Kin selection or resource partitioning for growing with siblings: implications from measurements of nitrogen uptake

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Abstract

Aims Kin selection and resource partitioning have been proposed to explain interactions between plants growing with siblings (from the same mother). These mechanisms have been examined by measurements of fitness, phenotype or allocation traits, but have seldom been tested with N acquisition traits.

Methods We determine if kin selection and resource partitioning are occurring using two annual species (*Sorghum vulgare* and *Glycine max*) with a short-term ^{15}N experiment. A mixture of ammonium, nitrate and glycine (1:1:1) was injected into soils around plants after they grew for 47 days. Only one nitrogen (N) form was ^{15}N labeled in each labeling solution.

Results *S. vulgare* increased root allocation when growing with strangers (from the different mother), but not

increase their N uptake. Although *G. max* strangers did not increase their root allocation, they significantly increased uptake of total N and the most abundant N form (nitrate) and decreased uptake of the least abundant (glycine).

Conclusions *G. max* siblings reduced competition due to chemical resource partitioning while *S. vulgare* showed kin selection. We concluded that processes related to kin selection and resource partitioning can occur simultaneously, resulting in different competitive ability. These findings can improve our understanding of plants growing with siblings or strangers.

Keywords Sibling competition · Kin recognition · Kin selection · Resource partitioning · Competitive ability

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Introduction

Plant competition strongly affects individual performance, population evolution and ecosystem structure and function. Plants compete for nutrients to meet their demands, and this competition occurs both within and between species (File et al. 2011). Many plant species are likely to grow close to siblings due to their limited dispersal distance and siblings are indeed experiencing competition (Masclaux et al. 2010). However, it remains unknown how these siblings growing together compete (Cheplick 1992) for available nutrients from soils. Clarifying this question will be beneficial for a better understanding of the consequence of its evolutionary and ecological significance (File et al. 2011; Simonsen et al. 2014).

Numerous studies have suggested that plants have various foraging strategies to access patchy resources more efficiently, e.g., spreading their roots through different soil layers to maximize access to resources (Cahill et al. 2010) or associating with microbial symbionts to access different forms of nutrients (Reynolds et al. 2003; Bever et al. 2010; Simonsen et al. 2014). Such strategies have been observed for interspecific pairings which often reduce the competition through consuming different nitrogen (N) forms (McKane et al. 2002; Xu et al. 2011). This is called “resource partitioning” (Cheplick and Kane 2004). Fierce competition should occur within species because they have more niche overlap between species (Murphy and Dudley 2009).

However, recent studies have shown that the competition between plants is more complicated than previously thought (Chu et al. 2008; Milla et al. 2009). Dudley and File (2007) demonstrate that *Cakile edentula* increases allocation to roots to increase competition when two individuals are strangers (from different mother plants of the same species), but not when they are siblings (from the same mother plants). Further studies about the competitive traits such as biomass allocation or fitness showed that kin recognition can occur in many plant species and facilitate growth of nearby siblings (Murphy and Dudley 2009; Biedrzycki and Bais 2010; Biedrzycki et al. 2010; Masclaux et al. 2010; Bhatt et al. 2011; Simonsen et al. 2014). Such kin recognition is assessed by the theory of kin selection based on Hamiltons’ rule: $r \cdot b > c$; r is the relatedness of the individuals, b is the benefit, and c is the cost of the altruistic trait expression (Hamilton 1964).

Initially, many studies have measured effects of growing with siblings or strangers on fitness (Cheplick and Kane 2004; Milla et al. 2009) to understand mechanisms of kin selection and resource partitioning. Subsequently, some studies have investigated effects of growth with siblings or strangers on morphological and allocation traits (Dudley and File 2007; Murphy and Dudley 2009; Biedrzycki et al. 2010). For example, plants growing with strangers increase allocation to roots to increase competition (Dudley and File 2007). When *Arabidopsis thaliana* growing with strangers, they produce more lateral roots and thus change root structure (Biedrzycki et al. 2010). Further study showed that root exudates mediate kin recognition through unknown signals (Biedrzycki et al. 2010). Recently, competitive ability was used to explain the siblings growing together (Masclaux et al. 2010; File et al. 2011).

However, no one has examined effects on N-acquisition traits of growth with siblings or strangers. Such investigations will improve our understanding of plant interactions in plant communities.

Most previous studies determined the consequences of the fitness to identify kin selection and resource partitioning as potential driving forces for plants growing with siblings (File et al. 2011). In this study, we consider a new index in a resource context to evaluate the competition derived from root interactions. Of various nutrients, N is a major limiting one for plant growth in most terrestrial ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008). The amounts and forms of available soil N depend on the balance of N loss and N retention, which strongly influence the competition between plants. Under N-limited conditions, plants have to compete strongly for available N for survival. Numerous studies have suggested that N partitioning contributes to species coexistence in grasslands (McKane et al. 2002; Xu et al. 2011; Gao et al. 2014). Therefore, N is an ideal nutrient used to examine root interactions when the same species plants grow together. According to the resource theory, the niches of relatives have more overlap in sibling groups than in stranger groups. As a result, stranger groups should outperform sibling groups. To test this, we grew two annual species *Sorghum vulgare* and *Glycine max* in pair-wise experiments under field condition with the presence of neighbors that are siblings or strangers. After growing for 47 days, we labeled these plants with ^{15}N tracers to quantify their N uptake. We aimed to test the following hypotheses: If kin selection occurs, siblings will outperform stranger groups. Otherwise, if strangers and siblings prefer different N forms, it will support resource-partitioning hypothesis.

Material and methods

Site

This study was conducted at the State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, located in northwestern Hebei province (40°N, 115°E). Annual precipitation averaged 370 mm and the mean annual temperature was 9.6 °C. The average temperature and precipitation in August was about 24.4 and 107.5 mm respectively. Soil

characteristics of the top 15 cm at this site are shown in Table 1.

Seeds

Seeds of *G. max* and *S. vulgare* were collected from a farm in Shandong Province. The cultivars used for *G. max* and *S. vulgare* were Ludou No. 2 and Luliang No. 1, respectively. Seeds for each species were collected from two individuals of the same cultivar, which grew in the same plot with a distance of about three meters. Seeds from the same individual shared the same mother, while those from different individuals were from different mothers.

Treatments

Treatments for *G. max* and *S. vulgare* were as follows: The groups were either siblings (from the same mother plant) or strangers (from different mother plants). Paired micro-plots were used to evaluate siblings grown together, but there were no barriers to root growth. Paired micro-plots for the sibling treatments received two seeds from the same mother. Paired micro-plots for the stranger treatments received two seeds from different mothers. All the seeds were planted on July 25, 2013, and they were then maintained by periodic weeding and watering. Some seedlings were removed by hand, leaving only a single seedling in a micro-plot. These paired micro-plots with 10 cm distance between two individuals were micro-units (Fig. 1). These micro-units were divided into four groups for three N forms and the control, with 5 and 8 replicates for *G. max* and *S. vulgare*, respectively.

Table 1 Properties in the top 15 cm soil at the study site ($n=16$ to 18)

Property	Mean	Standard deviation
pH	8.80	0.14
Soil organic C (g kg^{-1})	6.82	1.16
Total N (g kg^{-1})	1.42	0.00
$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	1.71	0.23
$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	6.08	2.45
Glycine-N (mg kg^{-1})	0.55	0.08

Labeling

After plants grew for 47 days, they were labeled with K^{15}NO_3 (99.19 atom% ^{15}N enrichment), $(^{15}\text{NH}_4)_2\text{SO}_4$ (99.14 atom% ^{15}N enrichment) or glycine (99.04 atom% ^{15}N enrichment). All three tracer solutions include ammonium, nitrate and glycine (0.0625 g L^{-1} for each N form), but only one form was ^{15}N labeled. Eight mL of tracer solution was injected into 5 cm depth at points around plants and finally each micro-plot received 1.5 mg total N. To ensure the tracer equal distribution, four points with 5 cm from the plant stems were injected with 2 mL solution. The ^{15}N solutions were added as uniformly as possible horizontally and vertically.

Sampling

One hour after ^{15}N injection, plants and soils were harvested from each micro-plot. Shoots and roots were washed carefully with distilled water and then dried at 65°C for 48 h for biomass. After then, they were ground with ball mill (MM2, Retsch, Haan, Germany) for measurements of N contents and $^{15}\text{N}/^{14}\text{N}$. Fresh soil samples were immediately brought to the laboratory and sieved to through 2 mm and stored at 4°C for measurements of available N within 2 days.

Analysis

The N contents and $^{15}\text{N}/^{14}\text{N}$ for plant materials were determined on an isotope ratio mass spectrometry (MAT253, Finnigan MAT, Bremen, Germany) connected to an Elemental analyzer (EA 1112, CE Instruments, Milan, Italy). A pH analyzer was used to measure the supernatant with dry soil-water ratio of 1:2. Total soil N was measured by dry combustion (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany). Soil organic C was measured by the dichromate digestion method (Kalembasa and Jenkinson 1973). Fifteen grams of fresh soil was extracted with 60 mL 0.05 M K_2SO_4 for 1 h at 70 rev/s using a shaker. Soil NH_4^+ and NO_3^- in extracts was measured on an auto analyzer (AA3, Bran-Luebbe, Germany). Soil glycine concentrations were measured by high-performance liquid chromatography (Waters 515, Waters Inc., USA) on the same extracts (Näsholm et al. 1987).

Fig. 1 Rectangle micro-units were used as an experiment design for *Glycine max* and *Sorghum vulgare* growing with a sibling or a stranger in the field. The different plant shapes represent different

siblings. Lines of these micro-units in the figure only indicate the distance between two plant individuals for different combinations, not the barriers for root growing

Calculation and statistics

Amount of N uptake by plant was calculated multiplying N% by atom percentage excess (APE) and total biomass. Nitrogen uptake rate was calculated dividing the amount of N uptake amount by time and root biomass. APE is the ^{15}N difference between ^{15}N -treated and control plants. The plant N uptake ($U_{\text{unlabeled}}$) was estimated considering the native concentrations of corresponding N forms as follows: $U_{\text{unlabeled}} = U_{\text{labeled}} * (m_{\text{unlabeled}}/m_{\text{labeled}})$, where U_{labeled} is the quantity of ^{15}N per plant uptake from the soil, $m_{\text{unlabeled}}$ is the native content of corresponding N form in control treatments, extracting with 0.05 M K_2SO_4 , and m_{labeled} is the total mass of injected ^{15}N to plants (McKane et al. 2002; Xu et al. 2011). The contribution of each N form was estimated as its percentage of total uptake for all three N forms.

Biomass was always normally distributed and thus not transformed. T-tests were used to compare behavior of plants competing with siblings or strangers on aboveground- and belowground- biomass. Three-way ANOVA was used to test the effect of species (*G. max* and *S. vulgare*), kin (sibling and stranger), N forms (glycine, ammonium, and nitrate), and their interactions on plant N uptake. Also, t-tests were performed to compare the effect of sibling or stranger groups to plant total N uptake in a specific species. All statistic analysis was performed with SPSS 16 (SPSS Inc., Chicago, IL, USA) and considered significant at the $P < 0.05$ level.

Results

Biomass

Whether grown with a sibling or a stranger, both *G. max* and *S. vulgare* did not show significant differences in aboveground biomass. Belowground biomass was not different between sibling and stranger groups for *G. max*, but *S. vulgare* growing with a sibling showed significantly lower belowground biomass than with a stranger (Fig. 2).

Plant N uptake

There was a significant effect of N form on plant N uptake, but there was no species effect. Among three N forms, NO_3^- -N was the dominant N resource, NH_4^+ -N was the second and glycine-N was the least (Table 1). The effect of sibling on plant N uptake tended to be significant, with higher uptake for stranger groups than for siblings. Interactions between siblings and N form significantly affected plant N uptake. By comparison, the interactive effect of species, sibling, and N form showed a marginally significant effect on N uptake ($P = 0.08$, Table 2). *G. max* and *S. vulgare* not only acquired NH_4^+ and NO_3^- , but also took up organic N in the form of glycine. Both crops took up more NO_3^- than glycine or NH_4^+ , with higher NH_4^+ than glycine uptake (Fig. 3).

NH_4^+ uptake was not significantly different between sibling and stranger groups for *G. max* and *S. vulgare* (Figs. 3 and 4). As to the uptake of glycine and NO_3^- ,

Fig. 2 Above- and below-ground biomass when *Glycine max* and *Sorghum vulgare* growing with a kin or a stranger. The values were mean $\pm$ SE of five to eight replicates. Different letters under the bars indicate the significant difference between sibling and stranger treatments for either of species

sibling and stranger groups differed significantly for *G. max*. Siblings had higher uptake of glycine while stranger showed higher uptake of NO_3^- . Compared to sibling treatments, stranger increased total N uptake for *G. max*. However, such differences were not observed for *S. vulgare* (Figs. 3 and 4).

Among three N forms, NO_3^- -N made the greatest contribution to total N uptake for *G. max* and *S. vulgare*

Fig. 3 Uptake of three N forms as well as total N uptake for *Glycine max* and *Sorghum vulgare* growing with a kin or a stranger. The values were mean $\pm$ SE of five to eight replicates. Different letters above the bars indicate a significant difference between sibling and stranger treatments within an N form

(Fig. 5). Sibling and stranger groups for *G. max* showed significant niche shifts for glycine (Fig. 5).

Table 2 ANOVA results of the effects of species, siblings, N forms and their interactions on plant N uptake

Effect	df	F	Sig.
Species	1	1.488	0.23
Kin	1	2.74	0.10
N form	2	88.31	<0.001
Species * Siblings	1	2.28	0.13
Species * N form	2	1.20	0.30
Siblings * N form	2	3.47	0.03
Species* Siblings * N form	2	2.59	0.08
Error	127		
Total	139		
Corrected Total	138		

Number in bold indicates that the effect was significantly different at a $P < 0.05$ level

Discussion

Kin selection and resource partitioning have been proposed to explain interactions between plants growing with siblings. These mechanisms have been extensively examined by measurements of biomass allocation and fitness. Here we tested biomass allocation and the competition for nutrients to improve our understanding of plant interactions in plant communities. We first demonstrated that *G. max* and *S. vulgare* have the capacity to take up organic N in form of glycine, but at very low rates compared to inorganic N (Fig. 3). A possible explanation is that glycine availability was too low in our soil. NO_3^- uptake contributes 80 % to total N uptake. This clearly reflects that two agricultural species prefer NO_3^- over NH_4^+ /glycine in the investigated soil.

Fig. 4 Uptake rates (mean $\pm$ SE) of three N forms as well as of total N for *Glycine max* and *Sorghum vulgare* growing with a sibling or a stranger. Different letters above the bars indicate a significant difference between sibling and strange treatments for a single N form

This could be ascribed to high soil pH of 8.8, because previous studies showed that plants prefer NO_3^- rather than NH_4^+ in high pH soil. Another reason is that the investigated soil had high gas diffusion and could lead

to strong nitrification and thus produced more nitrate (Zhang et al. 2013). Under this condition, plants could prefer the dominant N form.

Furthermore, we examined the theories of kin selection and resource partitioning invoked for interactions between siblings growing together within a nutrient context. On the basis of biomass allocation, *S. vulgare* was observed to increase root allocation in response to strangers compared with siblings. This indicates that kin recognition occurs among *S. vulgare* siblings, supporting the kin selection theory (Dudley and File 2007, 2008; Murphy and Dudley 2009). Related individuals will behave more altruistically based on signals of root secretions (Dudley and File 2007; Biedrzycki et al. 2010). In contrast, *G. max* did not demonstrate kin recognition based on biomass allocation. This reflects that it may be not universal among self-incompatible plants (Masclaux et al. 2010), but this needs further investigation. Another explanation is that increased root biomass might be not necessary for kin recognition (Dudley and File 2008), e.g., *Impatiens pallid*, a shade plant, increases aboveground allocation to acquire more light when growing with strangers (Murphy and Dudley 2009). In this study, we did not observe increased aboveground allocation for *G. max*.

According to N uptake data, we observed a completely different pattern as suggested by biomass allocation. Numerous studies have suggested that greater root allocation should enhance belowground competitive ability (Dudley and File 2007). In this study, when grown with strangers, *S. vulgare* did not enhance their N uptake (Figs. 3 and 4), although it increased root allocation (Fig. 2). A possible explanation for this foraging

Fig. 5 Chemical niche shifts for *Glycine max* and *Sorghum vulgare* when growing with a kin or a stranger. The axes of x, y and z represent the contribution of ammonium, nitrate and glycine

to total N uptake (as %). Asterisks indicate significant difference ($P < 0.05$ level) between sibling and strangers

strategy (Chu et al. 2008; Cahill et al. 2010) is that plants increased root allocation through root elongation or larger overall surface area to occupy larger soil volumes (Morgan and Connolly 2013). For *G. max*, although increased root allocation was not observed when growing with strangers, they significantly increased total N uptake as well as the uptake of the dominant N (i.e., NO_3^-) and decreased the uptake of the less abundant N (i.e., glycine) (Figs. 3 and 4). When growing with siblings, *G. max* showed niche shift, increasing uptake of glycine but strongly decreasing uptake of NO_3^- . This kinship decreases total N uptake due to more niche overlap (Fig. 5), but also reflects resource partitioning between strangers and siblings (Cheplick and Kane 2004). We also suggest that kinship strongly decreases competition for dominant N through cooperation, while strangers strongly compete for the dominant N form (Figs. 3 and 4). However, two theories (i.e., kin selection and resource partitioning) cannot fully explain the cooperation and competition (Chu et al. 2008; Biernaskie 2011) between the siblings of these two crop species.

We found that *G. max* siblings did not exhibit kin recognition based on biomass allocation, but they significantly altered their competition for available N. Although *S. vulgare* invested more biomass to roots, this cost was not paid for enhancing the competition for available N. We suggest that this cost may be paid for space or water usage, but this requires further investigations. Masclaux et al. (2010) found biomass difference occurred when growing with different accessions, showing the interaction outcomes based on relative competitive ability. Andalo et al. (2001) suggested that intricate competitions may offset effects of kin selection between kin growing nearby. Here we conclude that interplay between facilitation and competition could lead to different competitive ability, providing better explanations for this (File et al. 2011).

In this study, plants grew in the field without any applied limitation to root growth (Fig. 1). Such design avoids the influence of pot size inequality and can separate complex roots clearly (Klemens 2008; Chen et al. 2012), but could decrease interactions between plants due to more space available for growth. Plants respond differently to kin neighbors and have contrasting results according to above- or belowground allocation and fitness (Cheplick and Kane 2004; Dudley and File 2007). The discrepancies may be attributed to different competitive traits which are expressed as kin selection or resource partitioning among different plant

species. Nonetheless, this is the first report to show interactions between siblings for available N based on ^{15}N tracers. This provides a new insight through the process of competing N as siblings growing, instead of using fitness, phenotype or allocation traits singly.

In conclusion, different interaction mechanisms (e.g., kin selection and resource partitioning) may occur simultaneously for siblings growing together (File et al. 2011), finally affecting plants competitive ability. Many previous experiments assumed that kin selection and resource partitioning occur independently, so that their discussions could be not comprehensive. Our findings reflect that interactions between siblings could be more complicated than previously expected. Although we only investigated two plant species, this aspect of kin selection may be a common phenomenon.

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