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Soil fertility and heavy metal pollution (Pb and Cd) alter kin interaction of *Sorghum vulgare*

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*Author for correspondence: Xingliang Xu, e-mail: xuxingl@hotmail.com; frwzym@aliyun.com Research highlights

- The first study to see heavy metal effect of plant kin recognition
- Use plant NUE as indicators of plant kin recognition

▶ Plant morphological traits, physiological traits and plant nutrient use efficiency (NUE) and heavy metal concentrations of Pb and Cd (N) were measured to determine plant kin interactions.

Significant soil fertility, kinship, heavy metal, and the interactive effects were observed on measured indicators in our study

Abstract

Plant kin recognition has been confirmed in some species, but it remains unclear how kin recognition is related to soil conditions such as fertility and heavy metal stress (Lead or Cadmium). A three-factor experiment based on soil fertility, neighbor's kinship and heavy metal addition was conducted using *Sorghum vulgare* as a target plant. Plant morphological traits, physiological traits and plant nutrient use efficiency (NUE) and heavy metal concentrations of Pb and Cd (N) were measured to determine plant kin interactions.

Significant soil fertility, kinship, heavy metal, and the interactive effects were observed on measured indicators. Kin plants showed higher SLA, root activity, the NUE of phosphorus (P) and sulphur (S) than strangers in nutrient-poor soil. When in nutrient-rich soil, lower SLA and the NUE of nitrogen (N) and sodium (Na) were observed in kin than strangers. Under Pb stress, decreased stem elongation and the NUE of potassium (K), whereas increased MDA and proline content were observed in kin than strangers in nutrient poor-soil. On contrast, kin plants increased the stem elongation, SLA, root activity, and the NUE of K compared to strangers in nutrient-rich soil; Under Cd stress, kin plants decreased the total biomass, the NUE of P, as well as the content of chlorophyll and proline, while increased the MDA and Cd concentration compared to strangers in nutrient-poor soil. Kin plants increased the SLA, root activity, MDA content and Cd concentration, while decreased the NUE of S compared to strangers in nutrient-rich soil. Under Pb plus Cd stress, compare to strangers, kin plants produced higher biomass and the NUE of P in nutrient-poor soil.

whereas exhibited lower stem elongation, less proline, Pb and Cd concentration than strangers in nutrient-rich soil.

We conclude that kin recognition of *S. vulgare* was soil fertility and heavy metal stress depended. Kin recognition occurred in nutrient-poor soil, while kin recognition and competition co-occurred in nutrient-rich soil. Pb stress alone made co-occurrence of kin recognition and competition, while Cd stress alone supports kin competition. By comparison, the Pb plus Cd stress supports kin recognition of *S. vulgare*. Plant NUE for specific elements, combined with morphological and physiological performance, can be used as sensitive response indicators for kin recognition in complicated soil conditions.

Keywords: kin recognition, nutrient use efficiency, physiological response, Pb and Cd

Introduction

In nature, the relatives of many plant species are likely to live together based on the seed dispersal mechanisms (Masclaux et al. 2010). Cooperation and competition between plants can severely shape plant community structure, diversity and dynamics (Callaway, 2007; Brooker et al., 2008). When considering interactions between these related individuals, there exist two major contrary theories which predict contrasting outcomes about how plant relatedness affects competition among neighbors: (1) The niche partition theory represents that species divide a niche to avoid competition for resources. Therefore, stronger competition can occur between more close related individuals because they share the same resources and have more niche overlap (Young 1981; Tilman 1982; Platt and Bever 2009), and (2) The kin selection theory describes the concept of inclusive fitness and the cost-benefit rule. In the theory, kin selection is regarded as an evolutionary strategy that favours the reproductive success of an organism's relatives, even at a cost to the organism's own survival and reproduction (Hamilton 1964). The theory predicts cooperation among close related relatives through kin recognition. So far, numerous studies focused on interactions among plant relatives obtain diverse outcomes (File et al. 2012).

Soil fertility can strongly mediate plant interactions through nutrient competition in plant community (Raynaud & Leadley 2004). Soil fertility modulates plant kin recognition or/and competition because nutrient availability plays a critical role in establishing plant root architecture (Berntson 1994) and the nutrient uptake (Yoneyama et al. 2007; Dakora &Phillips 2002). As a result, different nutrient levels could affect kin recognition through altering cost and benefit of their cooperation (Palmer et al. 2016). For example, compared to less stress, facilitation occurs among intraspecific individuals of *Nothofagus pumilio* under a moderate stress (Fajardo & McIntire 2011). Such cooperative behaviors are also observed in other plant species (Farrer & Goldberg 2011; Cavieres & Penaloza 2012; Castellanos et al. 2014). The resource competition and use strategies also affect plant interactions, e.g., interspecific plants usually develop strategies to access multiple soil resources by consuming various nutrient forms (McKane et al. 2002) or extend roots into both horizontal and vertical soil layers (Cahill et al. 2010). Despite intraspecific plants with similar nutritional niche (Silvertown 2004), kin plants may reduce plant competition through improving their nutrient use efficiency (NUE) (Maestre et al. 2009; Li et al. 2017). Except nutrients, soil heavy metal stress could be a potential factor to affect plant kin recognition. Soil stress resulted from heavy metal, e.g., lead (Pb) and Cadmium (Cd) can affect plant physiological performances of transpiration, photosynthesis and antioxidant system (Ting et al., 1991; Wahid et al., 2010; Cao et al., 2013), as well as morphological growth, biomass and reproduction (Sanchez-Martin &Sanchez-Camazano, 1993; Goodnight, 1985; Benavides et al., 2005; Gao et al., 2010; Huang et al., 2010) due to toxic effects of heavy metal acting on plant roots. However, it remains unclear how kin recognition is affected by soil fertility and heavy metal stress.

Plant kin recognition is generally expressed through reduced competitive performances (Dudley & File, 2007; Bhatt & Dudley, 2010; Biernaskie, 2011; Caffaro et al. 2011; Crepy & Casal, 2015) and increased plant fitness (Tonsor, 1989; Argyres & Schmitt, 1992; Biedrzycki et al., 2010; Biernaskie, 2011) among kin neighbors. However, these morphological and fitness indicators are usually affected by multiple environmental factors, which could then influence kin recognition results (Fajardo & McIntire 2010; Lepik et al. 2012; Milla et al. 2009; File et al. 2011). Therefore, plant functional process such as nutrient acquisition, which is important for plant growth, could be important potential driving force for kin recognition. (James et al. 2005; File et al. 2011, 2012; Simonsen et al. 2014; Li et al. 2017). Moreover, kin recognition responses may be additionally revealed by physiological performances, especially under heavy metal conditions (Pakkasmaa & Laurila, 2004; Biedrzycki & Bais, 2010). E.g., the changes of chlorophyll content can reflect the strength of the plant leaf photosynthesis function by inhibiting the chlorophyll acid ester reduction and the synthesis of amino ten ketone valeric acid (Stohart et al. 1985), which is also used to characterize aging of plant tissue and organs under heavy metal stress (Lambers et al. 2005; Melis 2009). Photosynthesis is likely to be changed if carbon use efficiencies are affected by neighboring plants (Richards 2000; McCormick et al.

2006; Lendenmann et al. 2011), plant root activity is often used to evaluate the absorption of water and mineral salts ability (Islam et al, 2007). Moreover, heavy metals in soil can strongly affect plant root activity and photosynthesis through osmotic adjustment (Tamura et al. 2003). Plants commonly accumulate osmolytes to avoid osmotic stress (Rampino et al. 2006) such as proline as and plants tolerance reactions (Rampino et al. 2006), along with arising malondialdehyde (MDA) concentration as the indicator of oxidative stress (Moran et al. 1994; Sairam et al. 2000; Esfandiari et al. 2007). These physiological changes could well reflect plant response to kin recognition under heavy metal pollution conditions. This indicates that kin recognition could be achieved by increasing the NUE of those soil elements among relatives for growth and reproduction (Cheplick 1992; Murphy et al. 2000; Zhang et al. 2016; Li et al. 2017). Thus, plant NUE and physiological performances could be proper indicators to evaluate kin recognition and conduce to interpret the kin recognition performances of morphological and fitness. However, such studies are still lacking (Li et al. 2017).

Sorghum vulgare, an annual crop species planting widely in the world, is most likely to have relatives living together (Hess & de Kroon 2007; File et al 2011). *S. vulgare* should be an ideal plant species for assessing kin recognition because their growth or yield is largely dependent on soil fertility and potentially affected by soil heavy metals (Biedrzycki & Bais 2010; Chen et al. 2012; Bais 2015). Because nutrient concentrations in annual plants can well reflect their NUE over their living period (Shaver & Melillo 1984; Birk & Vitousek 1986), plant nutrient content on a mass basis is used to represent plant NUE in this study. We aim to examine the following hypotheses: (1) Plants respond to kin recognition by increasing their NUE in nutrient poor soil based on the stress gradient hypothesis; (ii) Kin recognition could occur in nutrient rich soil by increasing the biomass growth while deducing root competition; and 3) Kin recognition responses of *Sorghum vulgare* differs under three types (Pb, Cd and Pb plus Cd) of heavy metal polluted conditions.

Material and methods

Seeds

Seeds of *S. vulgare* 'Luliang No. 1' were collected from a farm in the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China (IGDB, CAS). The seeds were collected from four mother plants that grew in the same plot about 10 m apart. Those mother plants were bagged before pollination to prevent pollens from others. The seeds collected from the same mother (siblings) were referred to kin, while those from different mothers were regarded as strangers.

Treatments

A three-factor design including neighbour's kinship (kin versus strangers), soil fertility (relatively poor versus rich) and heavy metal (Pb versus Cd) of S. vulgare was conducted (Fig. 1). For the kinship treatment, two seedlings either kin or strangers from four mother plants per cylinder pot were planted as kin and stranger pairs. The distance of the two seedlings was 8 cm and no barriers for root contact. The cylinder pots were 16 cm high and 16 cm in diameter; For the soil fertility treatment, two types of soil either relatively nutrient- rich or nutrient- poor soils were conducted for kin and stranger pairs. The soils were sterilized under high temperature and changed the fertility only by adding roseite to avoid pH changes. Composition and characteristics of the two soils were shown in Table 1. For the heavy metal treatment, we added either Pb or Cd in each soil fertility treatment. The addition mode including only adding Cd (Cd treatment) or Pb (Pb treatment), adding both Cd and Pb (Cd + Pb treatment). For heavy metal addition, the complex solution of Cd^{2+} and Pb^{2+} were prepared by dissolving appropriate quantities of Cd (NO₃)₂ •4 H₂O and PbCl₂ at pH 4.5. We then added 50 mg kg⁻¹ Cd (NO₃)₂ •4 H₂O in Cd treatment (Cd₅₀); 500 mg kg⁻¹ PbCl₂ in Pb treatment (Pb₅₀₀); 50 mg kg⁻¹ Cd (NO₃)₂ •4 H₂O and 500 mg kg⁻¹ PbCl₂ in Pb plus Cd treatment ($Pb_{500} + Cd_{50}$). This adding dosage of Pb_{500} and Cd_{50} were based on our preliminary experiment, which was conducted to measure biomass effect of S. vulgare under solely Pb, solely Cd, as well as combined pollutions of three concentrations. Based on the preliminary experiment results, we selected solely Pb 500, solely Cd 50, and combined concentration of Pb 500 plus Cd 50 for kin recognition experiment under heavy mental stress. This is because under those concentrations, the plant biomass significantly decreased compare to the no-adding (CK) groups, suggested obvious heavy mental stress. There were 12 replicates for each kin and stranger pairs under each soil fertility and heavy metal stress soil treatments. In total, there were 192 pots (12 replicates $\times 2$ kinship $\times 8$ soil condition treatments), and 384 seedlings (two seedlings in each pot) in the experiment.

Growth conditions

This study was conducted in the greenhouse of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China. On May 15, 2016, the seeds were germinated in petri dishes filled with deionized water and grown at 22°C during the day and 18°C during the night, with the photoperiod of 16 h light and 8 h dark (photosynthetic photon fluency rate 180 μ M m⁻² s⁻¹) and 70% relative humidity. After 6-day-old, healthy and similarly sized seedlings were selected to eliminate size bias, and then transplanted into pots. The pots were watered every 2 days to maintain soil moisture at 70% soil water holding capacity by weighing. The plants were not fertilized during the experiment.

Morphological traits and biomass measurements

When the plants growing about 3 months, plant height was measured directly with a rule. The stem elongation was calculated as the ratio of plant height to stem dry mass. Two medium-sized and fully expanded healthy leaves from each individual were measured leaf area using a scanner (Epson Perfection V700 Photo, Long Beach, CA, USA) to obtain the averaged leaf area. The specific leaf area (SLA) was calculated as the ratio of averaged leaf area to leaf dry mass. The roots were carefully washed, and their length was measured. Shoots and roots were harvested separately, dried in an oven at 65°C for 48 h, and weighed for biomass measurements. Allocation to leaf, stem and root was calculated as ratios of leaf mass, stem mass or root mass to plant total biomass, respectively.

Physiological measurements

After plants grew for about 70 days before heading, plant root activity, chlorophyll content, malondialdehyde (MDA) and proline concentration of siblings and strangers were measured by photocolorimetric method using a spectrophotometer. Root activity was determined by the triphenylte trazolium chloride (TTC) reduction method (Islam et al., 2007). Briefly, plant roots were clearly washed with deionized water, cut into pieces and reacted with 0.1 mM phosphate buffer solution (pH 7.0) and 0.4% TTC respectively for 1 h in 37 °C in test tubes. Adding 1 M H₂SO₄ for chromogenic and read the absorbance of which at 485 nm. The root activity was expressed by dehydrogenase activity. Plant leaves were ground in mortar and the Chlorophyll was extracted by acetone at 80%. Then, the absorbance of the extracted Chlorophyll was read at 645 and 663 nm. The content of chlorophyll was calculated according to the equation 20.2A₆₄₅ +8.02A₆₆₃ (Lichtenthaler et al. 1987). Plant MDA concentration was contracted with a mixture solution of 0.07% NaH₂PO₄•2H₂O, 1.6% Na₂HPO₄•12H₂O, 20% trichloroacetic acid containing 0.5% thiobarbituric acid under 100 °C for 30 min. The absorbance of the supernatant was read at 532 nm and 600 nm and the concentration of MDA was expressed as n mol g^{-1} DW (Heath & Packer 1968). The free-proline concentration was extracted by sulfosalicylic acid at 3% bathing at 100°C. The extract was then reacted with ninhydrin acid reagent (ninhydrin, phosphoric acid 6 M, glacial acetic acid 60%) and glacial acetic acid at 100°C, which then extracted by benzene (Irigoyen et al. 1992). The absorbance of the organic phase was measured at 520 nm and the proline concentration was expressed as ug g⁻¹.

Element concentration measurements

Element concentrations in plant were expressed on a mass basis, which are presented as concentrations per unit plant dry mass. The dried plant individuals were ground with a ball mill (MM2, Retsch, Haan, Germany). Ground plant materials were weighed to measure total N and S contents using Elementar Analysensysteme (Vario Max; GmbH, Hanau, Germany). Samples were weighed to determine P, K, Ca, Mg, and Na content according to the method described by Zarcinas et al. (1987). Concretely, the samples were placed in a 100 mL microwave jar and digested with 5 mL of nitric acid for 1 h, followed by the addition of 2 mL of hydrogen peroxide (H₂O₂). After the digestion, they were allowed to cool to room temperature and then transferred into 15-mL volumetric flasks; their volumes were adjusted by adding ultrapure water to equal levels. The total concentration of macro-elements in the digests was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) according to the method described by Fassel et al. (1978). The Cd and Pb content in plant tissues were determined by coupled plasma- mass spectrometry (ICP-MS) according to the method of Fassel et al (2008). All values were expressed in mg kg⁻¹ DW plant.

Calculations and Statistics

All data were analysed with SPSS16 (SPSS Inc., Chicago, IL, USA). After the normal distribution of the residuals and the homoscedasticity of the residual variance were tested, a three-way ANOVA was used to test the effects of relatedness, soil nutrient, heavy mental and their interactions for each indicator. Plant performance between siblings and strangers for each tested trait was compared between each fertility and heavy metal soils. All significance was considered at P < 0.05.

Results

S. vulgare living in nutrient-poor soil showed significantly lower biomass compared to plants in nutrient-rich soil. Individuals growing in two fertility soils had higher biomass than plants living in heavy metal soils (Table 2). Significant soil fertility, kinship, heavy metal, and the interactive effects were observed on measured phenotypic and physiological traits, the NUE of nutritive elements, and the Pb and Cd accumulations (Table 2; Fig. 2, 3, 4).

The SLA of kin was higher (P = 0.009) in CK of nutrient-poor soil, whereas lower than strangers (P = 0.048, Fig. 2b) in CK of nutrient-rich soil. Kin plants showed higher root activity than strangers in nutrient poor soil, compared to nutrientrich soil (Fig. 3b). Compare to nutrient-rich soil, kin siblings showed lower P (P = 0.014) and S (P = 0.007) concentrations in nutrient-poor soil than strangers. By comparison, higher N (P = 0.021) and Na (P = 0.040) concentrations of kin plants were observed than strangers in nutrient-rich soil compare to nutrient-poor soil (Fig. 4a).

Pb stress decreased stem elongation (Fig. 2a), MDA and proline contents (Fig. 3b), while increased K concentration (Fig. 4b) of kin plants compared to strangers in nutrient poor soil. When in nutrient rich soil, kin plants increased the stem elongation (Fig. 2a), the SLA (Fig. 2b) and root activity (Fig. 3b), but decreased chlorophyll content (Fig. 3a) and S concentration (Fig. 4b) compared to strangers.

Cd stress decreased total biomass (Fig. 2c), chlorophyll content (Fig. 3a), proline content of kin plants (Fig. 3d), while increased MDA content (Fig. 3c), concentrations of P and Cd (Fig. 4c) compared to strangers in nutrient poor soil. In nutrient rich soil, kin plants increased the SLA (Fig. 2b), root activity (Fig. 3b), MDA content (Fig. 3c), S and Cd concentrations (Fig. 4c) compared to strangers.

Compared to strangers, kin plants produced higher total biomass (Fig. 2c), while lower P concentration (Fig. 4d) in nutrient poor soil under Pb plus Cd stress. In nutrient-rich soil, kin plants exhibited lower stem elongation (Fig. 2a), lower proline (Fig. 3d), as well as lower Pb and Cd concentration (Fig. 4d) than strangers.

Discussion

More and more studies have confirmed that kin recognition can occur in nature, but morphological and fitness-based studies often demonstrate equivocal conclusions (File et al. 2012). The discrepancy could be caused by soil conditions (Palmer et al. 2016). Here, we provide sound evidence for the effect of soil fertility and soil pollution (Pb_{500} or/ and Cd_{50}) on kin recognition. The decreased plant biomass, chlorophyll content (related to photosynthesis) and root activity (related to belowground absorption), together with increased MDA and proline content in heavy metal soils compared to no-adding heavy metal soils suggested obvious heavy metal stress of *S. vulgare* (Carlson & Bazzaz 1977; Islam et al. 2007; Zhang et al. 2008). This allowed us to evaluate how soil fertility interacts with heavy metal pollution to

affect kin recognition of *S. vulgare* through measuring phenotypic traits and biomass (Murphy & Dudley 2009; Biedrzycki et al. 2010; Biernaskie 2011), as well as nutrient utilization.

Numerous studies have shown that kin recognition can be reflected by root changes because roots are involved in soil resource capture (Dudley & File 2007; Bhatt & Dudley 2010; Biernaskie 2011; Caffaro et al. 2011). Root interaction can increase plant fitness of a kin competitor because this process may maximize resource acquisition but reduce the resource supply to the non-kin competitors (Fransen et al. 2001). A previous study also found that *S. vulgare* showed kin recognition through decreased root allocation when growing with siblings compare to strangers (Zhang et al. 2016). However, in our study we did not observe kin response neither on root biomass nor on root length in each soil conditions. This is mainly own to the root space effect. Compare to field growing in the experiment of Zhang et al. which allows root expansion to be the sensitive and powerful competitive trait in plants interactions, while pots growing in our study forms a relatively limited root space, which may confined the root maximize growth strategy (Schenk 2006) and lacks of root response (Masclaux et al. 2010).

Even if no phenotypic response, roots could mediate kin recognition through altering their acquisition of soil nutrients (Zhang et al. 2016). Concentrations of essential elements (e.g. N, P, S, K, Ca, Mg and Na) on the basis of plant dry mass could represent NUE of annual plants (Shaver & Melillo 1984; Birk & Vitousek 1986). High element concentration indicates low plant NUE. We find that element concentrations in *S. vulgare* were dependent on soil fertility and heavy metal stress but varied between kin and strangers. For example, concentrations of P and S in kin siblings are significantly lower than in strangers, denotes that kin siblings increased the NUE of P and S in nutrient-poor soil. In contrast, higher N and Na concentrations in kin siblings than strangers indicate the decrease in the NUE for N and Na in nutrient-rich soil. This corroborates our first hypothesis that the NUE of soil nutritive elements could be potential indicators of plant kin interactions because they mediate plant growth and competitive performances (Maestre et al. 2009). However, the NUE

response of kin interaction varied on elements. This may be due to different roles of nutrition elements and their distinct thresholds in plant growth (Marrs et al. 1983; Myster & Fernández 1995; Myster 2006). As a result, kin recognition responses are related with soil conditions (Marrs et al., 1983; Myster and Fernández, 1995; Myster, 2006). Previous studies showed that limited N supply might lead to stronger N competition (Hodge et al. 2002) and affect the vegetative growth (Weiner, 2001; Biedrzycki and Bais, 2010). It is contrary in excess N conditions (Keddy, 1989; Wilson and Tilman, 1993). Moreover, the contents of soil elements are different. Based on the Liebig's law of minimum, the elements with lower thresholds in high fertility soil are more sensitive and more likely to produce kin recognition responses. However, this needs further investigations, and plant NUE observed from element concentrations in plants can be a powerful tool to examine kin recognition as plant functional trait.

Kin recognition of S. vulgare was modulated by soil fertility (Fig. 4 a, b). It is suggested that kin plants usually increase root allocation to capture more nutrients particularly in nutrient-poor soil (Robinson et al. 1999; Gersani et al. 2001; O'Brien et al. 2005; Dudley & File 2007; Mommer et al. 2011; File et al. 2012). However, in our study, kin recognition and competition co-occurred for S. vulgare, being dependent on soil fertility. In nutrient-poor soil, higher SLA of kin plants indicates increased mutual shading than strangers in aboveground (Ballaré et al. 1994; Griffith and Sultan 2005; Lepik et al. 2012). Meanwhile, decreased root activity of kin plants suggested lower root uptake ability, leading to less P and S uptake compared to strangers. However, plants grown with kin were able to use P and S more efficiently and achieved the same biomass and reproductive output as plant grown with strangers, despite presumed more intense competition for nutrients among kin according to niche partitioning theory. Therefore, plants benefit their kin though increased their NUE and do not reduce root allocation to ensure the nutrient supply to above ground parts under limited soil nutrients. On the contrary, kin benefit interactions in nutrient-rich soils were more pronounced - kin groups achieved significantly higher biomass production than stranger groups. Besides, lower SLA of siblings reflects decreased plant mutual shading compared to strangers. This suggests kin recognition through decreased aboveground competitive ability and increased biomass of siblings (Biedrzycki et al. 2010; Biernaskie 2011). However, increased N and Na concentrations in kin plants indicate lower NUE than strangers. This could be ascribed to plant growth strategy of reducing the investment belowground to produce more biomass for potential reproduction of annual plants in nutrient-rich soil (Weiner et al. 2001), instead of increasing nutrient use efficiency, compared to in nutrient-poor soil. In this case, even kin groups reduced NUE, but it did not compromise their biomass. Moreover, although the reduced NUE of N and Na in kin plants compare to strangers, the contents of which in plant body were significantly higher in kin than stranger groups. This indicates that it is not necessary for plants to increase their NUE to achieve kin recognition in nutrient-rich soils compared to nutrient-poor soil conditions. This is reasonable for our results showing that kin plants performed better in plant vegetative biomass in nutrient-rich than in nutrient-poor soil due to their stronger aboveground response. Concretely, kin plants managed to produce more biomass with low NUE through decreasing their SLA and thus less leaf overlap and shading, leading to increased efficient light capture (Semchenko et al., 2017). Therefore, these findings support our first hypothesis that plants respond to kin recognition by increasing their NUE in nutrient- poor soil, while by increasing efficient light capture of aboveground in nutrient- rich soil, respectively. Based on the above, our results denote that whether/how plants respond to kin recognition could result from their successfully integrated information regarding both neighbor identity and nutrient availability (Cahill et al. 2010).

Except soil fertility, kin recognition responded variously in different types of heavy metal stress (Fig. 4). Under Pb stress alone, kin benefit was expressed by decreased physiological stress e.g. MDA and proline (Wang et al. 2008; Chen et al. 2016; Jones &Pierce 2013), but it co-occurred with decreased the NUE of K element in nutrient- poor soil (Fig. 4 c). Increased stem elongation of plant is regarded as an initiative avoiding action to get more lights when sensed mutual shading. Thus, decreased stem elongation of kin plants indicates less mutual shading compare to

strangers. In nutrient- rich soil, kin benefit was reflected in belowground by increasing efficiency of water and nutrient absorption e.g. root activity (Islam et al., 2007) and the NUE of S nutrient, but it co-occurred with increasing of aboveground competitive ability for light and space (increased SLA). This increased competitive ability may result from more mutual shading (increased stem elongation), which leads to less chlorophyll content and then lower light photosynthesis efficiency of kin plants compared to strangers (Fig. 4 d). In solely Cd soil, compared to the strangers, significant decrease in biomass and chlorophyll suggest lower growth performance of kin plants, which may resulted from lower NUE of P and higher accumulation of Cd in plant body in nutrient- poor soil. Moreover, higher Cd accumulation leads to more MDA production (Esfandiari et al., 2007), suggesting severer cell peroxide damage of kin plants. However, kin plants produced less proline, indicating less amino acid metabolism damage (Jones & Pierce 2013) than strangers (Fig. 4 e). In nutrient- rich soil, compare to strangers, the root activity increased, consistently increased the Cd concentration and then the MDA content of kin plants. Moreover, increased SLA and S concentration of kin indicate decreased resource capture or use efficiency of light and S nutrient (O'Brien et al. 2005). Kin plants bear severer physiological stress, which affects the further growth of plants and finally exhibit biomass effect as shown in poor nutrient soil (Dakora & Phillips 2002) (Fig. 4 f). As to Pb plus Cd stress, kin superiority occurred through increased biomass growth and the NUE of P element in nutrient- poor soil (Fig. 4 g). In nutrient- rich soil, decreased aboveground competitive ability (decreased stem elongation), the Pb and Cd concentration, along with the proline content were decreased, which indicate potential kin benefit (Fig. 4 h).

This gradient of Pb (Pb₅₀₀) and Cd (Cd₅₀) stress leads to different kin interaction results of *S. vulgare*. Both kin benefit and negative effect are found among kin plants under Pb stress alone, while Cd stress alone suggests a negative kin response. However, the Pb plus Cd stress leads to obvious and potential kin benefit. We found kin effect of Pb poison did not lead to biomass response, for the similar Pb accumulation of kin and strangers in solely Pb soils. In contrast, the negative kin

effect on biomass and physiological poison could due to high toxicity of more Cd accumulated of kin plants than strangers in solely Cd soils. It is commonly suggesting a synergetic effect on plant of Pb and Cd in relatively high concentrations. However, we did not observe this kin synergetic effect in Pb plus Cd soils, the mechanism of positive kin effect may due to less Cd concentrations, this may result from Cd dilutive effect by existed Pb in soil and increased Cd concentrations in plant body (Masaharu et al. 2008; Salazar et al. 2012). This thereby is mainly thought to be a rescue behavior for less biomass loss of kin plants under Pb plus Cd stress. Moreover, for plant growth, this Pb and Cd treatments factually form a stress gradient based on plant biomass and physiological performances. Although no obvious biomass difference was observed, higher chlorophyll, root activity and proline content indicate more amino acid metabolism in disorder (Jones &Pierce 2013), while less MDA content indicate less membrane lipid peroxidation damage on the cell membrane (Wang et al. 2008) suggests less physiological stress in plants living under solely Pb than solely Cd stress.

In summary, the plant NUE for observed elements and physiological responses between kin and strangers are complementary indicators of kin interaction. We conclude that kin recognition of *S. vulgare* is dependent on soil fertility and heavy metal stress. Kin benefit in nutrient-poor soil was achieved by increasing the NUE of light and soil nutrient. In nutrient-rich soil, kin benefit was achieved by decreasing mutual shading, while co-occurred competition was represented by decreasing efficiency use of soil nutrients. Pb sress alone enables co-occur of kin recognition and competition, while Cd stress alone suggests a negative kin response. The Pb plus Cd stress supports kin recognition of *S. vulgare*. These findings suggest that plant NUE for specific elements, combined with morphological and physiological performance, can be used as sensitive response indicators for kin recognition in complicated soil conditions.

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Droporty	Rich		Poor			
Property	Mean	SD	Mean	SD		
pH (H ₂ O)	6.8	0.241	6.5	0.111		
Total C (g kg ^{-1})	12.46	1.162	6.11	2.019		
Total N (g kg ⁻¹)	2.24	0.098	1.02	0.075		
Available N (g kg ⁻¹)	0.142	0.114	0.085	0.233		
Total S (g kg ⁻¹)	1.365	1.022	0.680	1.020		
Total P (g kg ^{-1})	0.262	0.175	0.129	0.171		
Available P (g kg ^{-1})	0.022	0.159	0.017	0.236		
Total K (g kg ⁻¹)	8.145	0.286	3.702	0.284		
Available K (g kg ^{-1})	0.109	0. 540	0.055	0.099		
Total Ca (g kg ⁻¹)	1.749	0.124	0.871	0.126		
Total Mg (g kg ⁻¹)	2.182	0.531	1.122	0.532		
Total Na (g kg ⁻¹)	0.158	0.147	0.058	0.140		
Total Pb (mg kg ⁻¹)	23.14	0.424	10.99	0.123		
Total Cd (mg kg ⁻¹)	0.135	0.019	0.061	0.008		

Table 1 Properties of soil in the study (n=10)

	df	Soil	Kinshi	Heavy S*K		с * П	V * U	С* К *П	
Effect			р	metal	2 K	5 п	К'П	9.K.U	
		1	1	2	3	5	5	11	
Stem	F	35.33	3.62	5.063	6.235	10.236	8.236	15.225	
elongation	Р	< 0.001	0.471	0.203	0.189	0.015	0.031	0.009	
ST A	F	12.606	11.068	11.002	6.505	5.332	10.330	23.652	
SLA	Р	0.001	0.018	0.020	0.001	0.110	0.025	0.031	
Doot langth	F	15.327	4.446	3.958	8.232	5.260	4.448	2.844	
Koot lengui	Р	0.008	1.152	1.526	0.128	0.257	0.320	1.003	
Plant total	F	40.118	0.175	5.009	4.350	14.199	3.001	13.700	
biomass	Р	< 0.001	0.298	0.130	0.664	0.010	0.998	0.022	
Chlorophyll	F	1.23	7.775	1.785	8.252	2.001	1.869	36.313	
content	Р	0.253	0.021	0.192	0.018	0.195	0.255	< 0.001	
Root activity	F	6.326	1.236	4.210	3.022	3.965	4.880	8.032	
	Р	< 0.001	1.112	0.009	0.015	0.011	0.005	< 0.001	
MDA	F	4.789	18.369	3.189	19.023	25.010	22.100	39.988	
content	Р	0.226	0.046	0.293	0.040	0.032	0.038	0.025	
Proline	F	6.998	8.025	7.023	8.023	9.005	3.123	10.995	
content	Р	0.044	0.012	0.035	0.029	0.014	0.122	0.010	
Ν	F	0.616	6.236	0.823	0.786	5.110	0.286	5.963	
concentration	Р	0.149	0.035	0.368	0.137	0.047	1.006	0.041	
Р	F	3.595	0.599	1.637	2.008	2.782	5.534	2.536	
concentration	Р	0.029	0.442	0.198	0.041	0.066	0.005	0.083	
S	F	4.927	1.110	4.623	3.404	3.312	1.520	6.536	
concentration	Р	0.032	0.1 24	0.038	0.045	0.050	0.103	0.006	
К	F	6.909	0.040	0.558	1.611	0.347	1.03	1.612	
concentration	P	0.011	0.842	0.574	0.099	0.707	0.36	0.203	
Ca	F	3.030	3.031	1.546	2.322	0.44	1.985	0.415	
concentration	Р	0.059	0.121	0.217	0.096	0.645	0.141	0.661	
Mg	F	1.770	0.179	0.002	2.404	0.319	1.172	0.97	
concentration	Р	0.255	0.467	0.998	0.123	0.727	0.313	0.382	
Na	F	5.968	2.380	4.052	3.830	6.476	0.561	0.89	
concentration	Р	0.023	0.128	0.02	0.039	0.002	0.572	0.413	
Pb	F	5.422	0.526	8.250	1.322	4.522	1.036	4.785	
concentration	Р	0.026	0.152	0.002	0.088	0.033	0.211	0.030	
Cd	F	2.236	0.380	1.985	0.830	0.752	0.666	1.580.	
concentration	Р	0.010	0.210	0.028	0.100	0.121	0.138	0.021	

Table 2 ANOVA results of the effects of siblings, soil conditions and their interactionson plant morphological traits and subarea biomass. Number in bold indicates that theeffect was significantly different between kin and strangers at a P < 0.05 level.

Adding Cd ²⁺ and/or Pb ²⁺				Kinship (Kin or Strangers)								
Ric						Kin				Stranger		
0L						А	А	А	А	Α	А	V 16
oor	R+Cd	R+Pb	R+Cd+Pb	R		А	А	А	В	C	D	16 cm
y (I				-		→ B	В	В	В	В	С	
rtilit						В	В	В	С	D	D	
l fe	P+Cd	P+Pb	P+Cd+Pb	Р		С	С	С	А	Α	А	
Soi					С	С	С	В	C	D		
R: R	R: Rich nutrients P: Poor nutrients				D	D	D	В	В	С		
R+Cd: Rich nutrients adding Cd ²⁺				D	D	D	D	D	D			
R+Pb: Rich nutrients adding Pb^{2+}												
R+Cd+Pb: Rich nutrients adding Cd^{2+} and Pb^{2+}				AA/ BB/ CC/ DD- Kin (Siblings)								
P+Cd: Poor nutrients adding Cd ²⁺				AB/ AB/ AC/ AD/ BC/ BD/ CD- Strangers								
P+Pb: Poor nutrients adding Pb ²⁺				A, B, C, D- Seedings from different mother								
P+Cd+Pb: Poor nutrients adding Cd ²⁺ and Pb ²⁺												

Fig. 1 Experiment design of three-factors including kinship (kin versus strangers), soil fertility (relatively poor versus rich) and heavy metal (Pb, Cd, Pb plus Cd) of *S. vulgare*.



Fig. 2 The means \pm SE (24 replications) of plant traits including (a) stem elongation, (b) SLA, (c) root length, (d) total biomass of kin and strangers in each soil fertility and heavy metal treatment are presented as scatter or bar graphs, respectively. The different letters on the scatter bars indicate the significant difference between the kin and strangers in each treatment (P < 0.05).



Fig. 3 Chlorophyll content (a), root activity (b), MDA (c) and proline (d) concentration between kin and strangers under CK, Pb treatment, Cd treatment and compound heavy metal (Pb + Cd) treatment in poor and rich soils. The means \pm SE of each attribute are presented as bar graphs. The different letters on the bars indicate the significant difference between the means of kin and strangers in corresponding soil treatment(s) (*P* <0.05)



Fig. 3 Plant N, P, S, K, Ca, Mg, Na, Pb and Cd concentrations in nutrient-poor and nutrient-rich soil (a), and the concentrations of each element of kin and strangers in Pb treatment (b) and of that in Cd treatment (c), and of that in Pb+Cd treatment, respectively. Because the large variation among means of the elements, we draw all the elements of each treatment in one radar-chart using two types of units. The coordinate units of N, P, S, K, Ca, Mg and Na were g kg⁻¹, the coordinate units of Pb and Cd were mg kg⁻¹. The logarithm means of each attribute of 12 replications are presented in the corresponding coordinates of radar chart. The asterisk (*) on the coordinates indicate the significant difference between the kin and strangers (P < 0. 05).



Fig. 4 Plant kin recognition mechanisms of *S. vulgare* in nutrient-poor soil (a) and nutrient-rich soil (b), Pb stress in nutrient-poor soil (c) and of that in nutrient-rich soil (d), Cd stress in nutrient-poor soil (d) and of that in nutrient-rich soil (e), Cd stress in nutrient-poor soil (e) and of that in nutrient-rich soil (f), as well as Pb+Cd stress in nutrient-poor soil (g) and of that in nutrient-rich soil (h). The dots with different colour on behalf of different elements. Blue dots: phosphorus (P); Brown dots: Sulphur (S); Green pots: Nitrogen (N); Pink dots: sodium (Na); Yellow pots: cadmium (Ca); Violet pots: Lead (Pb).