Effects of various nitrogen regimes on the ability of rapeseed (Brassica napus L.) to suppress littleseed canarygrass (Phalaris minor Retz.)

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Abstract

Background: Littleseed canarygrass (*Phalaris minor* Retz.) is one of the most troublesome invasive weeds infesting winter crops in Yunnan Province, China. Our previous study found that rapeseed (*Bassica napus* L.) was a logical candidate crop to control littleseed canarygrass in agroecosystems. Nitrogen (N) could impact plant community composition by altering competitive interactions, however, the effects of different N regimes on weed control efficacy of rapeseed were unknown. Here, we report the effects of different N regimes on the competitiveness of rapeseed with littleseed canarygrass and accompanying differences in photosynthetic characteristics.

Results: The results showed that the rapeseed yield and its control efficacy on littleseed canarygrass were significantly affected ($P<0.05$) under different N regimes, and the control efficacy of littleseed canarygrass by rapeseed increased first and then decreased with the increase of basal nitrogen rates, while increasing topdressing N rates increased control efficacy of littleseed canarygrass by rapeseed. In fact, yield and weed control efficacy of rapeseed was most ideal when both basal and top-dressing N was 90 kg·ha^{-1}. We also found that N significantly impacted the competitive ability of rapeseed to littleseed canarygrass and rapeseed had the highest competitive ability when both basal and top-dressing N was 90 kg·ha^{-1}. With the increase of basal nitrogen rates, competitive balance index (CB) of rapeseed increased initially but decreased beyond an optimal level. CB continually increased with increasing of topdressing N rates. Our research also showed level and period of N application had a significant effect ($P<0.01$) on the photosynthetic rate (Pn) and chlorophyll content (Chl) of both rapeseed and littleseed canarygrass. Under the same N application regime, the Pn and Chl of littleseed canarygrass were higher than that of rapeseed in December, while the Pn and Chl of rapeseed was higher than that of littleseed canarygrass in February. Our study indicated that photosynthetic characteristics of rapeseed and littleseed canarygrass in different growth stages differ in their sensitivity to N regimes, creating a dynamic competitive relationship.

Conclusions: Together, our results demonstrated that optimal application of fertilizer N could help rapeseed produce higher yields and greater weed control efficacy, suggesting that future modeling or experimental studies on utilizing crops to control invasive weeds should carefully consider both timing and placement of N.

Background

Invasive weeds in agro-ecosystems reduce the yield of crops, seriously affecting the export trade of agricultural products, and even threatening ecosystem safety and human health [1, 2]. In order to meet these challenges, and to provide alternatives to control by herbicides or mechanical methods environmentally friendly approaches, which have been explored extensively over the past several decades. Ecological control using high value species (e.g., crop species grown locally, native species and/or cash crops) has emerged as a viable option for management of invasive alien plant species [3–5]. Compared with mechanical or chemical control methods, ecological control has generally been considered safer, economical, eco-friendly, and sustainable [6]. However, due to increasing demand for food, nitrogen (N) application to croplands has been on the increase [7], which may affect ecological control efficacy. Some studies have shown that increasing N application rates affect growth and control efficacy of native species on invasive weeds [8–10]. For example, increased N application was found to increase the invasiveness of the plant, *Ambrosia artemisiifolia*, as well as its ability to be suppressed by competition with native species [8]. Response to N levels varies widely by species, which explains although increased N influences *A. artemisiifolia* favorably, other plants have yet stronger responses to N. Furthermore, increases in biomass due to increased N can make other nutrients less accessible. Given N is an indispensable nutrient for agricultural production, studies on the effects of various N regimes on the ecological control efficacy of native plant for managing invasive weeds are critical for future management of invasive weeds in agroecosystems.

Littleseed canarygrass (*Phalaris minor* Retz.), an annual weed native to the Mediterranean region [11, 12], is one of the most harmful weeds of winter crops in the world. This weed has spread across Eurasia, South and North America, East and South of Africa and Australia [12, 13]. This weed came to China from Mexico via the introduction of wheat in the 1970s and is presently among the most destructive invasive plants in temperate field cropping systems of Yunnan Province, Southwest China [14–16]. Numerous methods have been developed to manage this weed, but the most effective control is generally achieved using herbicides [17–19]. Environmental issues stemming from the use of herbicides, along with the frequent occurrence of herbicide resistance, provide a good rationale for the implementation of alternative control measures [20]. As a promising alternative to traditional control methods, the use of rapeseed to control the invasive weed *P. minor* has recently been reported in China [21].

Rapeseed ranks second among almost all oilseed crops worldwide and is the first-ranked oilseed crop in Yunnan Province [22]. Rapeseed competes strongly with weeds and also exhibits allelopathic activity, facilitating ecological control of weeds in farmland by rapeseed [16, 21]. For example, one study found that *Alopecurus japonicus* Steud., *Myosolon aquaticum* (L.) Mocnch. and *Lapsana apogonoides* Maxim were significantly inhibited by rapeseed in farmland [23]. Our previous field surveys found that population densities of littleseed canarygrass in rapeseed fields were significantly lower than those in other fields [17]. Subsequent studies examined the effects of rapeseed on littleseed canarygrass growth and reproduction [16]. In mixed culture, rapeseed exhibited greater competitive ability than littleseed canarygrass, and the total biomass, branch, panicles numbers and seed numbers of littleseed canarygrass were suppressed significantly by rapeseed [16, 21]. As one of the key factors affecting crops, weeds and their interactions, N fertilizer has been widely applied in farmland to increase rapeseed yield. However, the effects of various N regimes on the efficacy of rapeseed in controlling littleseed canarygrass are not known.

Building on our previous studies [15, 16, 17, 21], the current research examined the influence of various N application rates on control efficacy of rapeseed and littleseed canarygrass in a field experiment in Yunnan Province, China. Our overall goals were to elucidate mechanisms by which rapeseed competes with littleseed canarygrass under different N regimes and explore more sustainable management methods for littleseed canarygrass in agro-ecosystems. Specifically, our objectives were to: (1) Evaluate impacts of different N regimes on rapeseed yield and its weed control efficacy; and (2) Measure competitive effects and photosynthetic characteristics of rapeseed and littleseed canarygrass under different N regimes.

Results

The impacts of littleseed canarygrass on the growth and yield of rapeseed under various nitrogen regimes

The results showed that the aboveground biomass and yield of rapeseed in all mixed culture treatments were significantly lower ($P < 0.05$) than that in all monoculture treatments under the same N application rate (Table 1). Effects of littleseed canarygrass on the growth and yield of rapeseed were significantly different ($P < 0.05$) under various N regimes. In monoculture, the aboveground biomass and yield of rapeseed increased with increasing N application rates. In mixed culture, the biomass and yield of rapeseed were highest for moderate basal N application rates, but the biomass and yield of rapeseed was highest for the highest topdressing N application rate. Under different N regimes, littleseed canarygrass could reduce rapeseed yield by 22.95% -54.60%, and yield loss was greatest (over 50%) with basal application rates of BN3.

. **Table 1.** Impacts of littleseed canarygrass on the growth and yield of rapeseed under various nitrogen regimes, including either basal nitrogen (BN) or (TN) topdressing nitrogen treatments.*

Treatments		Total aboveground biomass of rapeseed (g plant^{-1})		Rapeseed yield (Kg ha^{-1})		Yield reduction rates of rapeseed (%)
BN	TN	Monoculture	Mixed culture	Monoculture	Mixed culture	
BN_1	TN_1	57.1 ± 0.45I(a)	36.6 ± 0.73G(b)	1405.3 ± 40.1I(a)	891.5 ± 29.6G(b)	36.34 ± 3.16B
	TN_2	65.4 ± 0.91H(a)	46.4 ± 0.77F(b)	1678.5 ± 23.5H(a)	1101.5 ± 17.2F(b)	34.35 ± 1.16B
	TN_3	72.7 ± 0.54G(a)	54.1 ± 0.97E(b)	1843.5 ± 16.4G(a)	1228.8 ± 26.2E(b)	33.30 ± 2.04B
BN_2	TN_1	78.9 ± 1.25F(a)	59.6 ± 0.59D(b)	2256.3 ± 19.9F(a)	1651.3 ± 32.5C(b)	26.79 ± 1.71C
	TN_2	87.1 ± 0.46E(a)	70.7 ± 0.94B(b)	2471.4 ± 18.2E(a)	1874.8 ± 18.5B(b)	24.15 ± 0.30C
	TN_3	92.6 ± 0.97 D (a)	76.9 ± 0.81A(b)	2623.3 ± 33.4D(a)	2021.3 ± 35.5A(b)	22.95 ± 0.91C
BN_3	TN_1	96.8 ± 0.70C(a)	52.9 ± 0.78E(b)	2768.1 ± 21.8C(a)	1256.2 ± 23. 9 E (b)	54.60 ± 1.22A
	TN_2	102.9 ± 1.09B(a)	63.4 ± 0.99C(b)	2864.2 ± 13.7B(a)	1318.1 ± 33.8E(b)	53.97 ± 1.28A
	TN_3	109.7 ± 1.0A(a)4	75.6 ± 1.04A(b)	3024.4 ± 42.7A(a)	1434.2 ± 38.8D(b)	52.57 ± 1.25A

*Data are expressed as means ± standard error. Basal nitrogen (BN) rates were 30, 90 and 150 kg·ha^{-1}; Topdressing nitrogen rates (TN) were 30, 60, and 90 kg·ha^{-1}. The upper case letters indicate that the comparison of rapeseed yield or aboveground biomass under different nitrogen application rates treatments; different upper case letters represent significant differences at $p < 0.05$. The lower case letters in brackets indicate that the comparison of rapeseed yield or aboveground biomass between monoculture and mixed culture treatments at the same nitrogen condition; different lower case letters represent significant differences at $p < 0.05$.

The inhibitory effects of rapeseed on littleseed canarygrass under various nitrogen regimes

The aboveground biomass and seed number of littleseed canarygrass were significantly inhibited ($P < 0.05$) by rapeseed under various N regimes (Table 2). In monoculture, the aboveground biomass and seed number of littleseed canarygrass increased with increasing N application rates. In mixed culture, the seed number was significantly decreased by the presence of rapeseed but seed production increased with increasing basal N application rates. Seed production in mixed culture by littleseed canarygrass decreased with increasing topdressing N application rates, however. Aboveground biomass of littleseed canarygrass did not differ between BN_1 and BN_2 treatments, but under the basal N application rate BN_3, aboveground biomasses of littleseed canarygrass were significantly higher than for lower basal N rates (Table 2). Rapeseed exhibited high control efficacy on littleseed canarygrass at low basal N rates but decreased with increasing basal N application rates. In mixed culture, seed number of littleseed canary grass was lowest at intermediate basal N application rates, whereas at highest basal N application rates control efficacy declined as little seed canary grass was the highest. Furthermore, within the highest basal N rates, seed production of the weed increased with increasing top dressing rates.

Table 2
Effects of various nitrogen regimes on rapeseed control littleseed canarygrass, including either basal nitrogen (BN) or (TN) topdressing nitrogen treatments.*

Treatments		Total aboveground biomass of littleseed canarygrass (g plant^{-1})		Seed number of littleseed canarygrass		Control efficacy (%)
BN	TN	Monoculture	Mixed culture	Monoculture	Mixed culture	
BN_1	TN_1	33.6 ± 0.79I(a)	17.4 ± 0.71C(b)	4171.3 ± 35.6I(a)	2094.3 ± 40.5D(b)	49.8 ± 0.73F
	TN_2	36.9 ± 0.60H(a)	18.5 ± 0.63C(b)	4491.3 ± 34.4H(a)	1952.8 ± 41.8E(b)	56.5 ± 1.18E
	TN_3	41.0 ± 0.64G(a)	19.7 ± 0.75C(b)	4808.5 ± 66.4G(a)	1864.5 ± 67.1E(b)	61.2 ± 0.88D
BN_2	TN_1	43.7 ± 0.78F(a)	17.6 ± 0.88C(b)	5227.8 ± 74.4F(a)	1732.8 ± 27.7F(b)	66.9 ± 0.63C
	TN_2	47.0 ± 0.61E(a)	18.8 ± 0.60C(b)	5557.3 ± 92.5E(a)	1301.0 ± 29.5G(b)	76.6 ± 0.56B
	TN_3	49.4 ± 0.76D(a)	19.3 ± 0.92C(b)	5989.8 ± 103.4D(a)	1211.5 ± 33.9G(b)	79.8 ± 0.63A
BN_3	TN_1	53.7 ± 0.53C(a)	35.3 ± 1.06B(b)	6223.3 ± 82.1C(a)	3314.8 ± 34.9C(b)	46.7 ± 0.73G
	TN_2	59.0 ± 0.72B(a)	38.7 ± 0.63A(b)	6558.1 ± 66.1B(a)	3623.1 ± 30.18B(b)	45.2 ± 0.35G
	TN_3	62.3 ± 0.69A(a)	40.8 ± 0.86A(b)	6979.5 ± 133.9A(a)	3852.5 ± 52.0A(b)	44.8 ± 1.06G

*Data are expressed as means ± standard error. Basal nitrogen (BN) rates were 30, 90 and 150 kg·ha^{-1}; Topdressing nitrogen rates (TN) were 30, 60, and 90 kg·ha^{-1}. Control efficacy is based on seed number. The upper case letters indicate that the comparison of aboveground biomass, seed number or control efficacy of littleseed canarygrass among different nitrogen fertilization treatments under the same planting mode(monoculture or mixed culture); different upper case letters represent significant differences at $p < 0.05$. The lower case letters in brackets indicate that the comparison of aboveground biomass or number of seed between monoculture and mixed culture treatments under the same nitrogen condition; different lower case letters represent significant differences at $p < 0.05$.

Competitive Interactions of rapeseed and littleseed canarygrass

The RY of rapeseed and littleseed canarygrass was significantly less ($P < 0.05$) than 1.0 in mixed culture, showing that the intraspecific competition between the two plants was less than their interspecific competition under different nutrient levels (Table 3). The CB (competitive balance) index of rapeseed was significantly greater than zero in mixed culture, except at basal N level of BN_3, which indicates that rapeseed was more competitive than littleseed canarygrass at low and moderate basal N levels, but sometimes less competitive at high basal N levels.

Under the same topdressing condition, with increasing basal N application rates, the RY and CB index of rapeseed first increased and then decreased, and the RY of littleseed canarygrass first decreased and then increased. However, with increasing topdressing N application rate, the RY and CB index of rapeseed increased, the RY of littleseed canarygrass declined.

Table 3
Relative yield (RY) and competitive balance (CB) index of rapeseed and littleseed canarygrass in mixed culture with either basal nitrogen (BN) or (TN) topdressing nitrogen treatments.*

BN	TN	Rapeseed RY	Littleseed canarygrass RY	CB index for rapeseed
BN_1	TN_1	0.641 ± 0.016d**	0.517 ± 0.009b**	0.216 ± 0.008e**
	TN_2	0.709 ± 0.002c**	0.501 ± 0.012bc**	0.348 ± 0.015d**
	TN_3	0.744 ± 0.008b**	0.479 ± 0.014c**	0.440 ± 0.019c**
BN_2	TN_1	0.756 ± 0.005b**	0.401 ± 0.013d**	0.635 ± 0.037b**
	TN_2	0.812 ± 0.009a**	0.399 ± 0.008d**	0.710 ± 0.009a**
	TN_3	0.831 ± 0.004a**	0.391 ± 0.013d**	0.755 ± 0.031a**
BN_3	TN_1	0.547 ± 0.007e**	0.658 ± 0.014a**	-0.186 ± 0.012h**
	TN_2	0.616 ± 0.007d**	0.655 ± 0.006a**	-0.061 ± 0.005g**
	TN_3	0.690 ± 0.016c**	0.654 ± 0.008a**	0.052 ± 0.016f**

*Data are expressed as means ± standard error. Basal nitrogen (BN) rates were 30, 90 and 150 $kg{\cdot}ha^{-1}$; Topdressing nitrogen rates (TN) were 30, 60, and 90 $kg{\cdot}ha^{-1}$. The different letters within same row mean significant differences at $P < 0.05$. The t-test was used to compare each value with 1.0 and 0; * and ** indicate significant differences at 0.05 and 0.01 levels, respectively.

Photosynthetic characteristics

In December, net photosynthesis rate (Pn) and chlorophyll content (Chl) of littleseed canarygrass were significantly higher than those of rapeseed ($P < 0.05$) in either monoculture or mixed culture (Figs. 1 and 2). With the increasing N application rates, the Pn and Chl of littleseed canarygrass increased; but Pn and Chl of rapeseed first increased and then decreased in the mixed culture treatment. Although the Pn and Chl of rapeseed or littleseed canarygrass in mixed culture were lower than these parameters in monoclutures, there were generally no significant differences for either species. However, for BN_3 treatments, the mixed culture rapeseed exhibited significant lower Pn and Chl levels by comparison to the other treatments(Figs. 1 and 2).

In February, the Pn and Chl of rapeseed were significantly higher than that of littleseed canarygrass ($P < 0.05$) in both monoculture or mixed culture (except the basal N application rate of BN3) (Figs. 1 and 2). In monoculture treatments, the Pn and Chl for both rapeseed and littleseed canarygrass gradually increased with increasing N application rates. In mixed culture treatments, Pn and Chl of rapeseed were highest at moderate basal N application rates, whereas Pn and Chl of littleseed canarygrass gradually increased as rates increased. However, Pn and Chl for both rapeseed and littleseed canarygrass in mixed culture gradually increased with increasing topdressing N application rates (Figs. 1 and 2).

Discussion

Utilizing crops to control invasive weeds are regarded as an environmentally friendly approach to the management of invasive weeds in agro-ecosystems [6, 25]. At the same time, due to the high demand for food, it is important to develop weed control methods to sustain crop yield. N is essential for plant growth and development and is widely used to improve crop vigor and productivity in agro-ecosystems [26]. Our previous studies found that rapeseed may be used to control littleseed canarygrass in agro-ecosystems [17]. It was generally hypothesized that increasing N rates could improve rapeseed yield and enhance the economic benefits [24]. Furthermore, we theorized that different types of N application, such as basal or top-dressing applications might influence responses of the crop and the weed as tested here. Crop and weed responses to N differ and are affected by competition, influencing the outcome of interactions between rapeseed and littleseed canarygrass [16]. We found that increased topdressing N rates decreased rapeseed yield loss in mixed culture, but the yield losses in rapeseed were even more influenced by basal N levels. For basal N application rates of BN_3 (150 $kg{\cdot}ha^{-1}$), the yield loss of rape was more than 50%. However, the yield loss of rapeseed was less than 30% when for basal fertilizer rate BN_2 (90 $kg{\cdot}ha^{-1}$). Our results suggested that weed control efficiency of rapeseed was greatly affected by N application levels, with basal N levels playing a leading role. Although at higher basal rates rapeseed yield was seriously reduced, intermediate N application rates may reduce the yield loss of rapeseed and could be successfully manipulated in favor of rapeseed,

Competitiveness of crops plays an important role in determining the likelihood of success in the control of invasive weeds [4, 5, 21]. Although increased N levels can improve the competitiveness and plant growth of both crops and invasives [8, 9], the relative effects vary depending on the particular species and other conditions, and adaptation for N uptake and utilization are related to the evolutionary history of invasive species and the artificial selection of crops. .Our evaluation of competitiveness via seed production of littleseed canarygrass provided another view of the effectiveness of various treatments. Just as seen in the biomass measurements, rapeseed showed the highest control efficacy at intermediate basal N rates, while at the highest N rates littleseed canarygrass produced substantially higher seed numbers, resulting in a control efficacies in the 40% range, as compared to 67–80% at intermediate basal N rates, depending on accompanying top-dressing rates, with increased topdressing N rates improving the competitive ability of rapeseed. Thus the N level and type of fertilization must be regulated carefully to favor rapeseed over littleseed canarygrass.

As an essential nutrient element for plant growth, N is integral for plant photosynthesis [27]. Higher rates of photosynthesis can lead to increased growth rates, biomass accumulation and overall production [28]. Our current study showed that net photosynthesis rate (Pn) and chlorophyll content (Chl) of littleseed canarygrass were significantly higher than those of rapeseed ($P < 0.05$) in December. At that point in the season, the Pn and Chl of littleseed canarygrass increased with increased N whereas by comparison Pn and Chl of rapeseed did not respond as well to the higher fertilizer levels. However, in February, the Pn and Chl of rapeseed were significantly higher than those of littleseed canarygrass ($P < 0.05$). At this point, increasing basal N continued to produce higher littleseed canarygrass Pn rates, but only increased rapeseed rates over the transition from BN1 to BN2; however, topdressed N consistently increased Pn and Chl in both species. Thus, in the seedling stage, N demand of littleseed canarygrass was stronger than that of rapeseed, with the result that higher N levels did not increase rapeseed productivity as much as the invasive plant littleseed canarygrass. However, by the reproductive stage, increasing topdressing N improved did improve the productivity of rapeseed.

Differences in nutrient requirements of plants at different growth stages is a common phenomenon [29]. If carefully matched to N demands at various plant growth stages, fertilizer availability could also be manipulated to provide ecological management of invasive alien plants. In our study we found that the net photosynthesis rate (Pn) of rape at seedling stage is weaker than that of littleseed canarygrass, while it is stronger at propagation stage. Our finding of a relatively low net photosynthesis rate (Pn) of rapeseed at the seedling stage, the efficacy of rapeseed in controlling littleseed canarygrass could be improved by timing the fertilizer application appropriately. By using fertilization strategically, e.g., by utilizing topdressing methods, N could be applied to deliberately to improve the competitive ability of rapeseed over invasive plants like littleseed canarygrass. Control indices are useful to developing clear measures for evaluating invasive plant control efficacy [30]. Our work was the first study to examine rapeseed yield and littleseed canarygrass weed seed number to evaluate the effect of N regime on their competitive relationship. Our measure of invasive plant control efficacy involved pitting yield of the crop (rapeseed) against the seed production of the weed (littleseed canarygrass), where yield is the measure of crop success and weed seed is essential for the establishment and spread of weed populations. Also key to our approach was showing how the two plants competed at different densities using a De Wit replacement series experiments [25] using biomass to develop an index to evaluate plant interspecific competition [31] as well as yield measures. The data from the De Wit replacement series enabled us to calculate competitive balance, which revealed that interspecific competition had a large effect on both species, larger than the effects of intraspecific competition in both cases. It was also clear that top-dressing N application favors rapeseed over littleseed canaraygrass, whereas basal N applications only favor rapeseed to an intermediate level, but at higher levels the competitive balance is tipped in favor of littleseed canarygrass.

With invasive weeds continuing to threaten food safety and agro-ecosystem sustainability, ecological control using crops or native species may provide safe, economical, and environmentally sustainable solutions for invasive weed management [24, 31]. Therefore, choosing crop species with strong competitiveness, high economic value and suitable for large-scale planting have formed the core issues of this study [5]. However, the interspecific competition between plants is dynamic. Environmental factors such as N availability may affect the interspecific relationship between alternative crops and invasive weeds as demonstrated in our study.

Conclusion

Our results demonstrated that optimal application of fertilizer N allowed rapeseed to produce strong yields and provide high efficacy in the control of littleseed canarygrass. We found that interspecific competition and photosynthetic characteristics of rapeseed and littleseed canarygrass could be greatly affected by different nutrient regimes. Littleseed canarygrass growth was more sensitive to basal N application than topdressing applications. Rapeseed had the strongest competitive ability for intermediate basal and topdressing N rates of 90kg·ha^{-1}. Our study also indicated that photosynthetic characteristics of rapeseed and littleseed canarygrass at different growth stages varied in their sensitivity to varying N regimes, which showed that optimally planned nutrient regimes may provide a strategic tool for the ecological control of littleseed canarygrass. Finally, we recommend that future modeling or experimental studies on utilizing crops to control invasive weeds in agroecosystems should simultaneously consider impacts of N application on both crops and weeds to calculate optimum fertilizer rates to maximize crop competitiveness.

Methods

Study Species

Littleseed canarygrass (*Phalaris minor* Retz.) is widely distributed in subtropical and temperate regions in Yunnan Province, Southwest China [14, 17]. Since 2013, littleseed canarygrass seeds have been collected from wheat fields in Songming County of Yunnan Province and propagated in the glasshouse of the Agricultural Environment and Resource Research Institute, Yunnan Academy of Agricultural Sciences, China. The average weight of 1000 seeds was 1.49 ± 0.05 g and the germination rate using culture dish filter paper method was 91.8%, as tested before the experimentation.

Rapeseed (*Brassica napus* L.) is a dominant oil crop in subtropical and temperate regions of Yunnan Province [22]. Our previous studies showed that rapeseed variety Yunyou No. 2 had strong competitive ability and could be used to control littleseed canarygrass in agroecosystems [24]. In this experiment, the seeds of Yunyou No. 2 were obtained from the Food Research Institute, Yunnan Academy of Agricultural Sciences (YAAS)

Experiment design and data collection

We conducted a field experiment from September, 2018 to April, 2019 at the Agricultural Environment and Resource Research Institute, Yunnan Academy of Agricultural Sciences, Kunming China (25°12'–25°39' N, 102°76'–102°89' E) in the same field as Xu et al. [17]. This area is characterized by a subtropical and temperate monsoon climate. Rainfall averages 1000–1300 mm per year and the annual mean temperature is 14.1°C [17]. The soil type of test site is yellow-brown, with a total N content of 1.32g·kg^{-1}, Olsen P content of 29.5 mg·kg^{-1}, and available K content of 86.4 mg·kg^{-1}, respectively.

The experiment was a split-plot design with basal N fertilization rates as the whole-plot factor (four replications for each N application rate treatment), topdressing N fertilization rates and plant ratios were split-plot factors, utilizing a de Wit replacement series method [32]. The rapeseed and littleseed canarygrass were sown on 24 September 2018 in the greenhouse. On 27 October 2018, similar-sized seedlings of both species(rapeseeds with 6 true leaves and and littleseed canarygrass with 4 leaves) were transplanted into 9 m^2 plots (3 m × 3 m) and subjected to different N fertilization treatments. Three basal N fertilization rates (30 (BN_1), 90 (BN_2) and 150 (BN_3) kg·ha^{-1}) were applied in the form of urea at pre-plant (26 October 2018), and three topdressing N fertilization rates (30 (TN_1), 60 (TN_2) and 90 (TN_3) kg·ha^{-1}) were also applied in the form of urea at bolting stage (26 January 2019). In each plot, the amount of other fertilizers used in the whole growth period was the same, P_2O_5 90 kg·ha^{-1}, K_2O 120 kg·ha^{-1}, B 1.6 kg·ha^{-1}, respectively.

We utilized a de Wit replacement series incorporating three ratios of rapeseed and littleseed canarygrass densities and nine different N regimes in replicated 9 m^2 plots. A combination of three ratios (1:0 (180:0 plants), 1:1 (90:90 plants) and 0:1 (0:180 plants)) of rapeseed and *P. minor* were studied at nine N fertilization rates (BN_1TN_1, BN_1TN_2, BN_1TN_3, BN_2TN_1, BN_2TN_2, BN_2TN_3, BN_3TN_1, BN_3TN_2, BN_3TN_3). A total of 180 plants per plot were grown at three ratios of rapeseed and *P. minor* while maintaining a constant planting density of 20 plants m^{-2} (0.25 m × 0.20 m space) in each plot. All plants were distributed uniformly within the plot. All plots were arranged in a complete randomized block design with four replicates per ratio and per nutrient level (total n = 4 replicates × 3 ratios × 9 nutrient levels = 108). A 1.0 m border was constructed between plots and each plot was fenced with 0.5 m high glass panels to avoid being disturbed by herbivores The net photosynthetic rate (Pn) measurements on leaves for rapeseed and littleseed canarygrass conducted using a Portable Photosynthesis System (LI-COR Biosciences LI-6400XT, Lincoln, Nebraska, USA), between 10:00 am and 16:30 pm, with a 6400-02 LED source and 1000 μmol m^{-2} s^{-1} photosynthetically active radiation [33]. During sampling, CO_2

concentration in surrounding air, air temperature and relative humidity (RH) in the chamber were measured. In December 2018, two months after transplanting, 40 fully expanded leaves (flag leaf and the top second leaf) of each species were randomly sampled from each plot and immediately scanned using an LI-6400XT [34]. Then, the leaves were cleaned for chlorophyll content determination. We cut 0.1g pieces from fresh leaves avoiding the main vein, and soaked the leaf fragments in 25 ml 95% ethanol at room temperature for 48 h. The detector was set at 665 nm in order to calculate the total chlorophyll content (Chl) [28]. In February 2019, four months after transplanting, twenty plants of each species were selected randomly and forty fully expanded sun leaves (flag leaf and the top second leaf) of each species were sampled for net photosynthetic rate and chlorophyll content, following the same method as above. Plants were manually uprooted and then cut at ground level for determination of aboveground biomass. Fresh plants were heated for 30 min at 105°C to halt metabolic processes, and then dried at 80°C in a forced-draft oven until reaching a constant weight before weighing. Rapeseed yield and littleseed canarygrass seed number were determined in a $2m^2$ area in each plot; rapeseed yield was adjusted to a moisture content of 10.0%.

Data analyses

To evaluate the effect of littleseed canarygrass on rapeseed yield under various N regimes, the yield losses of rapeseed were determined from rapeseed yield comparing monoculture versus mixed culture according to the formula: the yield reduction rate of rapeseed(%)=(1 yield in mixed culture / yield in monoculture)×100%. To evaluate the ability of rapeseed to compete effectively with littleseed canarygrass under various N regimes, we determined the efficacy of littleseed canarygrass suppression by comparing its seed numbers in monoculture versus mixed culture according to the formula: control efficacy of littleseed canarygrass (%)=(1 seed number in mixed culture / seed number in monoculture)×100%.

Relative yield (RY) per plant [35] and competitive balance index (CB) [36] were calculated from the final aboveground biomass obtained for each species in each plot. Relative yield per plant of species a or b in a mixed culture with species b or a was calculated as $RY_a= Y_{ab}/Y_a$ or $RY_b= Y_{ba}/Y_b$. Competitive balance index was calculated as $CB_a= \ln (RY_a/RY_b)$, where Y_{ab} is the yield for species a growing with species b (g/individual), Y_{ba} is the yield for species b growing with species a, Y_a is the yield for species a growing in pure culture (g/individual), and Y_b is the yield for species b growing in pure culture. Values of RY_{ab} measure the average performance of individuals in mixed cultures compared to that of individuals in pure cultures. An RY_{ab} of 1.00 indicates species a and b are both equal in terms of intraspecific competition and interspecific competition. An RY_{ab} greater than 1.00 means intraspecific competition for species a and b is higher than interspecific competition, and an RY_{ab} of less than 1.00 implies intraspecific competition of species a and b is less than interspecific competition [37]. Values of CB_a greater than 0 indicate that species a is more competitive than species b [38].

The rapeseed yield and its yield reduction rate, seed number of littleseed canarygrass and its control efficacy, aboveground biomass, physiological (Pn) and chlorophyll content of rapeseed and this weed were analyzed by analysis of variance (one-way ANOVA) using IBM SPSS 23.0 software (Armonk, New York, USA). The F and partial eta squared statistics were calculated considering density ratio and N level with their interaction as factors at a 5% level of significance. Relative yield from each mixed culture were compared to the value of 1.00 using t-tests (α = 0.05), and values of CB for deviation from 0 using a paired t-test.

Declarations

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Authors’ Contributions

L.Y.D. and F.D.Z. conceived and designed the experiments; G.F.X., S.C.S., S.S.Y., and Y.Z. performed the experiments; G.F.X. and S.C.S. analyzed the data and wrote draft; D.R.C. edited the manuscript for style; L.Y.D. and F.D.Z. commented on manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the present research are accessible from the Corresponding author on reasonable request.

Ethics approval and consent to participate

All aspects of the study comply with institutional, national, and international guidelines. All experiments were conducted on non-regulated organisms. The study site was rented by Agricultural Environment and Resource Research Institute, Yunnan Academy of Agricultural Sciences and no permits were required to take samples.

Consent for publication

Not applicable.

Competinginterests

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript and in the decision to publish the results.

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Figures

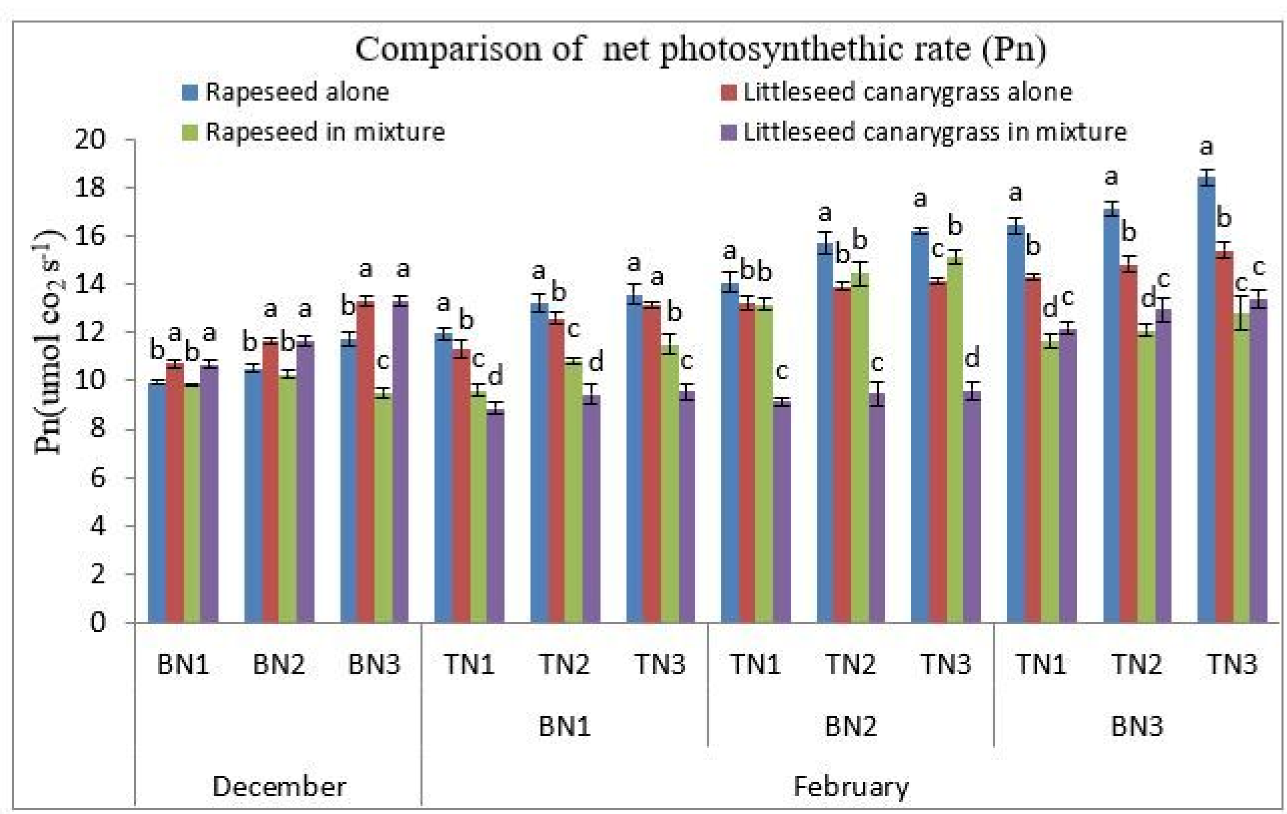

Figure 1

The effects of different nitrogen regimes on net photosynthetic rate (Pn) of rapeseed and littleseed canarygrass in December and February. Bars are means ± standard error, different letters represent significant differences under the same nitrogen condition at p<0.05,The same below.

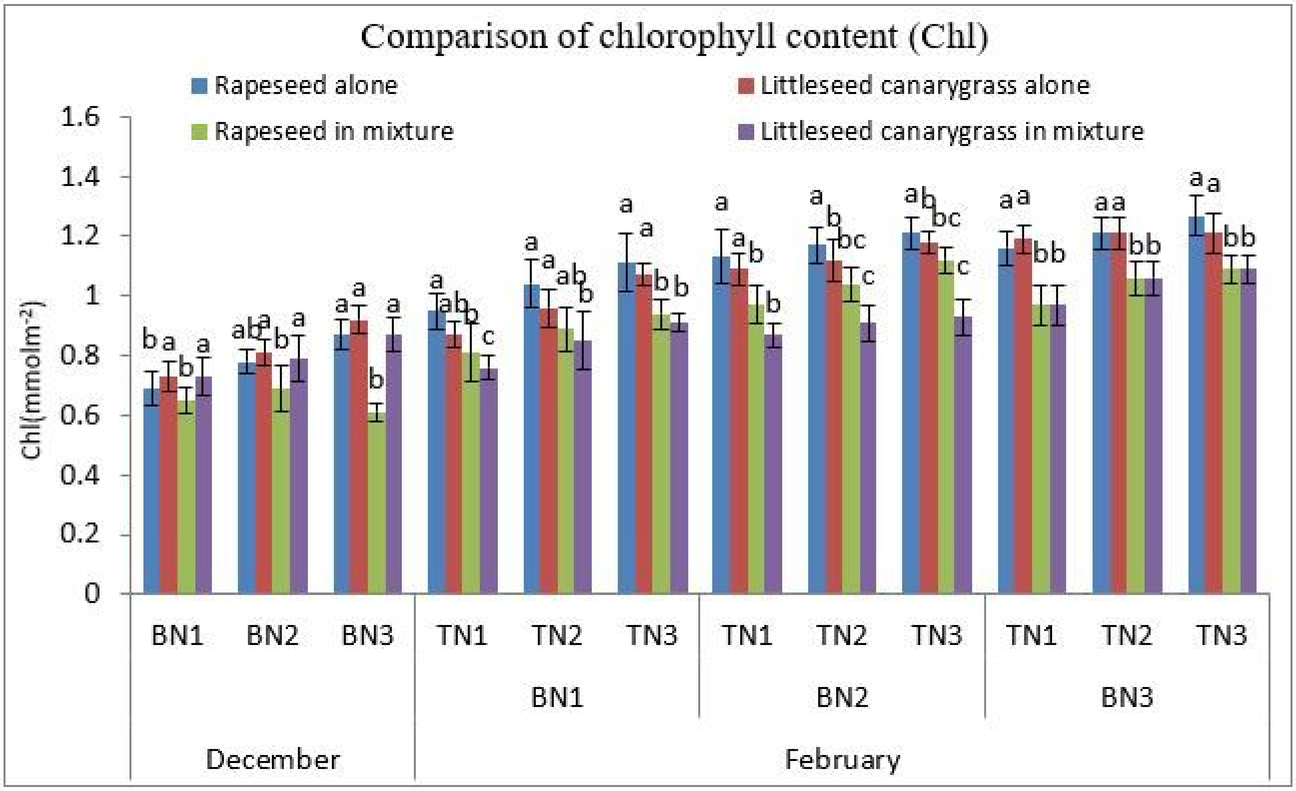

Figure 2

The effects of different nitrogen regimes on on chlorophyll content of the two species in December and February