

Establishment of alien invasive plant, yellow sweet clover (*Melilotus officinalis*) at a complex ecosystem distributed with farmlands and wasted lands

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Abstract

Alien invasive plants had a far-reaching impact on local vegetation and biodiversity, and the hazard of invasion was widely concerned. Field trial was conducted to discuss the establishment of yellow sweet clover and its interaction with local plant vegetation on a synthetical ecosystem patched with cultivated farmlands and wasted lands in northern China. The results showed the establishment for yellow sweet clover was quite different under the two land types. The density of seedlings in the wasted lands only reached up to 16.9% of that in the farmlands which flourished with annual large broad-leaf plants, like velvet weed (*Abutilon theophrasti*), jimson weed (*Datura stramonium*), and flower-of-an-hour (*Hibiscus trionum*) at a higher frequency contrast with thin-leaf species in the wasted lands, e.g. *Salsola collina*, *Humulus scandens*. The two types of lands had a community similarity ($C_J = 0.5135$), but both significantly decreased with the increasing density of yellow sweet clover. On details, biomass, plants per square meters, and diversity, they were all less or lower in wasted lands compared to farmlands. After its establishment, soil characteristics and soil texture were also modified due to yellow sweet clover itself or environmental factors, e.g. a higher level of available nitrogen and soil organic matter. The results suggested that it is not easy for yellow sweet clover to establish on wasted lands under the suppression of native plants and adverse environmental factors; but it may flourish in the farmlands with competitive advantages at initial stage, though presented a stagnation after generations.

Introduction

The invasion of alien species has a great impact on invaded habitats and ecosystems. It not only leads to changes in the composition and structure of the ecosystem, affects the biodiversity of the invaded region, and changes the ecological nature and function (Elton 1958; Ricciardi et al. 2013; Theoharides and Dukes 2007; Vila and Ibanez 2011; Wan et al. 2009). As leguminous nitrogen-fixing plant, yellow sweet clover (*Melilotus officinalis*), mixed with its congeneric species white sweet clover (*M. albus*), was well spread in more than 25 provinces in China, which was listed as invasive plants (Chen et al. 2017; Liu et al. 2006; Wang et al. 2011). Besides its original region in Asia-Europe, it also distributed in the North America, e.g. Canada, America, even Alaska included (Conn et al. 2011; Lesica and DeLuca 2000; Moyer et al. 2007), South America (Paiano et al. 2007), Australia (Evans and Kearney 2003), New Zealand and Africa (Rojas-Sandoval 2017). Sweet clovers, with wide adaptability and strong stress resistance, will cause obvious invasion harm once it invades into a new habitat (or ecosystem). In some areas in the north China, such as Agro-pastoral ecotone, sweet clovers have become dominant plants in some habitats, forming yellow and white bands distributed along the road during the flowering period (June to July) (Chen et al. 2014) or invading into farmland-grassland ecosystems (Chen et al. 2013). At present, sweet clovers have been naturalized in a variety of habitats and developed into dominant species, such as wasteland, roadside, farmland, grassland, floodplains and so on (Conn et al. 2011). Sweet clovers are easy to establish in new habitats and over-compete local plants because of their wide adaptability and strong competitive advantages (Lesica and Deluca 2000). When sweet clovers die, the residual plants still contain high nitrogen, which will be more conducive to the establishment and growth of a new generation of sweet clovers in the degraded habitat (Wolf et al. 2004). In addition, sweet clovers also have a strong effect of allelopathy, not only the water extract has a significant inhibitory effect on seed germination and seedling growth of other plants (Wu et al. 2010), but also the stubble (Moyer et al. 2007) or as green manure (Blackshaw et al. 2001), it can also effectively control the occurrence of weeds in the field, even the application of hay powder can significantly inhibit the growth of weeds in the field (Wu et al. 2010). Therefore, the study on the competitiveness or allelopathy of sweet clovers play an important role in understanding the competitive advantage and invasion mechanism of alien plants. At the same time, it is worthy of further research and in-depth content to further explore the changes of vegetation and soil characteristics in the invaded area after the establishment of sweet clovers, as well as the impact on the establishment of seeds in the next generation. As nitrogen-fixing legumes, yellow sweet clover has the potential to alter soil conditions, especially nitrogen cycling, and also may inhibit the establishment of native plants. Thus, vegetation structure and soil characteristics were all tested to explore the pattern of establishment and generation alternation of yellow sweet clover in the study.

Materials and Methods

The experiment was conducted at a complex ecosystem composed of farmlands and wasted lands in Beijing (Xiaotangshan National Experiment Station for Precision Agriculture, N 40°11'3.69" E 116°26'34.77"), with mean annual precipitation 569 mm and average annual temperature 13.0 °C. The wasted lands here used to be plowed as farmlands, but then left undisturbed for more than two years before this experiment. Two contrasting vegetation types were gradually formed herein, farmlands were continuously plowed for cultivation, but the wasted lands were flourished with weeds. In the experimental trial, different plant densities were set to simulate homologous invasion stages of yellow sweet clover. The designed plant densities were ranked as five levels, namely 0 (none, control), 10 (low density), 20 (moderate density), 50 (high density) and 100 (very high density) plants/m², respectively. The germination rate of yellow sweet clover was measured by culture dish before sowing, and then seed amount was calculated according to the germination rate and plot area. After sowing, fully irrigation was adopted for each plot, and the survival and growth of sweet clover was observed and quadrat investigation was conducted. Survey indicators include plant species, coverage, plant density, and biomass. Field survey was carried on both the first year of vegetative

growth and the second year of reproductive growth of yellow sweet clover. The experimental trials are mainly surrounded by tall grasses (*Miscanthus sacchariflorus*) and isolated in an enclosed condition in case of the spread of yellow sweet clover.

Simpson's diversity index and Shannon-Wiener index was calculated as follows,

$$D = 1 - \sum (n_i/N)^2;$$

$$H = - \sum P_i \log P_i$$

where, p_i = the proportion of individuals belonging to species i in the sample. If the total number of individuals in the sample is n , and the number of individuals belonging to species i is n_i , then $P_i = n_i/N$.

For the similarity of plant community, Jaccard index (C_J) was used to calculate the similarity between different treatments in both farmlands and wasted lands,

$$C_J = j/(a + b - j)$$

where, j is the common plant species of different plant communities, and a and b are the number of plant species in its community, respectively.

Soil samples at different depths were tested by conventional methods. The indicators included soil pH, total nitrogen, ammonium nitrogen and nitrate nitrogen, total phosphorus, available phosphorus, soil organic matter, and soil compaction. See advanced ecology and soil agrochemical analysis (2nd Edition) for specific determination methods, Elemental Analyzer method (EA, Vario MACRO Cube) are used in measuring total nitrogen (TN) and soil C, Continuous Flow Analyzer method (CFA, Bran + Luebbe, No:AA3) for ammonium nitrogen and nitrate nitrogen; Mo—Sb colorimetry (Olsen method) for total phosphorus and for available phosphorus; Potassium Dichromate Oxidation-Heating Method for soil organic matter, soil pH was tested by pH meter (Seven Compact, No: S210); soil compaction was tested by portable soil compact meter (No: JZ-JC).

Excel 2016 was used for data statistics and analysis. The values were expressed as mean $\pm$ standard deviation. The significance analysis level was set as $\alpha = 0.05$.

Results

Changes of plant density and biomass

The results showed it was easy for yellow sweet clover to establish and grow in the cultivated farmlands, where the actual plant density was much close to the designed density due to germination rate tested by culture dish; yet, it was relatively difficult for yellow sweet clover to establish in the wasted lands (Table 1). On average, plant density in the wasted lands reached only up to 16.9% of that in the adjacent cultivated farmlands, varied from 11.1–20.8%. Plant density was observed to decrease in the farmlands after two years' desolation since the first generation, which had a highest plant density for second generation of 44.0 ± 6.6 individuals per square meter under T4, $24.0 \pm 4.0/m^2$ under T3 and $15.0 \pm 3.6/m^2$ under T2, respectively. Reasonably, there were rare individuals under the treatments of T1 and CK in the farmlands, and less individuals under all the treatments in the wasted lands.

Table 1
Plant density of yellow sweet clover in the farmlands and wasted lands

Plant density (plants/m ²)		CK	T1	T2	T3	T4
Designed plant density		0.0	10.0	20.0	50.0	100.0
Actual plant density	Farmlands	0.0	11.7 ± 1.5	22.0 ± 2.4	53.0 ± 6.2	96.0 ± 6.6
	Wasted lands	0.0	1.3 ± 1.0	4.0 ± 1.5	9.3 ± 2.0	20.0 ± 5.8

On the aspect of biomass, the total biomass presented an increasing trend with the increase of sowed yellow sweet clover from T1 to T3 in the cultivated farmlands, but it decreased swiftly when yellow sweet clover became the dominant species of the community (the plant density was more than 50 plants/m²). Meanwhile, biomass of yellow sweet clover presented an increasing trend from a low percentage of 4.2–36.6%. Whilst in the wasted lands, the total biomass and biomass of yellow sweet clover increased significantly with the increasing of

sowed yellow sweet clover (Table 2). Interestingly, total biomass in the wasted lands gradually increased with plant density of yellow sweet clover, by a maximum ratio of 92.7% under T4, though yellow sweet clover itself accounted for a low percentage of the total biomass, about 5.2% under T4.

Table 2
Biomass under different densities of yellow sweet clover

Land type	Biomass (g)	CK	T1	T2	T3	T4
Farmlands	Total biomass	390.2 ± 82.4	413.8 ± 59.9	418.2 ± 88.8	443.5 ± 91.0	379.7 ± 69.8
	Yellow sweet clover	—	17.2 ± 5.9	36.3 ± 10.5	90.8 ± 18.5	139.0 ± 22.3
Wasted lands	Total biomass	448.6 ± 77.3	545.5 ± 72.1	705.2 ± 68.2	744.9 ± 75.3	864.4 ± 84.7
	Yellow sweet clover	—	4.0 ± 0.9	16.8 ± 5.2	44.9 ± 15.9	45.2 ± 15.1

Community characteristics and vegetation composition

According to the field survey, the result showed the plant species occurred in the two land types were quite different though the lands were adjacent to each other. It showed the similarity between the two communities decreased with the sowed density of yellow sweet clover, namely C_J varied from a relatively high level of 0.5135 under CK to a lowest level of 0.2059 under T3 (Table 3). Anyhow, the similarity of different treatments in the farmlands was higher than that in the wasted lands. On details, the lowest C_J was 0.6571 between T3 and T4 in the farmlands; but it was 0.3333 between CK and T4 in the wasted lands. On the aspect of vegetation composition, the dominant species were fast-growing annual plants in the farmlands, e.g. velvet leaf (*Abutilon theophrasti*), Jimsonweed (*Datura stramonium*), flower-of-an-hour (*Hibiscus trionum*), and goose grass (*Eleusine indica*), which were also alien plant species in the region; whilst in the wasted lands, it flourished with small-leaf native plants, for example, *Salsola collina*, *Cirsium setosum*, *Eclipta prostrata*, and so on.

Table 3
Similarity of plant communities under two land use patterns

C _J		Farmlands				
		CK	T1	T2	T3	T4
Wasted lands	CK	0.5135[□]	0.7714	0.7429	0.6667	0.8710
	T1	0.5714	0.3889	0.6579	0.7222	0.7647
	T2	0.4643	0.5714	0.3429	0.6944	0.7353
	T3	0.3704	0.3810	0.4444	0.2059	0.6571
	T4	0.3333	0.4737	0.4375	0.7500	0.2258

[□]Data in bold means the similarity of the same treatment under two land use patterns. When in the bottom-left corner, it presented the similarity of different treatments in the wasted lands; while in the upper-right corner, the results showed the similarity of different treatments in the farmlands.

In terms of species number per unit area, plant species per unit area had a peak under T1, then decreased with the increasing density of yellow sweet clover in cultivated farmlands, but the fluctuation range was small; whilst in the wasted lands, the number of plant species per unit area decreased significantly with the increased plant density of yellow sweet clover (Fig. 1).

Changes of plant diversity

Analysis on diversity index showed that Simpson diversity index and Shannon-Wiener diversity index presented a similar trend: with increased plant density of yellow sweet clover, the diversity index increased to a highest level under T2, but then decreased with plant density in the farmlands; yet, diversity index presented a downward trend in the wasted lands, though a little rebound under T2 (Table 4).

Table 4
Diversity index under different plant densities

Treatments	Diversity index			
	Simpson		Shannon Wiener	
	Farmlands	Wasted lands	Farmlands	Wasted lands
CK	0.7522	0.6867	0.8159	0.7265
T1	0.7683	0.6377	0.8506	0.6068
T2	0.8528	0.6962	0.9794	0.6267
T3	0.7923	0.6498	0.8405	0.5447
T4	0.7615	0.5306	0.7611	0.4177

Physical and chemical properties of soil

Soil properties were quite distinctive in the two land types, showing different patterns in the presence and absence of yellow sweet clover. The results showed that soil N (total N, nitrate nitrogen, ammonium nitrogen) was increased due to the establishment of yellow sweet clover, especially in the layer of top soil (0–10 cm) in the farmlands, with an increasement of 133.3% for nitrate nitrogen. Yet, it presented a opposite trend in the wasted lands, which the content of nitrate nitrogen decreased by 23.4% with the establishment of yellow sweet clover in the top soil. Anyhow, the content of nitrate nitrogen was higher in the layer of 10–20 cm, compared with that with no yellow sweet clover. Due to soil C, the characteristics was quite similar to soil N. Anyhow, total C and soil organic was accumulated after the establishment of yellow sweet clover in the farmlands, though in a different rate at different layers; whilst they both decreased with the establishment of yellow sweet clover in the wasted lands. Soil P (total P and available P) of all layers decreased with the establishment of yellow sweet clover at some extend in both the farmlands and wasted lands. Interestingly, pH of top soil presented a opposite result which was increased in the farmlands, but decreased in the wasted lands. Anyhow, soil compaction was quite distinguishable, which was much higher in the wasted lands (45.3 ± 5.4) compared to that in the farmlands (29.3 ± 4.7) (Table 5).

Table 5
Physical and chemical properties of soil in different depths

Land type	Yellow sweet clover Distributed	Soil depths (cm)	Total N (%)	NO ₃ ⁻ (mg/kg)	NH ₄ ⁺ (mg/kg)	Total P(mg/kg)	Available P (mg/kg)	Total C (%)	Organic (g/kg)	pH	Soil compaction (kg/cm ²)
Farmlands	Yes	0–10	0.118	6.79	1.48	844	7.5	1.161	16.7	8.17	28.7 ± 5.4
		10–20	0.080	2.83	1.14	826	4.3	0.939	12.5	7.79	–
		20–30	0.086	2.74	1.41	857	4.4	0.904	12.9	7.95	–
		30–40	0.083	1.09	1.51	781	4.1	0.725	10.3	7.77	–
	No	0–10	0.088	2.91	1.19	900	7.5	0.905	12.8	7.97	29.9 ± 4.5
		10–20	0.085	2.90	1.13	882	6.5	0.927	12.7	7.96	–
		20–30	0.076	2.40	1.57	862	6.1	0.858	14.1	7.82	–
		30–40	0.065	0.70	1.36	817	4.7	0.713	11.1	7.77	–
Wasted lands	Yes	0–10	0.101	7.23	1.73	846	6.6	0.951	14.6	7.95	47.4 ± 6.3
		10–20	0.080	5.04	2.05	882	6.1	0.982	13.4	7.73	–
		20–30	0.066	2.14	1.39	809	5.4	0.763	10.1	7.83	–
		30–40	0.077	0.86	1.56	676	7.1	0.726	10.6	7.62	–
	No	0–10	0.106	8.92	1.05	908	8.3	1.152	16.9	8.28	43.6 ± 4.7
		10–20	0.086	2.09	0.75	840	7.6	0.901	12.1	8.16	–
		20–30	0.079	2.11	0.88	907	5.2	0.872	10.3	7.88	–
		30–40	0.085	0.88	0.99	760	4.6	0.673	8.8	7.90	–

Discussion and conclusions

Yellow sweet clover (*M. officinalis*) was extensively introduced as forage or N-fixing crop, which had caused a negative effect on local habitats and was considered as invasive plant out of its origin in various of systems in the world range (Chen et al. 2014; Evans and Kearney 2003; Lesica and Deluca 2000; Paiaro et al. 2007; Wolf et al. 2004; USDA-NRCS 2008). As biennial fast-growing plant, yellow sweet clover had its own advantages to establish, spread, and even invade into a new habitat or system. According to related researches, it's reported that this plant can produce over 100,000 seeds per plant (Klein 2011). Our result showed its seed production can reach up to maximum 29,582 seeds for a single individual in the experimental trials (17663 ± 4824 seeds/plant, on the average). Besides, seeds can remain viable in the soil for many years (Klein 2011; USDA-NRCS 2008). Thus, abundance of soil seed bank, together with long seed life, may generate a far-lasting effect on the persistence of yellow sweet clover. Due to our field observation for two more generations, yellow sweet clover plants are still found to distributed in and near the trial site though in a low occurrence. And, it was well tolerant to salinity and alkaline conditions, researches showed that yellow sweet clover had a strong tolerance to salinity stress and extensive adaptability to pH variations (Ghaderi-Far et al. 2010; Klein 2011). As observed in the study, yellow sweet clover grows quite well in alkaline soils with pH more than 8.2. Besides, sweet clovers are highly tolerant of cold temperatures, due to their wide distribution in the world range, e.g., northern China, Tatarstan, and Alaska in the USA (Conn et al. 2008; Chen et al. 2017; Dubrovnaya et al. 2021).

Once established, yellow sweet clover may over-compete native plants with high competitiveness and extensive adaptability. Related researches showed that yellow sweet clover strongly decreased the abundance of other species and species diversity, causing a negative effect on native species, which also showed favoring on exotic species (Van Riper and Larson 2009), this is similar to our results that yellow sweet clover facilitates fast-growing annual alien broad-leaf plants in the farmlands in the study. Except its competitive advantages, related researches revealed that yellow sweet clover can increase nitrogen in biomass (Dickson et al. 2010) and release inorganic N to the soil (Lesica and DeLuca 2000). According to our results, nitrate nitrogen increased a lot after the establishment of yellow sweet clover with high plant density in the farmlands, especially in the layer of top soil (0–10 cm). That indicated yellow sweet clover can release inorganic N to the soil in some extend. Due to soil characteristics in the study, our results also indicated that the establishment of yellow sweet clover changed soil C and soil P, especially in the top soil, which in return may facilitate the establishment of next generation. Besides, allelopathic effect was also an important part in the establishment of yellow sweet clover, related studies showed yellow sweet clover can effectively suppress

weeds (Blackshaw et al. 2001; Wu et al. 2010; Chen et al. 2022). However, this was less discussed in the study, and it needs further study of the allelopathic effect on itself and plant community.

Local environmental and biological factors affect the establishment of yellow sweet clover in a way that promotes or hinders; also, it presents an effect of interaction at the mean time. Related studies showed yellow sweet clover were prone to establish and flourish on human-disturbed lands, e.g., spreading in the interface in a farmland-grassland ecosystem (Chen et al. 2013), and distributed along the roadsides from roadbed to adjacent grassland (Chen et al. 2014) in the northern China. As in the North America, sweet clovers are also found in disturbed soils, such as farmlands (Duke 1981), Rocky Mountain National Park (Wolf and Rohrs 2001), and floodplains (Conn et al. 2011). Human-disturbed lands changed resource utilization efficiency, e.g. soil texture, soil N, soil P, soil C, pH, and soil characteristics (Wolf and Rohrs 2001; Wolf et al. 2004), which were favoured by alien plants. As showed in the study, it was easy for yellow sweet clover to establish themselves in the cultivated farmlands, compared to wasted land. On the contrary, it also indicated local vegetation hindered the establishment and growth of yellow sweet clover by crowding native plants, like *Salsola collina*, and fast-growing creeping plant *Humulus scandens*. Anyhow, land use pattern and soil texture played a much important role on the process of establishment for an alien species. Due to different land types, the similarity of the two types were acceptable, but after yellow sweet clover established, it presented a similarity of the plant communities in low level. To our field survey, the establishment of yellow sweet clover was related to opportunistic chance in the wasted land, compared to that in the farmlands. However, the effect of land type may trigger the main difference from the beginning.

Yellow sweet clover was not always the successful invader, but acting as a weak competitor and had no consistent effect on other species, e.g., in western wheatgrass prairie (Van Riper and Larson 2009). According to our results, yellow sweet clover was found relatively difficult to establish in the wasted land crowded with weeds. Due to field survey, we also found less individuals of second generation established in the farmlands, much less in the wasted lands. Further, individuals of third generation were randomly found in the experiment area after two more years' desolation. Yet, seed germination of yellow sweet clover was sensitive to drought stress (Ghaderi-Far et al. 2010), though it's drought tolerant plant. Our field survey showed yellow sweet clover was not very resistant to flooding, most of the plants would die after a period of summer flooding more than two weeks in the region of northern China, though Baldrige and Lohmiller (1990) reported that sweet clover seedlings can tolerate 10 to 14 days of early spring flooding. Thus, final invasion may be determined by comprehensive factors, both environmental and biological factors.

Declarations

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Author contributions

KHM made the field survey and wrote the manuscript; TWJ and PZ performed the experiment and made the field survey; ZGF tested the soil samples; CC designed the experiment and revised the manuscript.

Declaration of competing interest

The authors reported no declarations of interest.

Data availability

All data included in this study are available upon request by contact with the corresponding author.

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Figures

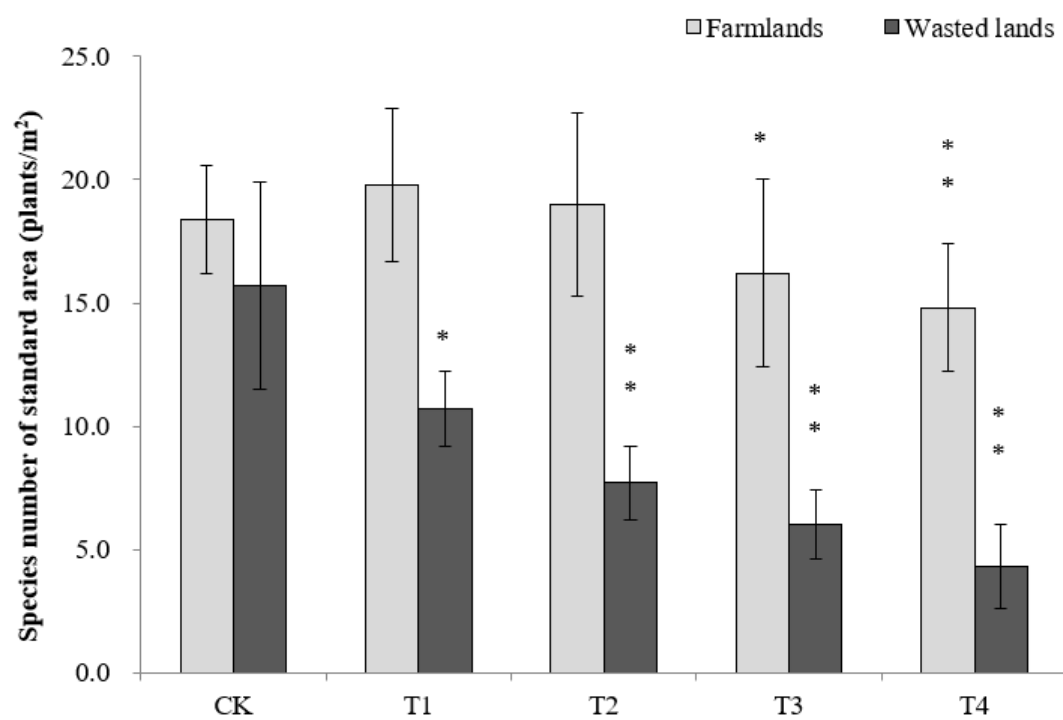


Figure 1

Plant species per unit area under different plant densities

* Means significant difference $P < 0.05$, while ** means very significant difference $P < 0.01$.