Local Grasses for the Control of the Invasive Vine *Mikania micrantha*

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Abstract

Aims: *Mikania micrantha* (Asteraceae) is an invasive vine found in tropical and southern subtropical Asian and the Pacific Islands. The current methods used to control this vine are inadequate, which warrants the development of ecologically sustainable methods. Therefore, we investigated the ability of four grass species to prevent the invasion of *M. micrantha*, with an ultimate goal of developing ecologically sustainable control methods for widespread application.

Methods: The clumps of native grass species from China (*Panicum incomtum*, *Pennisetum purpureum*, *Saccharum arundinaceum* and *Microstegium vagans*) were established. We sowed *M. micrantha* seeds and transplanted the seedlings into the grass clumps to examine whether the clumps could eliminate the new *M. micrantha* plants. In addition, we transplanted *M. micrantha* into existing grass clumps to examine whether the grass clumps could prevent the re-invasion of *M. micrantha*. Furthermore, we grew *M. micrantha* with *P. incomtum* and *P. purpureum* in the field to examine whether the grasses could outcompete *M. micrantha*.

Important Findings: *M. micrantha* seeds had difficulty germinating in the grass clumps, and all seedlings died within 3 months. It was difficult for the vine to survive in the existing grass clumps. Our field experiment showed that the coverage of *M. micrantha* was significantly lower than that of the grass species in the first year, and the vine was outcompeted after 2

years. To our knowledge, this study is the first to reveal that tall grasses, particularly *P. incomtum* and *P. purpureum*, have potential to serve as bio-control agents for *M. micrantha*.

Keywords: Local grass; *P. incomtum*; *P. purpureum*; Biological control; *Mikania micrantha*; Invasive

Graphical Abstract

1. Introduction

Information pertaining to the significance of alien plant species and the dangers of allowing the uncontrolled spread of invasive species has been increasing through both scientific and popular press articles. Various possible impacts of plant invasion have been reported, including alterations in biogeochemical cycling; disruption of food webs; alterations in plant–plant, plant–insect and plant–microbe interactions as well as reductions in biodiversity, which lead to detrimental effects on native plant communities and ecosystem processes (Hobbs *et al.*, 2009).

Mikania micrantha Kunth—a vine species of Asteraceae—is one of the worst invasive plant species of the world (Cronk *et al.*, 1995, Huang *et al.*, 2016, Lowe *et al.*, 2000), is becoming problematic in tropical and southern subtropical Asia, the Pacific Islands and northern Australia (Waterhouse, 2003, Zan and Li, 2010). In Southern China, the reports of damage due to this species can be traced back to the 1980s (Zan and Li, 2010, Zhang *et al.*, 2003). The vine blankets open lands and covers orchards, plantations and secondary forests (Zan and Li, 2010), thereby jeopardising the photosynthesis of underlying plants and eventually killing them, which ultimately transforms the site into an *M. micrantha* monoculture.

Typical methods for controlling invasive plants have been widely reported and practiced; these methods include mechanical (Britton *et al.*, 2000, Swamy *et al.*, 2010, Timmins, 2004), chemical (Paynter *et al.*, 2004, Shen *et al.*, 2013) and biological (Barreto *et al.*, 1995, Yu *et al.*, 2011, Mack *et al.*, 2000, Messing *et al.*, 2006) controls. Unfortunately, either mechanical or chemical measure may exaggerate invasions for nonspecific and unsustainable approaches (Mason *et al.*, 2007, Milligan *et al.*, 2003, Rob *et al.*, 1997).

Traditional biological control measures via the introduction of pathogens, parasites and predators against invaders probably pose risks to ecosystem integrity (Ding *et al.*, 2007, Jia *et /al.*, 2011). Consequently, more efficient and ecologically sustainable control methods are urgently needed to supersede the conventional approach. An emerging potential alternative method to suppress exotic plants relies upon the growth characteristics of one or more local plants, thereby simultaneously reducing the damage due to invasive species and improving the health of the local natural ecosystem by mitigating the spread of invasive plants (Gosper *et al.*, 2010, Li *et al.*, 2015, Lugo, 1997, Shen *et al.*, 2015, Yusong *et al.*, 2014). For example, Perry *et al.* (2003) reported that native *Carex hystericina* might suppress invasive grass *Phalaris arundinacea* under certain conditions of soil nitrogen content. Prider *et al.* (2009) suggested that the native parasitic plant *Cassytha pubescens* serves a-s the effective regulator of an invasive population by exerting a greater impact on the invasive plant than on the co-occurring native plant. Firn *et al.* (2010) reported that functionally similar Australian native grasses reduced the competitiveness of an African invasive grass. Corbin *et al.* (2004) found that in a California coastal grassland, established native perennial species were superior to Eurasian invasive annual grasses. These reports demonstrated the potential of certain plants to serve as biological control agents. In our previous studies, we have successfully controlled *M. micrantha* by restructuring the plant community after infestation (Zhou and Li, 2012). We found that *Heteropanax fragrans* and *Macaranga tanarius* were the two most effective species for creating an unsuitable habitat for *M. micrantha* and eventually outcompeted the vine (Zan and Li, 2010).

Our pilot studies showed some positive results in terms of the control of this invasive vine. Under the competition of *P. purpureum*, the *M. micrantha* biomass inside the grass clumps drastically -decreased within 2 m from the edge*; this reduction was less than that of an adjacent *M. micrantha* plot (Zhou and Li, 2012). The results implied that *M. micrantha* was unable to grow effectively with *P. purpureum*. Moreover, we found that all *M. micrantha* stems in the *P. purpureum* clumps originated from outside, indicating that no seedling of *M. micrantha* could establish in the clump. The results suggested that tall grass can be used as a bio-control agent.

On the basis of this finding, in the pursuit of ecologically sustainable methods for preventing the invasion of *M. micrantha*, we questioned what other tall grass species could control *M. micrantha* and with what effects. We selected four typical tall grass speci-es in tropical and southern subtropical Asia. Field experiments were performed to address the following questions:

(1) Can the grass species suppress *M. micrantha* seed germination and growth?

(2) Can they deter *M. micrantha* stems from re-entering the area?

(3) Can their presence render *M. micrantha* an inferior competitor?

2. Materials and methods

2.1 Grasses

Four common perennial grasses with tall stalks and large biomass were used in this study. These included three nati+ve species (*Panicum incomtum*, *Microstegium vagans* and *Saccharum arundinaceum*) and one introduced and widely cultivated forage species (*Pennisetum purpureum*) in South China.

Two-node segments of the four species were collected from a field at Huijiang Village, Guangzhou City, China (22°99′N, 113°28′E). The segments were then planted in sand beds and watered twice daily until they were transplanted into the study area.

2.2 Construction of grass clumps

Grass clumps were established to simulate natural grass clumps. An experimental plot in Shunde District, Foshan City, China (22°79′N, 113°25′E), was cleared by pulling out all weeds and spraying the plot with a short-lived herbicide (glyphosate) to eliminate any remaining weed propagules. The field was divided into 12 subplots of 3 × 3 m, each with 1-m spacing between any two adjacent subplots.

Each subplot was evenly planted with the segments of a single grass species—*P. incomtum* (200 segments), *M. vagans* (200 segments), *S. arundinaceum* (100 segments) or *P. purpureum* (100 segments)—according to their individual plant sizes and natural growth density. During the first 2 weeks, any dead segments were promptly replaced and watering was performed twice daily to ensure grass establishment. After 2 weeks, watering was performed as needed. Finally, 13-month-old grass clumps were established to further control the investigation. Three replicates were used for each grass species.

2.3 Cultivation of M. micrantha

Seeds and two-node stem segments of *M. micrantha* were used in this study. The seeds were collected from Qi'ao Island, Zhuhai City, China (22°40′N, 113°64′E). The seeds were air dried and maintained in sealed bags at room temperature. The stem segments were collected from a field at Huijiang Village. They were planted in nursery sand beds and watered twice daily until they were transplanted into the study area.

2.4 Experiment 1

Juvenile specimens of *M. micrantha* (1- and 3-month-old) were prepared by cultivating a two-true-leaf germinated seedling in a pot (diameter = 10 cm, height = 10 cm) in the greenhouse of Sun Yat-Sen University (23°12′N, 113°25′E), Guangzhou, China. After 1 month, 20 juvenile specimens and their soils were carefully removed from each pot and were transplanted into the bare ground among the grass stalks in each subplot. There were a total of four treatments (four different grass species) and three replicates for each treatment. In addition, 20 1-month-old seedlings were transplanted into an open area next to the three grass subplots and were used as controls (three replicates). The survivors among the transplanted seedlings were counted on a weekly or monthly basis. Because 3-month-old seedlings are larger than 1-month-old seedlings, to simulate the real field situation, 15 3-month-old seedlings were transplanted into each grass clump on the following day as described above and another 15 3-month-old seedlings were transplanted into open areas next to the grass clumps as controls. The surviving 3-month-old seedlings were counted on a weekly or monthly basis. All experiments were performed randomly and in triplicate.

We first sought to investigate whether grass clumps could effectively inhibit germination and/or destroy germinated seedlings, thereby disrupting seed dispersion and thus the invasion of *M. micrantha*. This experiment was designed to compare the ability of the clumps of candidate grass species to deter the wind-dispersed seed germination *M. micrantha* and its subsequent seedling growth.

After 13 months from the establishment of the grass clumps, at least 40-cm away from the edge of each subplot, five PVC pipes (diameter = 15 cm, height = 20 cm) were placed on the ground to space the grass stalks (Figure 1). Another five pipes were placed on the ground nearby as controls. The pipes were pressed slightly into the soil to ensure no gaps were present between the pipe and soil. A total of 50 seeds of *M. micrantha* were spread inside the pipe to simulate wind dispersion. Watering was performed every other day.

The ability of an established grass clump to suppress *M. micrantha* was evaluated based on the number of living *M. micrantha* seedlings that had germinated from the seeds. Seedling enumeration was performed on days 0, 7, 14, 28, 56 and 91.

2.5 Experiment 2

We investigated whether the grass clumps could prevent the creeping vine of *M. micrantha* from entering the grass clump and from encroaching upon the grass from the outside. In total, 15 3-month-old *M. micrantha* seedlings were transplanted into the ground at a distance of 50 cm from the edge of a grass clump (Figure 1). The growing stems of *M. micrantha* were manually guided to creep toward the grass clump. Once the tip of a creeping stem entered the grass clump, the stem was left to grow without any human intervention. The coverage and height of *M. micrantha* and the grass were measured and used as indicators to evaluate the control effect.

Natural grass clumps that were growing nearby and were still free from the invasion of *M. micrantha* were selected before this experiment. Two natural clumps of *P. incomtum* (areas = 30 m^2 each, height = 1.5 m, coverage = 98%) in Panyu District, Guangzhou City, China (22°95′N, 113°26′E), and a natural clump of *P. purpureum* (areas = 40 m^2, height = 1.5 m, coverage = 98%) in Shunde District were selected. The sites were then protected from non-experimental human intervention.

Half of the area of each clump was mown at a height of 15 cm above the ground. All nearby creeping stems of *M. Micrantha* were allowed to grow without any human intervention. The coverage and height of *M. micrantha* and the grass in the grass clumps were measured afterwards.

2.6 Experiment 3

Finally, we simulated the scenario of planting grass segments as a measure to control invasive plants in the field. The field plot experiment was performed at Huijiang Village. The experimental site was sprayed with glyphosate to kill existing weeds. The soil was turned and irrigated to induce the germination of the remaining weed propagules. All new emergent weeds were pulled out every week until use. The site was divided into 25 subplots of 1.5 × 1.5 m, with 1-m spacing between any two adjacent subplots (Figure 1). The field was protected from non-experimental human disturbances.

The normal sizes of *P. incomtum* and *P. purpureum* range between 60 and 100 cm; however, *M. micrantha* has a climbing or creeping growth. Pure grass or pure *M. micrantha* subplots were developed by planting 40 2-month-old seedlings of *M. micrantha* and 40 20-day-old seedlings of *P. incomtum* or *P. purpureum*. Mixed-culture subplots were developed by planting 20 seedlings of *M. micrantha* and 20 seedlings of either *P. incomtum* or *P. purpureum*. The cover and height of *M. micrantha*, *P. incomtum* and *P. purpureum* were measured after 8 months. All of the above treatments comprised five biological replicates.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the effects of different grass species on the number of *M. micrantha* seedlings in Experiment 1. To examine whether *M. micrantha* could enter existing grass clumps, we compared the cover and height of *M. micrantha* and the four grass species in their clumps. One-way ANOVA was used to explore the differences in the cover and height of *M. micrantha* and the four grass species. Tukey's multiple-range test was performed to determine whether significant differences occurred between the treatments based on the means at a significance level of 0.05 in Experiment 2. To evaluate the results of competition between *M. micrantha* and *P. incomtum* or *P. purpureum* in Experiment 3, we compared the cover and height of *M. micrantha* and these two grass species between the monoculture and mixed-culture plots using one-way ANOVA and Tukey's multiple-range tests.

All data analyses were performed using SPSS 19.0, and the figures were prepared using SigmaPlot 12.0.

3. Results

3.1 *Effects of the established grass clumps on M. micrantha seed germination and seedling survival*

As shown in Figure 2, the highest number (percentage) of seedlings exhibiting germination was 14 (or 28%) in the *S. arundinaceum* clumps, whereas the lowest was 5 (or 10%) in the *P. incomtum* clumps. In the clumps of the other two grasses, *P. purpureum* and *M. vagans*, a moderate proportion of 12%–16% at peak germination was observed. Only *P.*

incomtum and *P. purpureum* significantly decreased the number of *M. micrantha* seedlings (Table 1). Moreover, all germinated seedlings died within 3 months (Figure 2).

All 1- and 3-month-old seedlings transplanted into the pre-existing grass clumps grew poorly; none survived for >3 months. However, in the open area, the seedlings grew normally and reached a height of 16.3 m and 12.8 m for the 1- and 3-month-old seedlings that were transplanted into the grass clumps, respectively (Figure S1).

3.2 Effect of the established grass clumps on the creeping stems of M. micrantha

P. purpureum clumps reduced the *M. micrantha* coverage to the highest extent; the vine coverage was only approximately 1% with a height of 1.12 m, when the grass reached 100% coverage. In both *P. incomtum* and *S. arundinaceum* clumps*, M. micrantha* seedlings had the coverage of approximately 2%, although the differed between the grass clumps: 0.79 m and 1.43 m, respectively (Table 2). Altogether, the cover and height of the *M. micrantha* were all far lower than those of the four grasses in the established grass clumps.

In the unmown natural *P. incomtum* and *P. purpureum* clumps, the coverage and height of *M. micrantha* were far lower than those in the four grasses; in the mown *P. incomtum* and *P. purpureum* clumps, the *M. micrantha* coverage was significantly greater than that in the unmown grass clumps (Figure S2).However, *M. micrantha* did not cover the re-sprouted *P. incomtum* and *P. purpureum*, and the height of the vine was lower than that of the re-sprouted *P. incomtum* and *P. purpureum* (data not shown). There were practically no differences between the mown and unmown grass clumps once the clumps were expanded to an adequate scale. In both the mown and unmown grass clumps, *M. micrantha* was noticeably distributed only at the edges of the grass clumps (data not shown).

3.3 Competition between grass and M. micrantha in the field plot

We found that *P. incomtum* and *P. purpureum* significantly retarded the growth of *M. micrantha* based on approximately 75% and 80% decreases in its coverage, respectively (Figures 3A and S3, Table 3). Consequently, *M. micrantha* was clearly outcompeted by these two grasses as these thrived in either their monoculture or mixed-culture subplots, even in adjacent spaces. Furthermore, *M. micrantha* disappeared from all mixed-culture subplots that were occupied by these two grasses (Figures 3A and S3, Table 3). The increase in the height of *M. micrantha* in mixed-culture subplots resulted from its creeping along the grasses (Figure 3B, Table 3).

4. Discussion

As mentioned previously, our pilot study showed that under the competition of *P. purpureum*, the biomass of *M. micrantha* inside the clumps drastically decreased within 2 m from the edge and was much smaller than that of an adjacent *M. micrantha* plot (Zhou and Li, 2012). In the present study, we further explored the possibility of using local native grasses to control *M. micrantha* and revealed that the biomass as well as seed and seedling traits of *M. micrantha* were lower when grown in association with *P. incomtum* or *P. purpureum*.

An ideal bio-control agent specialises in suppressing the reproductive potential of an invasive species, including flowering characteristics, seedling dispersal and seed germination parameters, when these characteristics are associated with invasiveness (Lodge, 1993, Reichard *et al.*, 1997, Rejmánek, 1996, Van Kleunen *et al.*, 2010, Wilke and Irwin, 2010). The seedling is the most vulnerable stage in the life cycle of *M. micrantha*, and seedlings suffer a high level of mortality under natural conditions (Weiyin *et al.*, 2002). Although the mechanism requires further investigation, some phenomena highlight the traits of grasses that are critical in combating *M. micrantha.* These phenomena are related to the traits of high-density growth and propagation by tillers. The living and dead material of the grasses accumulate on the ground, thereby preventing the adventitious roots of *M. micrantha* from reaching the soil. Dense grass intercepts the light and nutrient availability and influences the reproductive output of *M. micrantha*, resulting in fewer flowers, a lower seed-setting percentage and shorter flowering duration. Therefore, one major factor reducing the reproductive output in *M. micrantha* observed in our study was the negative impact of local tall grasses on sunlight and nutrient availability; this is consistent with a commonly observed association between environmental conditions and growth rate (Wilke and Irwin, 2010).

Apart from this, soil nutrient and allelochemicals are critical for native plants in combating invasiveness; for example, allelochemical secretion, positive feedback between plant and soil and rapid absorption of soil nutrients influence plant growth (Pei *et al.*, 2019, Shameer *et al.*, 2021). The combination of the abovementioned traits ensured that the local grasses used in this study could gradually expand. *M. micrantha* was unable to encroach upon the grasses, and it was continuously pushed out to the perimeter of the grass clumps. Eventually, the vine will become an insignificant weed and may even be completely eradicated from this area.

The bio-control agents must ensure the eradication of the invasive plants and re-establishment of the native plant community (Díaz *et al.*, 2003, Flory *et al.*, 2009, Hulme, 2006); the re-established community then should resist the re-invasion of the invasive plants (Myers *et al.*, 2000).The results of the present study clearly indicate that *M. micrantha* is inferior to *P. incomtum* and *P. purpureum,* these two grass species thus present an effective phytocontrol strategy for *M. micrantha*; this finding is consistent with that of Corbin and D'Antonio (2004) who employed native perennial species to prevent the invasion of Eurasian invasive annual grasses in a coastal grassland in California. However, the clumps of these two grasses are difficult to expand. The results of our field plot experiment indicated that *P. incomtum* and *P. purpureum* primarily propagate through vegetative reproduction. Seabloom *et al.* (2003) has suggested the current rarity of native perennials is due to a recruitment limitation rather than competitive resource. This finding explained why the capacity of these two grasses to control *M. micrantha* was not previously detected in the field. It is necessary to propagate these two species by vast-area planting to use them as bio-control agents. To obtain the best result, some measurements might be beneficial, such as spraying herbicide to eliminate the existing *M. micrantha* propagules before planting grasses to ensure that the grasses have more time to grow.

5. Conclusion

Our results revealed that two, *P. incomtum* and *P. purpureum*, of the four local tall grasses used in the study significantly suppressed the growth of *M. micrantha* in terms of both seed germination and subsequent seedling growth retardation through the shading due to the high cover of these grasses. In addition, *P. incomtum* and *P. purpureum* are high value forage grasses; thus, we recommend planting these local grasses in areas infested by *M. micrantha*. The potential of utilising local grasses such as *P. incomtum* and *P. purpureum* to compete with an invasive plant may be applicable to other agronomic settings employing other management techniques (e.g., chemical, mechanical or classical biological controls) that are unreliable or are associated with environmental concerns. These findings indicate that the application of *P. incomtum* or *P. purpureum* in an area where the impact of *M. micrantha* is most severe, such as Guangdong province, is a robust and ecologically sound control method.

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Tables

Table 1. Results of the ANOVA test performed to examine the effects of different grass species on the number of *M. micrantha* seedlings.

Response variable	Grass species	*F* value	*P* value
Number of *M. micrantha* seeding (n)	*P. incomtum*	2.906	**0.043**
	P. purpureum	5.454	**0.002**
	M. vagans	0.761	0.521
	S. arundinaceum	1.235	0.306

Note: Bold numbers represent significant differences.

Table 2. Height and coverage of the creeping stems of *M. micrantha* in the pre-existing grass clumps

Site	Height (m)		Coverage (%)	
	M. micrantha	grass	*M. micrantha*	grass
P. incomtum clumps	0.79a	1.45b	2a	100b
P. purpureum clumps	1.12a	3.14b	1a	100b
M. vagans clumps	0.79a	1.44b	7a	100b
S. arundinaceum clumps	1.43a	3.52b	2a	100b

Note: Different letters indicate significant differences in the height or coverage of *M. micrantha* and grass within clumps.

Table 3. Results of the *t*- and ANOVA tests performed to examine the effects of different culture patterns on the coverage and height of *P. incomtum, P. purpureum and M. micrantha*.

Response variable	Culture pattern	
	t/F value	*P* value
Coverage (%) of *P. incomtum*	2.637	0.058
Coverage (%) of *P. purpureum*	1.753	0.154
Coverage (%) of *M. micrantha*	77.171	**<0.01**
Height (m) of *P. incomtum*	1.696	0.128
Height (m) of *P. purpureum*	−2.355	0.062
Height (m) of *M. micrantha*	28.821	**<0.01**

Note: Bold numbers represent significant differences.

Figure captions

Figure 1. Experiment 1 performed in the established grass clumps to evaluate *M. micrantha* seed germination and seedling survival.

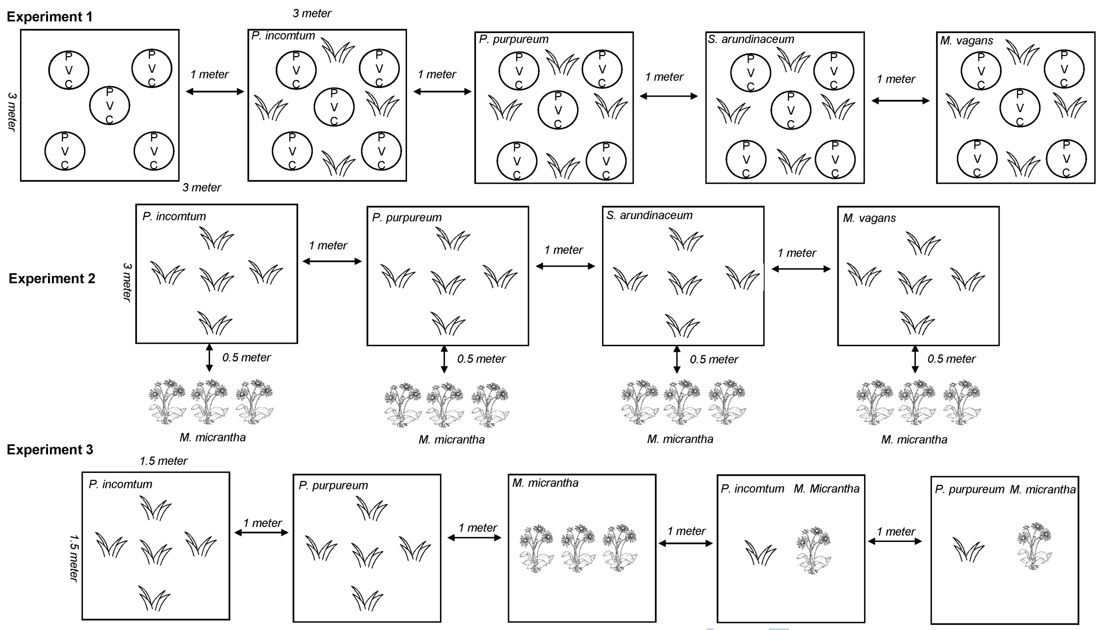
Experiment 2 performed in the established grass clumps to evaluate the survival of 1- and 3-month-old juvenile specimens of *M. micrantha.*

Experiment 3 performed in the established grass clumps to evaluate the creeping stems of *M. micrantha.*

Figure 2. Seedling survival of *M. micrantha* in the established grass clumps.

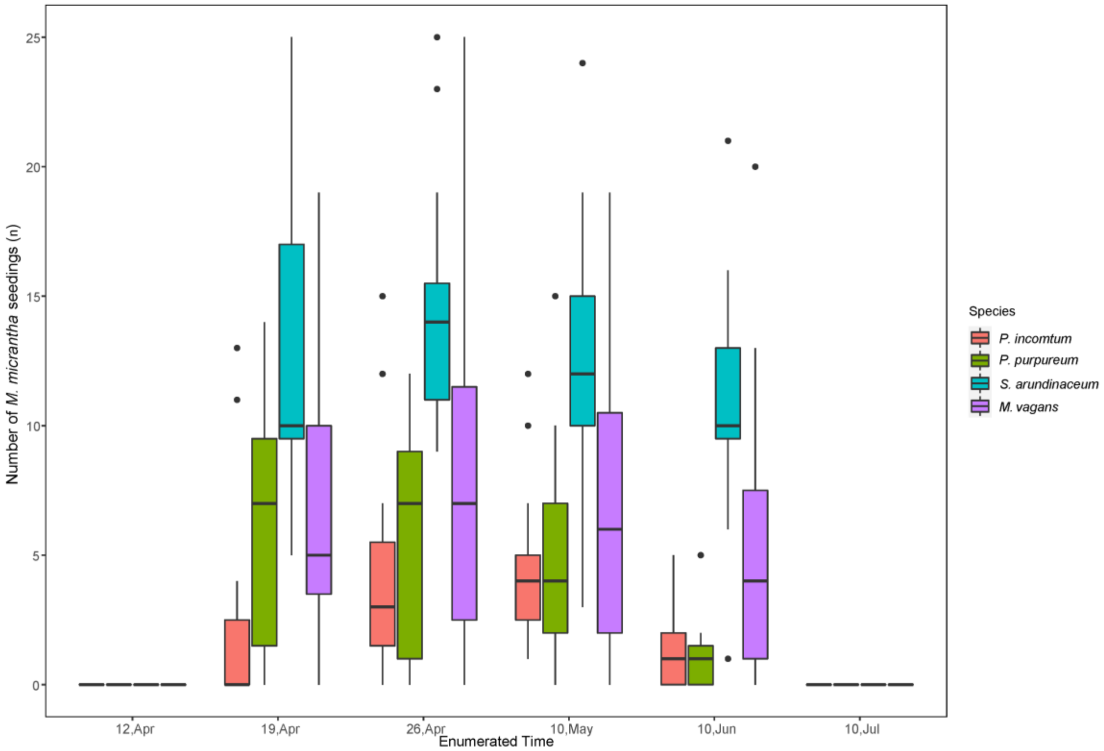
Figure 3. Coverage (A) and height (B) of *P. incomtum*, *P. purpureum* and *M. micrantha* under two competitive conditions. Different letters represent a significant difference between grass and *M. micrantha* in the mixed-culture subplot. Different letters above the columns indicate a significant difference within the same species under the different competitive conditions.

Figure 1



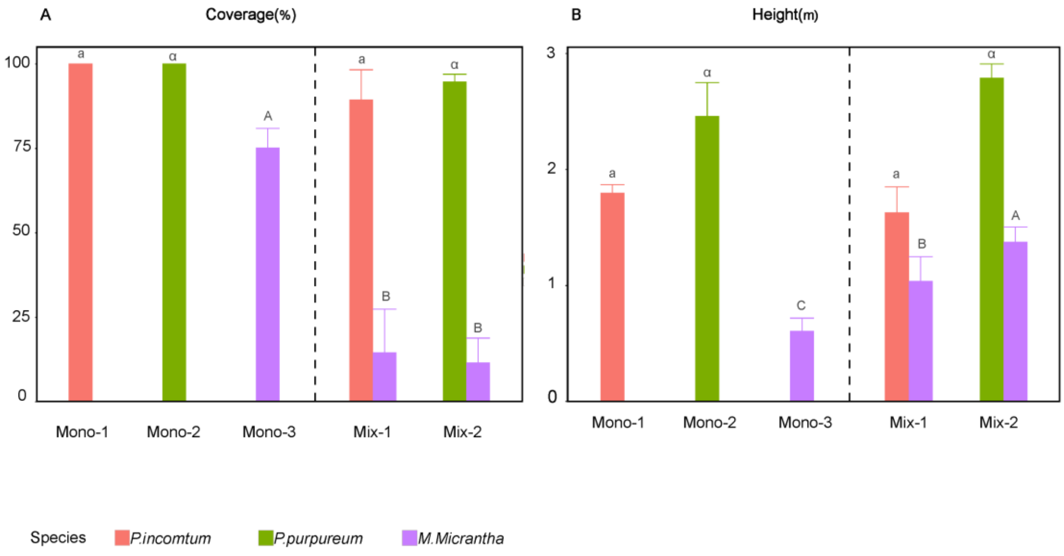
Experiment 1
3 meter
3 meter
3 meter
P. incomtum
P. purpureum
S. arundinaceum
M. vagans
1 meter
1 meter
1 meter
1 meter
PVC
Experiment 2
3 meter
P. incomtum
P. purpureum
S. arundinaceum
M. vagans
1 meter
1 meter
1 meter
0.5 meter
0.5 meter
0.5 meter
0.5 meter
M. micrantha
M. micrantha
M. micrantha
M. micrantha
Experiment 3
1.5 meter
1.5 meter
P. incomtum
P. purpureum
M. micrantha
P. incomtum M. Micrantha
P. purpureum M. micrantha
1 meter
1 meter
1 meter
1 meter

Figure 2



25
20
15
10
5
0
Number of M. micrantha seedings (n)
12,Apr
19,Apr
26,Apr
10,May
10,Jun
10,Jul
Enumerated Time
Species
P. incomtum
P. purpureum
S. arundinaceum
M. vagans

Figure 3


A
Coverage(%)
B
Height(m)
100
75
50
25
0
3
2
1
0
Mono-1
Mono-2
Mono-3
Mix-1
Mix-2
Species
P.incomtum
P.purpureum
M.Micrantha