important resources for human activity and, as a part of the global ecosystem, its stability has a significant contribution to the sustainable development of the

Earth resources (Tilman et al., 2002; Brussaard et al., 2007; Nielsen et al., 2015). Soils are under increasing pressures and soil biota particularly its diversity are substantially altered by human activities, threatening ecosystem function persistence insured by soil biodiversity as a buffering reservoir against the stresses and disturbances (Geisen et al., 2019). Recently, along with the negative impact of natural environmental fluctuations and anthropogenic activities on ecosystems, soil functional stability has received increasing attention (Girvan et al., 2005; de Vries et al., 2012; Griffiths and

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Philippot, 2013; Danneyrolles et al., 2019), which is described as the ability of functional response after disturbances (Orwin and Wardle, 2004). Stability has two components: 1) resistance, change range of soils after disturbances; 2) resilience, the soil's ability to recover after disturbance (Griffiths et al., 2001). However, due to the huge multiple facets of soil ecosystem function, the evaluation of its stability is still facing many challenges, such as finding better indicators to reflect the ecosystem property fluctuations than traditionally used static soil physical and chemical properties.

Soil biota is a determinant of soil health ecological functions (Doran and Zeiss, 2000; Orgiazzi et al., 2016), and not only interacts with soil fertilizer and environment, but also plays a pivotal role in soil functional stability under various stress (Coleman et al., 2004; Yang et al., 2018). Soil nematodes are the most abundant metazoans in the soil, ubiquitously distribute in a variety of habitats, notably occupy the detrital food web in a variety of niches, and play fundamental roles in soil ecological process (Bongers and Ferris, 1999; Yeates, 2003; Neher, 2010; Schratzberger et al., 2019). Many reports have suggested that nematodes were the most potentially useful bioindicators in soil ecosystem health following disturbance (Yeates, 2003; Li et al., 2020). Besides, soil nematodes have been confirmed to affect microbial community and diversity since decades ago (Griffiths et al., 1999; Bonkowski et al., 2000), and the nematode mediated biological interactions are central in regulating soil food web structure and functioning, particularly critical for the fine-tuning multiple ecosystem functions (Chen et al., 2007; Thakur and Geisen, 2019; Zhu et al., 2019; Delgado-Baquerizo et al., 2020). Also soil nematode communities respond quickly to different disturbance conditions, such as farming, pollution, acidification, insecticide, liming and fertilization (Fiscus and Neher, 2002; Ferris and Bongers, 2006; Zhang et al., 2012; Cortois et al., 2017; Pothula et al., 2019).

In recent years, the relationship between heavy metal contamination and soil nematodes has attracted increasing attention (Korthals et al., 1996; Li et al., 2006). Copper contamination, mainly because of mining and the application of Cu-containing fungicides, is one of the major soil heavy metal pollutants in soil and can perturb the soil microbial community and threaten soil biodiversity (Dussault et al., 2008; Hewitt et al., 2010). Also, in recent years, with the increasing frequency of global change, especially extreme climate, the stability of soil ecosystem is facing unprecedented environmental pressure, such as high-temperature even heat stress for soil ecosystems (Rein et al., 2016; Schwarz et al., 2017). Experimentally, copper and heat have been used as the model type of stresses in studies of soil resistance and resilience, and considered to be representative of a persistent and a transient stress, respectively (Griffiths et al., 2000). However, it is still not clear whether nematodes, or the interaction between soil nematodes and microorganisms, could promote the stability of soil ecological function after stress or disturbance.

In this investigation, we manipulated soil to have an

enriched abundance of bacterivorous nematodes and then subjected soil to copper or heat stress. We hypothesized that copper or heat stress could significantly reduce soil functional stability by reducing the resistance and resilience of decomposition, but that the presence of enriched bacterivorous nematodes would increase soil functional stability under stress conditions. To test the hypothesis, soil microcosms were subjected to copper contamination and heat stress, and soil functioning was assessed by the short-term decomposition of added plant material as used in previous studies (Griffiths et al., 2000; 2001; Kuan et al., 2006). Soil nematode and dominant bacterivore abundance, as well as microbial substrate utilization pattern assessed by BIOLOG-Eco microplate, were determined to monitor the role of the nematodes in soil functional stability.

2 Materials and methods

2.1 Soil and substrates

The soil used in this experiment was a sandy-loam alluvial soil collected from arable soil (0–20 cm horizon) in Banqiao town, Nanjing city, Jiangsu Province, China (Mao et al., 2006). The soil was composed of 57.5% sand, 26.6% silt and 15.9% clay, with 9.20 g kg⁻¹ of organic C, 0.89 g kg⁻¹ of total N, 7.24 µg g⁻¹ NH₄⁺-N, 9.39 µg g⁻¹ NO₃⁻-N, and the pH (H₂O) was 6.32. Fresh soil was sieved through a 2-mm mesh to remove stones, macrofauna, and discernible plant material. The pig manure, added into the soils for the enriched nematodes (C: N ratio of 18.6), was sundried and milled to sieve through a 2 mm diameter mesh before use.

2.2 Obtaining soil enriched nematodes

The approach to enrich nematodes in laboratory was similar to Mao et al. (2006) who had described in detail for getting bacterivorous nematodes from native soils. The nylon mesh of pore diameter 5 µm or 1 mm were filled with 400 g fresh soils mixed with 14 g pig manure, were placed in the center of a pot and surrounded by 650 g of same fresh soil. This would allow nematodes and soluble nutrients, which developed in the inoculum soil, to migrate through the 1 mm diameter mesh to the surrounding 650 g soil. While the 5 µm diameter mesh would prevent the migration of nematodes but still allow soluble nutrients to pass into the surrounding 650 g soil (Fig. 1). In this way, two different soils were established here, 1) the outer soil surrounding the 1 mm mesh bag would become enriched in nematodes, (Nema soil, enriched nematode abundance); 2) the outer soil surrounding the 5 µm mesh bag contained background nematode abundance (CK soil) (Fig. 1).

2.3 Establishment of soil microcosms

Two stresses, copper and heat, represented a transient and a persistent stress, were applied to microcosms (Griffiths et al., 2000; 2001). Briefly, each microcosm of the control treatment

Fig. 1 The plots designed for obtaining two different soils with different nematode abundance, one was Nema soil, enriched nematodes from native soils; another was CK soil, background nematodes from native soils.

(without any stress) was amended with 100 μL sterile distilled water. 100 μL $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution was added to each of the copper treatments to give 500 $\mu\text{g Cu g}^{-1}$ dry soil. To guarantee the copper was homogeneously distributed, all microcosms were gently shaken and mixed. For heat stress, the microcosms were added 100 μL sterile distilled water and then subjected to a heat shock at 40°C for 18 h in a constant temperature chamber. All stress treatments were applied to the soils at day 0 and the microcosms were subsequently incubated at 20°C in the dark, with a loose-fitting lid, in a moist atmosphere. To enable destructive sampling over time, each treatment replicate was established in six microcosms, giving a two-factor design, including 2 (with more or less nematodes) $\times$ 3 (no stress, copper or heat stress) $\times$ 4 (replicates) $\times$ 4 (sampling) = 96 microcosms in total. At days 1, 8, 28, and 56 after stress application, microcosms from each treatment were randomly selected and destructively sampled for further analysis.

2.4 Nematode parameters

Nematodes were extracted from 50 g fresh soil with a modified Baermann method using trays instead of funnels (Liu et al., 2008). After 48 h of extraction at room temperature, the nematode suspension was collected and the numbers of all nematodes counted under a dissecting microscope. Then the nematodes were heat-killed for 2 min at 60°C and preserved in 4% formaldehyde. Subsequently, all nematodes from samples were identified to genus. The dominant nematode relative changes were used to investigate the changes of different nematode taxon following the stress, which was calculated as:

$$\begin{aligned} & \text{Nematode relative changes}(\%) \\ &= (\text{nematode abundance}_{\text{stress}} / \text{nematode abundance}_{\text{control}}) \\ & \times 100. \end{aligned}$$

2.5 Functional parameters

Stability analysis was following the approach of Griffiths et al. (2000). The rate of carbon mineralization (as measured by the short-term decomposition of plant residues) was selected as a measure of soil function. At each sample date, two replicate aliquots of 10 g soil from each microcosm were added with 100 mg barley grass powder (equivalent to 456.8 mg C per gram, C:N 10:1) and mixed well, respectively. Then they were transferred into 100 mL respiration flask by alkaline solution absorption methods to determine CO_2 evolution over 24 h at 20°C. The concentration of CO_2 in alkaline solution (0.2 M NaOH) was titrated with 0.1M hydrochloric acid (HCl) and then CO_2 evolution was calculated.

Functional stability was calculated as the changes in short-term decomposition of the stressed soil compared with the unstressed control (as a percentage):

$$\text{Functional stability}(\%) = (\text{CO}_2_{\text{stress}} / \text{CO}_2_{\text{control}}) \times 100$$

Resistance was defined here as the instantaneous effect of the stress, i.e., stability measured on day 1, while resilience is defined as the recovery over time, i.e., the change in stability measured on days 1, 8, 28 and 56 (Griffiths et al., 2000).

The microbial functional diversities were analyzed using BIOLOG-Eco microplates (Biolog Inc., Hayward, CA) based on the utilization of 31 carbon substrates. Each of 5 g soil sample with 45 mL of sterile saline solution (0.85% NaCl, w/v) by shaking for 30 min. One thousand-fold dilutions were performed after the suspensions were allowed to settle for 5 min. The plates were inoculated with 150- μL aliquots using a multi-channel repetitive dispensing pipette. The color development of the wells at 590 nm was read using an automated Biolog Microplate™ Reader (Biolog Inc., Hayward, CA). The plates were then incubated at 25°C in darkness and read every 24 h over a period of 7 days. The absorbance measured after 120 h, was used to calculate the functional diversity.

2.6 Data analysis

All data were expressed on an oven-dry soil basis (24 h, 105°C). The stability of nematode and soil function was calculated as the percentage change in nematode or decomposition function parameters of the disturbed soil compared with the undisturbed control. The significance of treatment effects on nematode abundance, soil respiration and functional stability were tested by one-way analysis of variance (ANOVA) using the Fisher LSD test. All statistical analyses were performed using the Statistica software package (StatSoft Inc., Tulsa, Oklahoma, USA).

3 Results

3.1 Nematode abundance in two different soils before the stress applied

Before stresses were applied, the nematode abundances and communities from two soils used in this experiment were significantly different (Table 1). Nema soil had a nematode abundance of 103.4 per gram soil, which was nearly 5 times higher than in CK soil (Table 1). The dominant trophic groups in each soil were bacterivores, nearly 99.6% in CK soil, 94.8% in Nema soil, in which *Acrobeloides* and *Protorhabditis* were nearly 60% and 38% in CK soil respectively, and were 66% and 28% in Nema soil respectively. Soil nematode diversity, assessed by Shannon–Weiner index, was greater in Nema soil than in CK soil (Table 1).

3.2 Nematode abundance following stress

After the copper and heat stresses, nematode abundance dropped drastically. In CK soil, nematode abundance decreased from 23.5 to 2.0 per 100 g soils after copper stress

1 day and to 0.9 per 100 g soils after heat stress 1 day (Fig. 2A–C). In Nema soil, nematode abundance also dropped down from 104.0 to 8.6 per 100 g soils after copper stress and to 1.6 per 100 g soils after heat stress 1 day (Fig. 2A–C).

Stresses and sampling time had significant influences on nematode abundance ($p < 0.05$, data not shown). During 56 days' incubation, nematode abundance recovered faster in Nema soil than in CK soil. At the end of incubation, 10.8% and 19.9% nematode recovery rates were observed in CK soil and Nema soil with copper stress, respectively. The nematode recovery rate under heating stress was much slower than copper stress, 4.2% and 5.8% in CK soil and Nema soil respectively.

3.3 The relative changes of two dominant bacterivorous nematodes following stress

Acrobeloides and *Protorhabditis* were the most dominant bacterivores before stresses and performed differently to two stresses in the whole incubation period. The resistance (the 1 day's relative change, Fig. 3A) of *Protorhabditis* was significantly higher than that of *Acrobeloides*, but both were similar in two soils performed under copper stress. The resilience of *Acrobeloides* in CK soil and Nema soil was no any differences following copper stress until the end of the incubation time in Nema soil and CK soil, while the resilience of *Protorhabditis* was increasing dramatically following copper stress in Nema soil, rather than in CK soil (Fig. 3A).

Heat stress depressed the resistance of *Acrobeloides* and *Protorhabditis*, much lower than those under copper stress, higher in Nema soil than in CK soil (Fig. 3B). The resilience of these two nematodes was very slow during the whole incubation time in two soils. At the end of incubation, nematode abundances of these two dominant species did not recovery.

Table 1 Nematode assemblages in two different soils before stress applied.

Genus	Trophic groups	Abundance	
		CK Soil	Nema soil
<i>Paratylenchus</i>	He	-	6
<i>Filenchus</i>	Fu	-	10
<i>Aphelenchus</i>	Fu	-	46
<i>Acrobeloides</i>	Ba	148	698
<i>Eucephalobus</i>	Ba	-	40
<i>Protorhabditis</i>	Ba	94	242
<i>Plectus</i>	Ba	-	4
<i>Aporcelaimellus</i>	Om	-	10
<i>Mesodorylaimus</i>	Om	-	8
<i>Ironus</i>	Pr	-	2
<i>Anatonchus</i>	Pr	-	2
Genus		4	12
Abundance (100 g ⁻¹ dry soil)		242b	1068 a
Shannon–Weiner index		0.89 b	1.14 a

He: Herbivores, Ba: Bacterivores, Fu: Fungivores, Om: Omnivores, Pr: Predators. A different letter indicates significant difference ($p < 0.05$, $n = 4$).

Fig. 2 Total nematode and two dominant bacterivores abundance (*Acrobeloides* and *Protorhabditis*) in soils under control or stressed treatments (copper or heat) during the incubation period from 0 day to 56 days. Error bars represent standard deviations ($n = 4$).

3.4 Soil respiration and functional stability

The basal respiration, which represented soil absolute function, was higher in the Nema soil than in the Soil S, all decreasing during the incubation time ($p < 0.05$, data not shown).

The functional parameters showed differences under two stresses. One day after copper amendment, the resistance was no difference in Nema soil and CK soil (Fig. 4A, B). After incubation 8 days, Nema soil was the most resilient to copper than Soil S, with 45% functional capability relative to a non-perturbed control (Fig. 4A). After 28 days incubation, the resilience of Nema soil and CK soil was steeply increasing. While in heat stress, the resistance was higher in Nema soil

than CK soil, which was steeply decreasing on 8-day incubation. The resilience was then increasing slowly after 8-days in Nema soil, while was keeping decreased until 28 days in CK soil, then was increasing after 28 days incubation.

3.5 The microbial substrate utilization pattern

The soil microbial substrate utilization pattern could reflect different treatments of biological community differences after stresses. From the principal component analysis after 1 day (Fig. 5), the microbial communities from two initial CK soil and Nema soil were total different. Copper stress and heat stress changed significantly microbial communities compared to the control treatment in both soils. At the end the incubation period (56 d), soil microbial community differentiation was still larger

Fig. 3 The relative changes (nematode abundance_{stress} / nematode abundance_{control} × 100) of two dominant bacterivores *Acrobelloides* and *Protorhabditis* under stress (copper or heat). Error bars represent standard deviations (n = 4).

Fig. 4 Soil functional stability (the percentage change in respiration, CO₂ evolution over 24 h, CO_{2stress} / CO_{2control} × 100) after barley straw amended to soils under different treatments during the incubation period under stress (copper or heat).

under the copper or heat stresses no matter with enriched nematodes or not (Fig. 5).

4 Discussion

Soil ecosystem has high diversity and complexity of biological community. A large number of studies have indicated that the whole soil biodiversity, particularly in view of biotic interaction of soil food web, maintains ecosystem functional stability under disturbance conditions (Bardgett and van der Putten,

2014; Kardol et al., 2016; Delgado-Baquerizo et al., 2017). Soil nematodes, through stimulating microbial activity, changing microbial community structure and spreading microorganisms (Trap et al., 2016), could therefore contribute to the stability of ecosystem function (Yeates, 2007; Liu et al., 2012; Schratzberger et al., 2019). This study, using two soils with distinct bacterivorous nematode assemblages (i.e. enriched or not), demonstrated that bacterivorous nematodes could increase soil functional resilience to the copper or heat stress, which was consistent with our hypothesis. Also we found that

Fig. 5 Principal component analysis of absorbance data from BIOLOG-Eco MicroPlate™ at 1-day and 56-day sampling time. Treatments included control (CK●, Nema○), copper stress (CK■, Nema□), heat stress (CK▲, Nema△).

bacterivores only enhanced soil resistance under heat stress, while did not affect soil resistance under copper stress.

4.1 Nematode changes

The procedure of adding organic matter to soil of the enrichment of soil nematodes was aimed to promote nematode abundance in soil. With the approach to enrich nematodes in laboratory, nematode abundance differed from two experimental soils, providing a reliable different community compositions and diversities of nematode species, dominated by bacterivores. Similar results were found from Mao et al. (2006), that the 1-mm mesh gave more total and bacterivorous nematodes in the outer soil than the 5-μm mesh. Zhao and Neher (2013) also reported that most nematode genera responded positively to organic amendments. In the unstressed control microcosms, nematode abundance kept stable and slightly increased during the whole incubation time in 5-μm mesh soil, while increased until

8-day in 1-mm mesh soil and then decreased. The main reason might be the food resources for nematodes in microcosm, which indicated that higher nematode density resulted in more consumption of food.

After copper and heat stress, the absolute values of nematode abundance dropped dramatically at the beginning of the stress, but nematode abundance remained high following stress due to higher initial numbers or the tolerant species compared to less nematodes soils. Two dominant bacterivores showed different tolerant performance during the recovery stage, indicating the importance of key species in the soil. Ekschmitt and Korthals (2006) emphasized that dominance within feeding type (DoFT), used to quantify nematode importance as sentinels of heavy metals and organic toxicants in the soil, and they found DoFT by far the better choice. It was known that, even within the same genus, different species could exhibit contrasting responses to heavy metals (Sturhan, 1986). Therefore, it seems possible that tolerance to toxic substances, or the potential for selective adaptation, evolved repeatedly, independently, and differentially within the Secernentea based on a general pre-adaptation of this group. Korthals et al. (1996) examined copper effects at different pH levels after 10 years of exposure in an arable field which was under normal agricultural practice in the meantime. They found that nematode assemblage indicated sensitively copper pollution. The increasing copper concentration significantly decreased the number of *Acrobeloides*, *Acrobeles* (Korthals et al., 1996). From our short incubation experiment, *Acrobeloides* dropped dramatically after copper stress, and related change dropped to 4.0%–7.5% after copper stress, even lower after heat stress, consistent with Bongers and Bongers (1998). CP-2 species *Acrobeloides* was not sensitive for heavy-metal-induced stress. CP-1 species, *Protorhabditis*, in this case, behaved stable and contributed much to the soil nematode recovery.

4.2 Soil functional stability

For unstressed soils, soil functioning (short-term decomposition of plant residues) was not significantly increased in the CK treatment on day 1, 8 and 56, but was significantly increased by the enriched soil nematodes during the whole incubation period. From the distinguished separation of microbial functional diversities represented by substrate utilization pattern during the incubation period, it indicated the soil microbial communities were significantly different for two soils. Many studies have reported that nematodes stimulated key ecological processes such as decomposition, nutrient release, plant nutrient uptake and plant productivity (Ingham et al., 1985; Bardgett et al., 1999; Yeates, 2003). For example, Beare et al. (1997) found bacterivorous nematodes were estimated to contribute (directly and indirectly) about 8% of nitrogen mineralization in conventional farming systems. Zhu et al. (2018) reported that bacterivores could stimulate soil gross N transformation rates, but depended on different nematode species. Those studies indicated that bacterivores could shape soil microbial communities differently and could

stimulate multiple soil ecosystem functions (Chen et al., 2007).

In stressed soils, bacterivores promoted soil functional performance, especially functional stability under copper and heat stresses in this study. Under copper stress, the resistance of enriched nematode soil did not have advantage while significantly higher resilience compared to background nematode soil. Zhao and Neher (2013) summarized that normally heavy metals had harmful effects on nematode community, while low doses of copper showed positive effect on nematode community. Girvan et al. (2005) observed greater resilience to benzene application in soil with a higher natural biodiversity, as demonstrated by a quicker recovery in the mineralization rate of 2,4 dichlorophenol, than in lower biodiversity soil. For two dominant bacterivores, copper had positive effects on *Protorhabditis* than *Acrobeloides*. There were reports of nematode genera increase or decrease in abundance exposed to copper (Ekschmitt and Korthals, 2006). It was presumed mainly because of the specific life history traits of these two bacterivores. *Protorhabditis*, colonizer-persister (CP) value 1, differed from *Acrobeloides* with CP value being 2. Taxa with lower CP values were assumed to be recovered fast (i.e., resilient) after disturbance (Bongers and Ferris, 1999).

In stressed soils, differ to the persistent copper stress, the transient heat stress which might be the most commonly encountered stresses in the natural environment of nematodes (Jones and Candido, 1999), had a negative impact on soil nematode abundance, and soil resilience during 28 days in our study. Schwarz et al. (2017) found that warming combined drought could shape the energetic structure of soil food webs and threaten soil ecosystem function. In addition, Thakur et al. (2017) reported that a consistent negative effect of warming on nematode diversity especially in resource-limited environments, very similar to our study, of which heat stress had a more negative effect on background nematode soil than enriched nematode soil. Enriched nematode soils had more nutrients and supplied more soil microbial communities, so resistance was higher in heat stress. For the two dominant bacterivores, the resilience was higher in enriched nematode soils comparing to less nematode soils during all incubation time, showing strong recovery ability of soil nematode suffered to environment. However, it has been suggested that the resistance and/or resilience of soil communities to disturbances could be influenced by the initial biodiversity of a particular system. The differences in the responses of soil function to disturbance with or without bacterivores suggested that bacterivores could contribute to soil functional stability.

5 Conclusion

This experiment compared the functional behavior of an enriched soil community (enriched with nematodes) and a poor soil community (few nematodes) under simulated stresses. Changes in relative nematode abundance, espe-

cially some dominantly specific nematode species, such as bacterivores, *Protorhabditis* and *Acrobeloides* in this case, reflected the potential of nematode as an indicator following the environmental changes. The differences in the responses of soil function to disturbance with or without bacterivores suggest that bacterivores are conducive to soil community and functional stability. Furthermore from the differences between nematode species responding to the disturbance, it could help us to utilize different soil nematode as the soil bioindicators more efficient, convenient and comprehensive, also to manipulate both the biodiversity and composition for steering their functional potential to increase ecosystem stability.

Acknowledgment

This work was supported by the National Foundation of Sciences in China (No. 41877056) and China Agriculture Research System-Green Manure (No. CARS-22-G-10).

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