

Pruning from spheroidal to cubic canopy induced aphid outbreak by altering the plant performance in Box tree

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Research article

Keywords: aphid, herbivore, plant-animal interactions

Posted Date: July 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-45207/v1>

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Abstract

Backgrounds: Plant-animal interactions comprise the fundamental relationships of ecological research, and are sensitive to environmental change. However, The effects of pruning on animal-plant interactions have rarely been studied.

Methods: We conducted field experiments to examine the impact of artificially-pruned shapes (e.g. cubic and spheroidal canopy) on the performance of the Box tree and the resulting aphid abundance at three sites; on a university campus, at a road green belt, and in a residential area. The differences of aphid abundance and plant morphology were determined with ANOVAs and paired-sample tests. Relationships between the investigated parameters were detected with simple regression and structural equation model

Result: Abundance was higher in plants with a cubic canopy than with a spheroidal canopy. Plants with a cubic canopy had lower leaf dry mass content and inflorescence numbers, but greater fresh twig length than the plants with a spheroidal canopy. The aphid abundance was negatively correlated with the leaf dry mass content and inflorescence numbers, and positively correlated with the fresh twig length.

Conclusion: Our findings have proven that pruning shape can significantly affect the abundance of herbivores on the pruned plants. The results can provide data support for human actives can alter plant performance, and thereby to change insect preference.

Introduction

Plant-animal interactions comprise the fundamental relationships of ecological research (Blue et al. 2011, Li et al. 2011, Liu et al. 2011, Martin and Maron 2012, Fang and Huang 2014, Mu et al. 2014). Changes in the relationships between animals and plants can affect plant growth and reproduction (Liu et al. 2011, Fang and Huang 2014, Mu et al. 2014, Shao et al. 2017), aboveground biomass (Blue et al. 2011, Li et al. 2011), community composition (Strauss and Irwin 2004, Nogales et al. 2015), nutrient cycling and energy flux (Heimann and Reichstein 2008, Defossez et al. 2011). Most previous studies have concentrated on effects of environmental change on plant-animal interactions (Morris et al. 2008, Malhi et al. 2009, Li et al. 2011, Liu et al. 2011, de Sassi et al. 2012, Hoover et al. 2012, Martin and Maron 2012) and have found that these relationships could be affected by rising temperatures (Li et al. 2011, Liu et al. 2011), changes in precipitation regimes (Morris et al. 2008, Malhi et al. 2009) and differential nitrogen deposition (de Sassi et al. 2012, Hoover et al. 2012). However, the effects of human activity also affect plant-animal interactions (Romero et al. 2006, Rosin and Poulsen 2016), especially in the case of plant and pollinator mutualisms (Aizen and Feinsinger 1994, Kearns et al. 1998).

Pruning is one widespread human activity that directly affects a plant's ability to perform vital life processes, such as photosynthesis (Agrawal and Spiller 2004, Grechi et al. 2008). Plant traits, including nutrient content and physical or chemical defenses, may also be altered by pruning (Martinez and Wool 2002, Agrawal and Spiller 2004, Kumar et al. 2010, Saifuddin et al. 2010, Maltoni et al. 2012, Albarracín et al. 2017) and could subsequently affect a plant's accessibility or attractiveness to herbivores (Nykänen

and Koricheva 2004, Wen et al. 2006). However, the effects of pruning on the preference of herbivores are inconsistent in previous studies, and thus unknown. On one hand, light pruning may remove unhealthy tissue, and reduce pest damage. This is known as the “escape strategy” (Rattan 1992, Sivapalan 1999, Maltoni et al. 2012). On the other hand, pruning produces vigorous shoots and leaves, which could attract more herbivores. This is called the plant vigor hypothesis (Price 1991, Grechi et al. 2008). Furthermore, a plant undergoing a heavy pruning treatment produces more tender twigs and leaves, which leads to a higher water content in the plant than under undisturbed conditions. The tender twigs could attract fewer pests, according to the plant-stress hypothesis (White 1969), or it could attract more sap-feeders, according to the pulsed stress hypothesis (Huberty and Denno 2004). Actually, effects of defoliation, which is similar as pruning, have been widely studied. The results show that defoliation can affect the plant architecture, nutrient content, and defensive chemicals, and thus affect the community dynamics of herbivores (Mattson and Palmer, 1987; Leather et al. 1988; Leather et al., 1993; Leather et al., 1995; Riipi et al., 2005; Springer-Verlag et al., 2006). However, only few studies have tested these theories and investigated the difference in predator abundance between lightly- and heavily-pruned plants of the same species, or the influence of pruning on plant structure (e.g., leaf mass, twig length etc.) within a species. We will use these metrics to assess the impact of pruning on herbivorous insect abundance and to increase our understanding of the response mechanisms of trophic relationships to human activity. Considering both that heavy pruning induces more compensatory growth than does light pruning and that plant structure and insect attraction are tightly correlated, we hypothesized that heavy pruning would produce more vigorous shoot and leaf growth and thereby attract more sap-feeders.

Our study system consists of the aphid species (*Aphis gossypii* Glover) and one of its host species, the Box tree (*Buxus megistophylla* Levl.). The Box tree is a popular evergreen garden plant and is cultivated in most provinces of China. The Box tree is often pruned to one of two canopy shapes: a cubic canopy, like a hedge with heavy pruning and a spheroidal canopy, like a ball with light pruning. We investigated the abundance of aphids, and we measured plant structure with the leaf dry mass content, the length of the annual shoots and the inflorescence number of the differently-shaped plants. Our objectives were 1) to test whether the different shapes of the plants would affect the plant-animal interactions, and 2) to explore the response of animals to different branch pruning shapes in plants they often eat.

Materials And Methods

Study site

In 2015, three adjacent 1 km × 1 km sites were selected for this study, a university campus (Jinming campus district of Henan University, 34°49' 17"N, 114° 17' 57"E, 73 m), a road green belt (Dongjing Road, 34°49'25"N, 114°18'19"E, 73 m) and a residential area (Longxiang-Shangrila district, 34°49'33"N, 114°18'19"E, 73 m) in the northwest district of Kaifeng city in Henan Province, China. In the study area, the mean annual precipitation is 626 mm and the mean annual temperature is 14 °C (Zhang et al. 2009). The soil is sandy loam, according to the FAO classification system (FAO 1990). The vegetation on the three sites is of a manmade composition, with the Box tree (*Buxus megistophylla* Levl.) as the dominant

species. The plots were selected followed with located observation method (Miao et al. 2018). In each study site, 10 pairs of plots were randomly selected, where each pair contained *B. megistophylla* Levl. having both cubic canopy (CU) and spheroidal canopy (SP). A consistent frequency and timing of disturbances, which included pruning, irrigation, and application of pesticide, were maintained for each paired-plot within a single site. Each pair of plots was set apart from other pairs at a distance of greater than 10 m.

Study species

Buxus megistophylla Levl. was chosen as the plant study subject. *B. megistophylla* Levl. is a common evergreen garden shrub in China, often subjected to frequent pruning to maintain its manicured shape. The shrubs in this study were planted in years 2006, 2010, and 2013 at the university campus, the road green belt and the residential area, respectively. The shrubs often produce a large number of fresh twigs in the spring and were pruned irregularly from late spring to late summer at the study sites. Shrubs were pruned to have either a cubic or a spheroidal canopy at all three study sites (Fig. 1). Additionally, the shrubs were watered semi-monthly and sprayed irregularly with omethoate pesticide from late May to late September of each growing year. The distance between adjacent cubic and spheroidal shrubs was 3 m, 1 m, and 5 m at the university campus, road green belt and residential area sites, respectively. Large numbers of aphids were found on the shrubs with a cubic canopy in the year 2015, thus we selected this species.

Aphids are the most common pest of *B. megistophylla* Levl. They first appeared on the shrub each year in late April at our study sites. After capturing a sample of aphids, fixing them with 75% alcohol and observing them under a stereoscopic microscope with an eyepiece micrometer, we identified them as cotton aphids (*Aphis gossypii* Glover), according to the telltale length of their cornicles relative to their caudas, the shapes of their caudas, and their body color, as described in the Fauna of Hebei, China, Aphidinea (Qiao et al. 2009). The cotton aphid is a holocyclic species (Qiao et al. 2009). They often lay their eggs near the leaf bud of their overwintering host, which may be a grape vine (*Vitis vinifera* L.), pomegranate plant (*Punica granatum* L.), peach tree (*Amygdalus persica* L.) or Box tree (*B. megistophylla* Levl.), and they remain dormant during the cold winter until they hatch in early spring. They reproduce several generations and then migrate to cotton plants to breed again. Cotton aphids suck the juice of fresh leaves and twigs, often inhibiting plant growth.

Aphid abundance measurements

The investigation of aphid abundance was conducted on one day, April 26, 2015. Three individuals of cubic and spheroidal were randomly collected using random number tables, and three annual twigs representing both cubic and spheroidal were haphazardly collected from one individual in each plot. Three paper scrap were put above the selected individuals in a breezy day during the twig selection, the first twig the paper touched was selected as the study subjects. The abundance of aphids on each of the selected twigs was counted with the naked eye. The aphid abundance on the selected twigs was first averaged for both cubic and spheroidal plants on each individual, and then averaged in each plots. The

herbivore load was calculated to determine whether the difference in abundances could be attributed to either the difference in plant structure or to an attraction to one of the two shrub shapes. Herbivore load was calculated as the ratio between the number of the aphids and the length of the twig.

Think about the above investigation lack unpruned bushes which are seemed as control, a further investigation was conducted in the residential area, the only site which had three types of shrub including unpruned, cubically pruned, and spheroidally pruned shrubs in 2020. In these observations, six bushes of each treatment were randomly selected using random table, and ten twigs were randomly selected from each bush. Aphid abundance in each twig was counted 1 or 2 days a time during a half month period. In addition, to detected the differences of the aphid abundance among leaves with different age, aphid abundance in each pair of leaves were counted from the first pair which is nearest to the top bud and is the youngest, to the fifth pair which is farther than other four pairs and is the oldest in the selected leaves in the last observation.

Plant structural measurements

The twigs selected for the aphid abundance measurements were clipped from the shrubs and measured after the aphids were counted. The leaves on each twig were collected and weighed first at their fresh weight, and then again at their dry weight after being oven-dried to a constant weight at 65 °C. Leaf dry mass content was calculated as the ratio between the dry weight and the fresh weight as a metric for the tenderness of the leaf, which is closely correlated with attractiveness to aphids.

A month after the aphid abundance measurements, three other perennial twigs were selected in the selected individuals following the method described in the aphid abundance observation. The number of inflorescences in each selected perennial twig was recorded.

In the further investigation, the shrub was observed to have two types of fresh twigs. One kind of twigs sprouted out in early spring, and did not grow rapidly after middle April, which was named as primary fresh twigs. The other kind of twigs could keep growing rapidly before late May, which was named as secondary fresh twigs. The aphid was mainly observed on the secondary fresh twigs. Therefore the differences of the secondary twigs density were investigated. In each investigation, the number of secondary twigs in a 50 cm × 50 cm area in each individual was counted at the last time for the aphid observation. In addition, thinking about number of inflorescences were thought to be an important regulation on plant growth and aphid density from the results of the first investigation, inflorescence number were also investigated in the 50 cm × 50 cm area in each individual in the further investigation in 2020.

Statistical analysis

Normal distributions were tested for each parameter before the data analysis. Aphid abundance and twig length were log-transformed to meet the assumptions of ANOVA. Two-way ANOVAs were conducted to compare plant structure and aphid abundance at the different sites and with the different pruning shapes in the investigation 2015. Repeat measurement ANOVA was employed to determine the differences

among the three pruning methods with the time variation in 2020. One way ANOVAs were conducted to compare plant structure among the three pruning methods and to determine the difference of aphid abundance among the different rank of leaves in 2020. A paired-t test was employed to determine the effect of pruning shape on plant performance and aphid abundance and simple linear regressions were run to determine their relationship. All the above statistics were calculated using SPSS 19.0 software package (SPSS Inc., CHI, IL, USA).

In addition, a structural equation model (SEM) was employed to detect causal linkages from explanatory variables to aphid abundance, including plant structural parameters following a previous study (Sun et al. 2016). The strength of direct and indirect relationships among all the considered variables was estimated with linear regression results (Grace 2006). Pairs of variables were checked for bivariate relationships before establishing the linear models to eliminate confounding factors. The SEM were constructed based on the known effects and potential relationships among the aphid abundance and the plant modular parameters. Data was fitted to the models using the maximum likelihood estimation method. The χ^2 -test was used to evaluate the fitness of each model (Grace and Bollen 2005). All SEM analyses were performed with AMOS 18.0 (Amos Development Co., Greene, Maine, USA).

Results

Aphid abundance

Aphid abundance varied greatly with study site. The aphid abundance at the road green belt was significantly higher than at the campus or the residential area sites (ANOVA: $F = 36.3$, Table 1; Fig. 2a). Pruning shape was found to significantly affect aphid abundance at all three sites (ANOVA: $F = 358.8$, Table 1). At each site, more than 30 aphids were found on each stem from a cubic canopy individual, whereas fewer than 5 aphids were observed on each stem from a spheroidal canopy individual. Aphid abundance on the cubic canopy shrub was 89.9 times higher than it on the spheroidal canopy shrub across all the three sites. Specifically, aphid abundance on the cubic canopy shrub was 46.7, 113.5 and 37.5 times higher than on the spheroidal canopy shrub at the campus, road green belt and residential area sites, respectively (t-test: $t = 5.1$, $t = 7.9$, $t = 3.5$, Table 2).

Table 1

Results of two-way ANOVAs (F values) regarding the effects of pruning shape and site on aphid abundance, fresh twig length, leaf dry mass content (LDMC) and inflorescence number. ***, $P < 0.001$

	df	Aphid Abundance	Fresh Twig Length	LDMC	Inflorescence Number	HerbivoreLoad
Site	2	36.3***	547.1***	37.4***	23.5***	38.0***
Pruning Shape	1	358.8***	124.4***	430.7***	775.0***	103.9***
Site * Pruning Shape	2	12.3***	3.4*	11.1***	17.3***	35.1***

Table 2

Results of paired-t test (t values) on the differences of aphid abundance, fresh twig length, leaf dry mass content (LDMC) and inflorescence number among the different pruning shapes at each site, ***, $P < 0.001$.

	df	Aphid Number	Twig Length	LDMC	Inflorescence Number	Herbivore Load
Campus	9	5.1***	13.3***	15.2***	20.4***	5.7***
Road Green Belt	9	7.9***	12.4***	6.3***	15.1***	8.2***
Residential Area	9	3.5**	8.4***	20.8***	15.0***	3.7**

The herbivore load on the cubic canopy shrub was 22.9 times larger than on the spheroidal canopy shrub across the three sites (t-test: $t = 5.7$, $t = 8.2$, $t = 3.7$, Table 2; Fig. 2b). Additionally, the effect of pruning shape on the herbivore load varied significantly with the study site (ANOVA: $F = 35.1$, Table 1). The herbivore load of the cubic canopy shrub was 32.2 times larger than of the spheroidal canopy shrub at the road green belt site, but only 12.9 and 12.8 times larger than of the spheroidal canopy shrub at the campus and residential area sites, respectively.

Aphid abundance was also significantly differed among the three treatments in the further investigation (ANOVA: $F = 2858.7$, $P < 0.001$, Fig. 3a). Although the pruning effect significantly varied with time (ANOVA: $F = 635.0$, $P < 0.001$, Fig. 3a). The number in the cubic shrub kept increasing from 2 aphids in the begin to more than 900 aphids in the end of the investigation, whereas the aphid in the spheroidal canopy and unpruned shrub only appeared in less than five days, with maximum numbers of 1 individuals per twig (Fig. 3a). Aphid abundance in 2020 varied significantly with the location of the leaves (ANOVA: $F = 88.0$, $P < 0.001$, Fig. 3b). The nearer leaves from the top bud, the more aphids on it were observed in the investigation (Fig. 3b).

Plant modular Parameters

Twig length varied significantly with study site (ANOVA: $F = 547.1$, Table 1), with the greatest (9.60 cm) and shortest (3.85 cm) lengths occurring at the road green belt and residential area sites, respectively (t-test: $t = 12.4$, $t = 8.4$, Table 2; Fig. 4a). The twig length in the cubic canopy shrub was on average 6.2 cm longer than in the spheroidal canopy shrub across the three sites (Fig. 4a). Further, the effect of the pruning shape on twig length varied with study site (ANOVA: $F = 3.4$, Table 1). The twig length of the cubic canopy shrubs were 4.4, 10.6, and 3.7 cm longer than the spheroidal canopy shrub samples at the campus, road green belt and residential area sites, respectively.

The leaf dry mass content was significantly affected by the study site (ANOVA: $F = 37.4$, Table 1). The leaf dry mass content at the residential area site was lower than at the other two sites (Fig. 4b). The LDWC of the cubic canopy shrub samples (27.0%) was significantly lower than for the spheroidal canopy shrub samples (34.3%) across the three sites. The site and pruning shape interacted to affect LDMC in

the experiment (ANOVA: $F = 11.1$, Table 1). The LDWC of the cubic canopy shrub samples was 8.54%, 5.01%, and 8.55% lower in absolute change than of the spheroidal canopy shrub samples from the campus, road green belt and residential area sites, respectively (t-test: $t = 15.2$, $t = 6.3$, $t = 20.8$, Table 2).

The inflorescence number was significantly affected by study site (ANOVA: $F = 23.5$, Table 1), with the maximum and minimum values occurring at the campus and the road green belt sites, respectively (Fig. 4c). The inflorescence number varied significantly with the pruning shape (ANOVA: $f = 775.0$, Table 1). The inflorescence number on the cubic canopy shrub was 16.3 times lower than on the spheroidal canopy shrub (Fig. 4c). The effect of pruning shape on inflorescence number varied with study site (ANOVA: $f = 17.3$, Table 1). The inflorescences on the spheroidal canopy shrub numbered 16.5, 20.3 and 14.1 times greater than on the cubic canopy shrub at the campus, road green belt and residential area sites, respectively (t-test: $t = 20.4$, $t = 15.1$, $t = 15.0$, Table 2).

Secondary twig number (ANOVA: $F = 77.5$, $P < 0.001$, Fig. 5a) and inflorescence number (ANOVA: $F = 44.1$, $P < 0.001$, Fig. 5b) varied significantly with different treatments. Secondary twig number of the cubic canopy shrub was significantly higher than it in other two shrubs (Fig. 5a), but the inflorescence number (Fig. 5b) of the cubic canopy shrub was significantly lower than it in other two shrubs, respectively.

Relationships between aphid abundance and plant structural parameters

The aphid number decreased with increasing LDMC at all study sites (all $P < 0.001$; Fig. 6a-c). However, when the regression analysis was conducted for each the cubic canopy shrub and the SP separately, aphid abundance on cubic canopy shrub was the only factor negatively correlated with LDMC, and only at one site, the road green belt (Fig. 6e); no other relationships between aphid abundance and leaf DMC were detected at either study site (all $P > 0.05$; Fig. 6d; 6f-i).

The aphid abundance increased with increasing twig length linearly at all study sites (all $P < 0.001$; Fig. 7a-c). When the regression analysis was conducted for each the cubic canopy shrub and the spheroidal canopy shrub separately, the aphid abundance in the cubic canopy shrub was positively correlated with twig length at all the sites (all $P < 0.01$; Fig. 7d-f). However, the aphid abundance in the spheroidal canopy shrub was not correlated with twig length at any of the three sites (all $P > 0.05$; Fig. 7g-i).

Aphid abundance decreased linearly with increasing inflorescence number at all study sites (all $P < 0.001$; Fig. 8a-c). When the regression analysis was conducted for each the cubic canopy shrub and the spheroidal canopy shrub separately, the aphid abundance in the cubic canopy shrub was negatively correlated with inflorescence number at the campus and the road green belt sites (all $P < 0.05$; Fig. 8d-e), but not with inflorescence number at the residential area site (Fig. 8f). In addition, the aphid abundance in the spheroidal canopy shrub was negatively correlated with inflorescence number at all three sites (all $P < 0.05$; Fig. 8g-i).

The best SEM model ($\chi^2 = 2.797$, $P = 0.094$, $df = 1$) explained 91% of the variation in aphid abundance. The variation can primarily be attributed to the increase in fresh twig length (Fig. 9). Light pruning (from cubic canopy shrub to spheroidal canopy shrub) and the resulting negative effect on twig length led to a decrease in aphid abundance. Light pruning also positively and significantly affected leaf dry mass content and inflorescence number, although the SEM indicated that the increase in leaf dry mass content or inflorescence number barely accounted for the variation in aphid abundance.

Discussion

Both of our results of the only one-time observation and half-year investigation show that the plants with a cubic canopy had higher aphid abundance, lower leaf dry mass content and lower inflorescence number, but they also had greater fresh twig length than plants with a spheroidal canopy. These results support our hypothesis that heavy pruning can significantly augment an herbivorous population through the alteration of plant performance, and suggest that the pruning-induced changes of plant performance may result in the variations in herbivores.

Pruning is an important horticultural management method, and has been reported to alter canopy size, to elevate growth rate and to increase the number of shoots to compensate for structures lost in pruning (Albert et al. 2010). Different pruning strategies have different effects on plant morphology (Tworkoski et al. 2006, Mampionona 2011). For instance, Mampionona (2011) reported that trees with a long trunk and short primary branches that were pruned had longer lateral shoots compared to the post-pruning growth from a tree with a short trunk and long primary branches. Tworkoski, Miller, and Scorza (2006) reported that pillar trees exhibit more upright growth after pruning, but have fewer sylleptic branches than standard trees. In the current study, we have observed that plants with a spheroidal canopy or natural canopy allocate more resources to reproductive (e.g., inflorescence), rather than vegetative growth (e.g., twig length) after pruning than plants with a cubic canopy (Fig. 3, Fig. 4). These different performances could be attributed to the heavier pruning treatment necessary for plants to maintain a cubic canopy rather than a spheroidal canopy. The former can produce many epitomic shoots, according to the compensating theory (Guilett and Bergström 2006). After the apical tissue of the shoots was pruned, the plants with a cubic canopy maintained dense shoots at the surface. High-density plants, like these with a cubic canopy, must grow sufficiently upward to obtain enough light (Vile et al. 2006, Liu et al. 2012). Because the spheroidal canopy is similar in shape to the natural form of shrubs, plants with a spheroidal canopy are also not subject to light limitation for growth in the same way as shrubs with a cubic canopy. Most resources of shrub with a spheroidal canopy are spent on new outward growth, rather than the re-growth of twigs. As a result, plants undergoing the less intensive pruning regimen for spheroidal canopies are much weaker than plants with a cubic canopy. Our results are consistent with previous findings for litchi (Olesen et al. 2013) and blueberry (Lee et al. 2015), which indicate that different pruning strategies may redistribute the trade-off between vegetative and reproductive growth in many species.

This study has found that moderate pruning toward a spheroidal canopy shape results in a lower abundance of insect herbivores than heavy pruning toward a cubic canopy shape, which supports our

hypothesis. Our results are consistent with previous studies that have reported that pruning may attract more sap-feeders (Price 1991, Grechi et al. 2008). However, our results contrast the results of previous studies (Cornelius 2001, Simon et al. 2007, Mdellel et al. 2015) that have reported that pruning may reduce the herbivory behavior of insects (Cornelius 2001, Simon et al. 2007, Mdellel et al. 2015) due to the removal of terminal shoots (Martinez and Wool 2003) and the reduction of the insect eggs during pruning. The lower abundance of aphids on the spheroidal canopy shrubs can be largely attributed to the shorter, regenerated branches of the plants, when compared to shrubs with a cubic canopy. More aphid on the leaves nearby the top bud in our study have approved that the aphid often lives on tender buds, leaves, and twigs as a common sap-feeder (Chau and Mackauer 1997, Hu et al. 2017). No aphids were found on our plants with either canopy form immediately after either pruning treatment, thus any aphids found on the shrubs after pruning had migrated from other plants (Observed by Chen). Our results for herbivore load indicate that the twigs on stems in cubic canopies attracted more aphids than the stems in spheroidal canopies because the plants with cubic canopies had longer, fresher twigs and lower leaf dry mass content. Alternatively, the plant with the spheroidal canopy produced more sap, which may have attracted more aphids, as stated in the pulsed stress hypothesis (Huberty and Denno 2004). Shrubs with the cubic canopy may also have been preferential for the herbivores because the longer, fresher twig can also access more sunlight and thus maintain a higher, more comfortable temperature. Barton and Ives (2014) reported that aphid abundance should increase at higher temperature where predators are absent. No predator were found in our study, thus we can expected the longer, fresher twigs on the plants with a cubic canopy to attract a larger abundance of aphids than the plants with a spheroidal canopy. To test this hypothesis, we observed aphid abundance on plants with both a cubic and a spheroidal canopy in the road green belt both prior to and following the first pruning treatment. The aphids disappeared after the first pruning. This finding suggests that the initial observations of aphids are most accurate and that the aphids had a higher reproductive rate among the cubic canopies (Appendix S1: Fig. S1). Besides, previous studies have reported that plants with different structures contain different concentrations of secondary metabolites, such as flavonoids (Conde et al. 2009, Maudu et al. 2010), which could affect the survival of insects that consume plant parts (Lattanzio et al. 2000). However, we did not detect significant differences in the flavonoid content between the plants having cubic and spheroidal canopies (Appendix S2: Fig. S2), thus the influence of the flavonoids on the aphids was considered negligible in the study. Furthermore, arriving gynoparae during the autumn may also play an important role on the regulation of aphid abundance (Leather et al. 1993; Leather et al., 1995). However, according to our observation, no aphids and eggs were observed on the box trees before early April, but some aphids have been observed on the nearby grape vine and peach tree in late March (Observed by Chen). From these observations, we can conclude that the difference of aphid abundance is not correlated with the difference of arriving gynoparae and the eggs among different treatments.

Our results have demonstrated that the influence of pruning on aphid abundance significantly varies with study site, which indicates that significant interactions are at play between pruning and study site on aphid abundance. Two possible reasons may be employed to explain the interactions. First, the road green belt is more open than the other two sites, thus the growth rate of the aphid population at the road

green belt may be greater than at the other two sites. Actually, the aphid abundance in the cubic canopy stems at the road green belt was also much greater than at the other two sites, but the difference in aphid abundance in the spheroidal canopy stems among the three sites was smaller than for the abundance in the cubic canopy only. Secondly, the horticulture management (e.g., irrigation, pruning, and pesticide application) differed in frequency among the three sites. According to our observations, the horticulture management was less frequent at the road green belt than at the other two sites, which may have induced the larger population of aphids. Our results indicate that the pruning effects may be altered according to the environment and other horticulture management practices.

Conclusion

Our results suggest that (1) aphid abundance on plants with a cubic canopy is higher than on the plants with a spheroidal canopy and that (2) aphid abundance is mainly correlated with fresh twig length and inflorescence number. Our findings have proven that pruning shape can significantly affect the abundance of herbivores on the pruned plants. The results can provide data to support for the human activities can alter plant modular that affect insect preference.

Appendix

Figure S1. Aphid number on the plants with a cubic canopy (hollow circles) and with a spherical canopy (solid circles) in the road green belt before the first pruning, which was conducted from April 15th to May 5th, 2015.

Figure S2. Flavonoid content of the leaves on the plants with a cubic (CU) and a spheroidal canopy (SP) on the campus (CA), at the road green belt (RGB) and in the residential area (RA).

Declarations

Ethics approval and consent to participate

This work is only form a field investigation experiment, so no ethics approval was required. All samples collection was permitted by the administrative department.

Consent for publication

We understand that the information will be published without our/our child or ward's/my relative's (circle as appropriate) name attached, but that full anonymity cannot be guaranteed.

We understand that the text and any pictures or videos published in the article will be freely available on the internet and may be seen by the general public. The pictures, videos and text may also appear on other websites or in print, may be translated into other languages or used for commercial purposes.

We have been offered the opportunity to read the manuscript there is no financial interest to report

Availability of data and material

All data used in this study are included in the manuscript and supporting information.

Competing interests

The authors declare no conflict of interest

Funding

This study was supported by the National Natural Science Foundation of China (31670477, 31200375), the China Postdoctoral Science Foundation (2017M612389) and the outstanding youth training foundation of Henan University (yqpy20140031).

Author contributions

Yinzhan Liu designed the experiment and wrote the first version of the MS. Anqun Chen, Xiaolin Liu, and Juan Xuan collected the data. Anqun Chen conducted the data analysis and further investigation, and revised the manuscript. Chunlian Qiao and Renhui Miao revised the manuscript. Anqun Chen and Yinzhan Liu contributed equally in the work.

Acknowledgements

The authors wish to thank Xinqiang Xi and Yanchun Liu for their valuable suggestions regarding experimental design and manuscript revision. Thanks for Elizabeth Tokarz at the Yale University, who worked in the Mapleedit Company in Tianjin for the English revision.

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Figures



Figure 1

Photo of *B. megistophylla* Levl. with a cubic canopy (CU) and with a spheroidal canopy (SP)

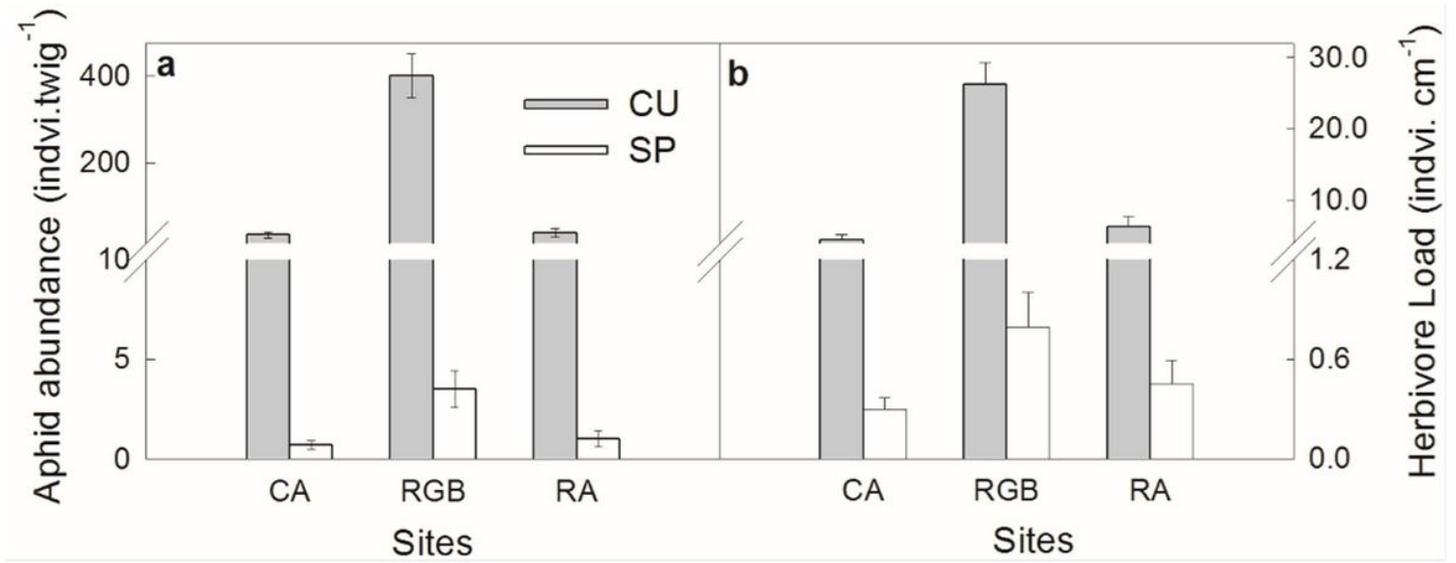


Figure 2

Aphid abundance (a) and herbivore load (b) of plants with cubic (CU) and spheroidal canopies (SP) on the campus (CA), at the road green belt (RGB) and in the residential area (RA) in 2015.

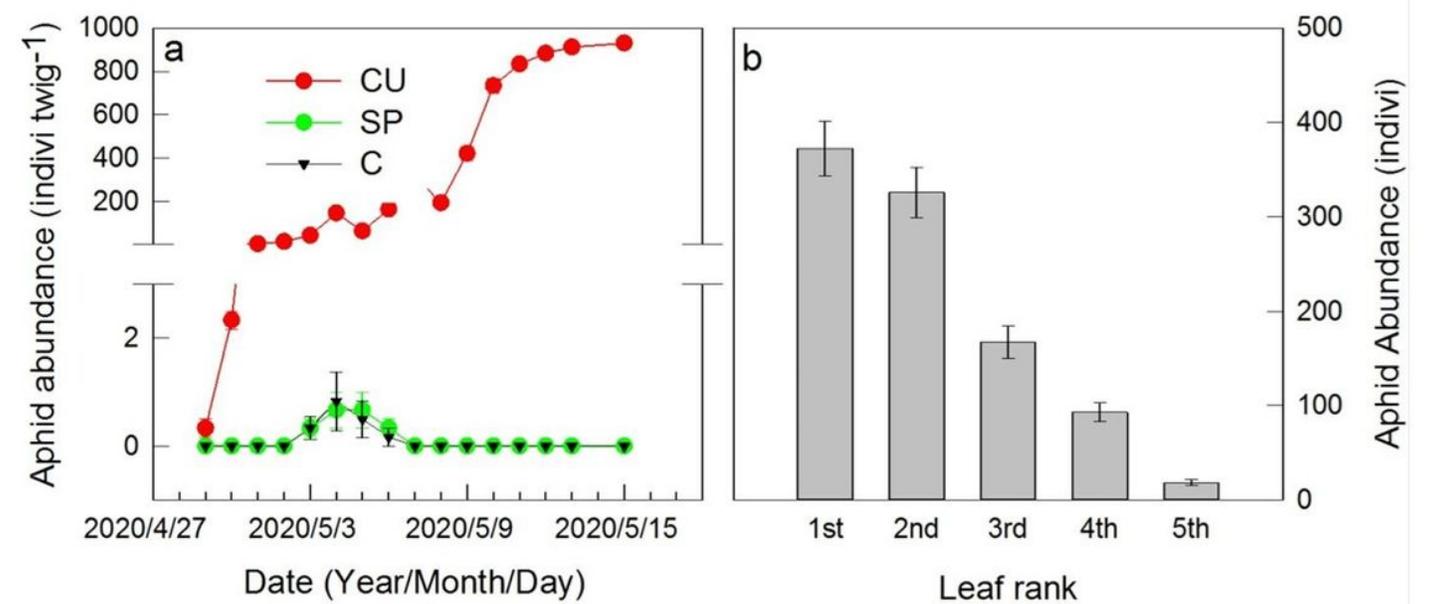


Figure 3

Aphid abundance on plants with cubic (CU) and spheroidal (SP) and natural (C) canopies (a) and on the leaves at different ranks on cubic canopy shrubs (b) in 2020.

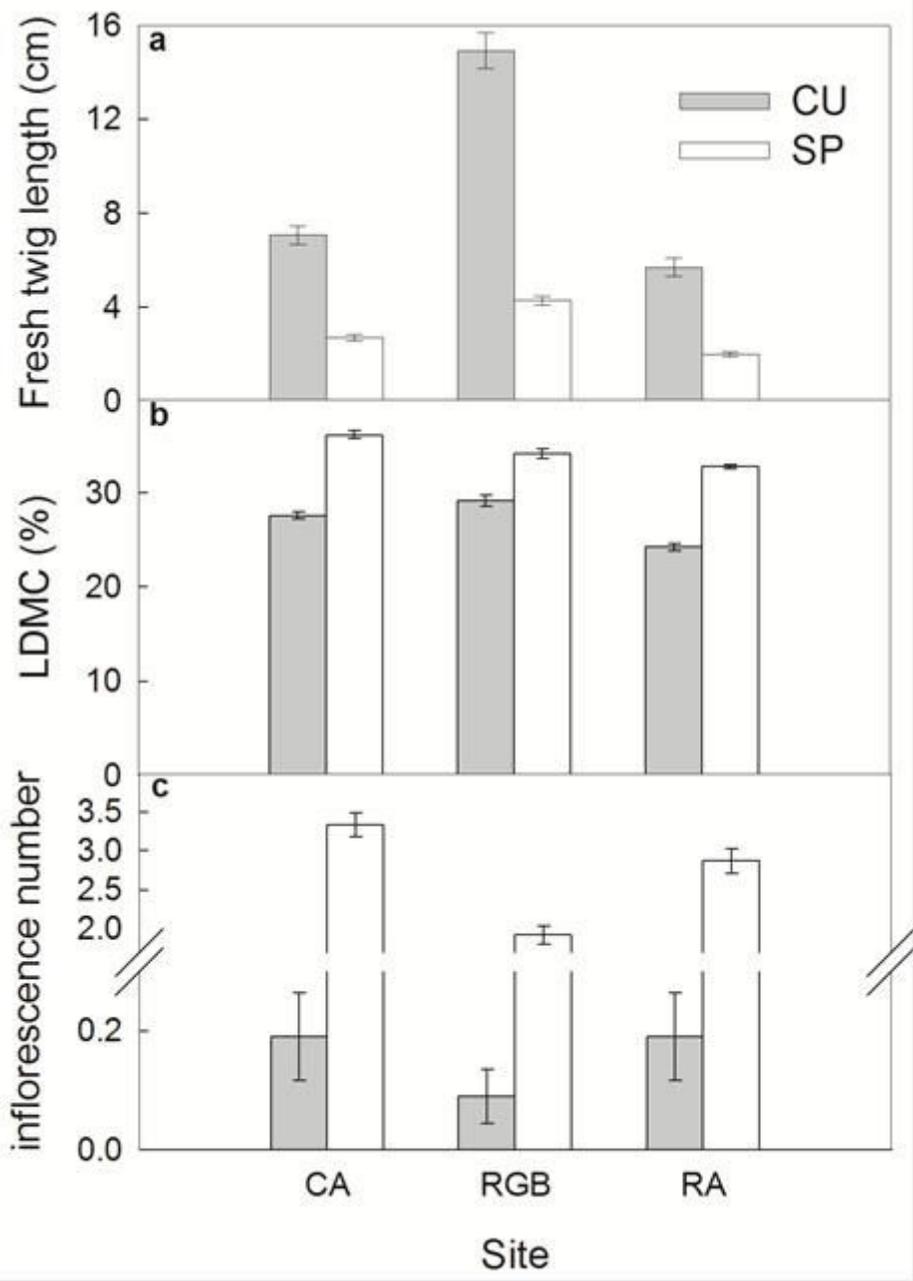


Figure 4

Fresh twig length (a), leaf dry mass content (LDMC, b) and inflorescence number (c) of plants with cubic (CU) and spheroidal canopies (SP) on the campus (CA), at the road green belt (RGB), and in the residential area (RA).

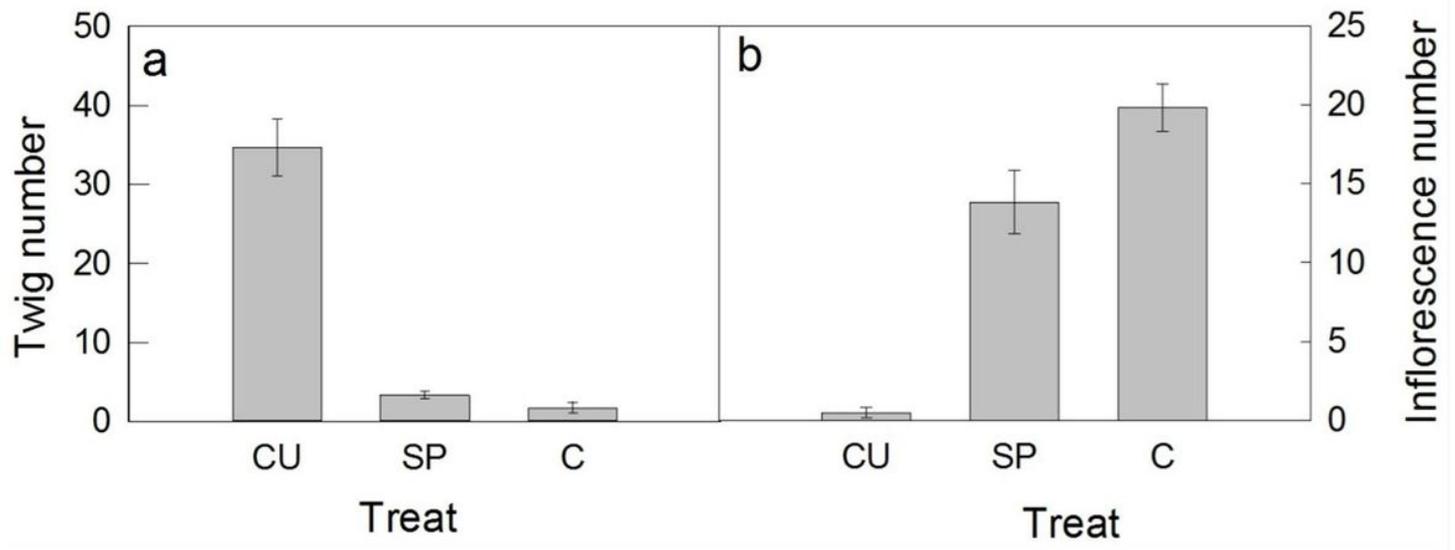


Figure 5

Twig number (a) and inflorescence number (b) on plants with cubic (CU) and spheroidal (SP) and natural (C) canopies in 2020.

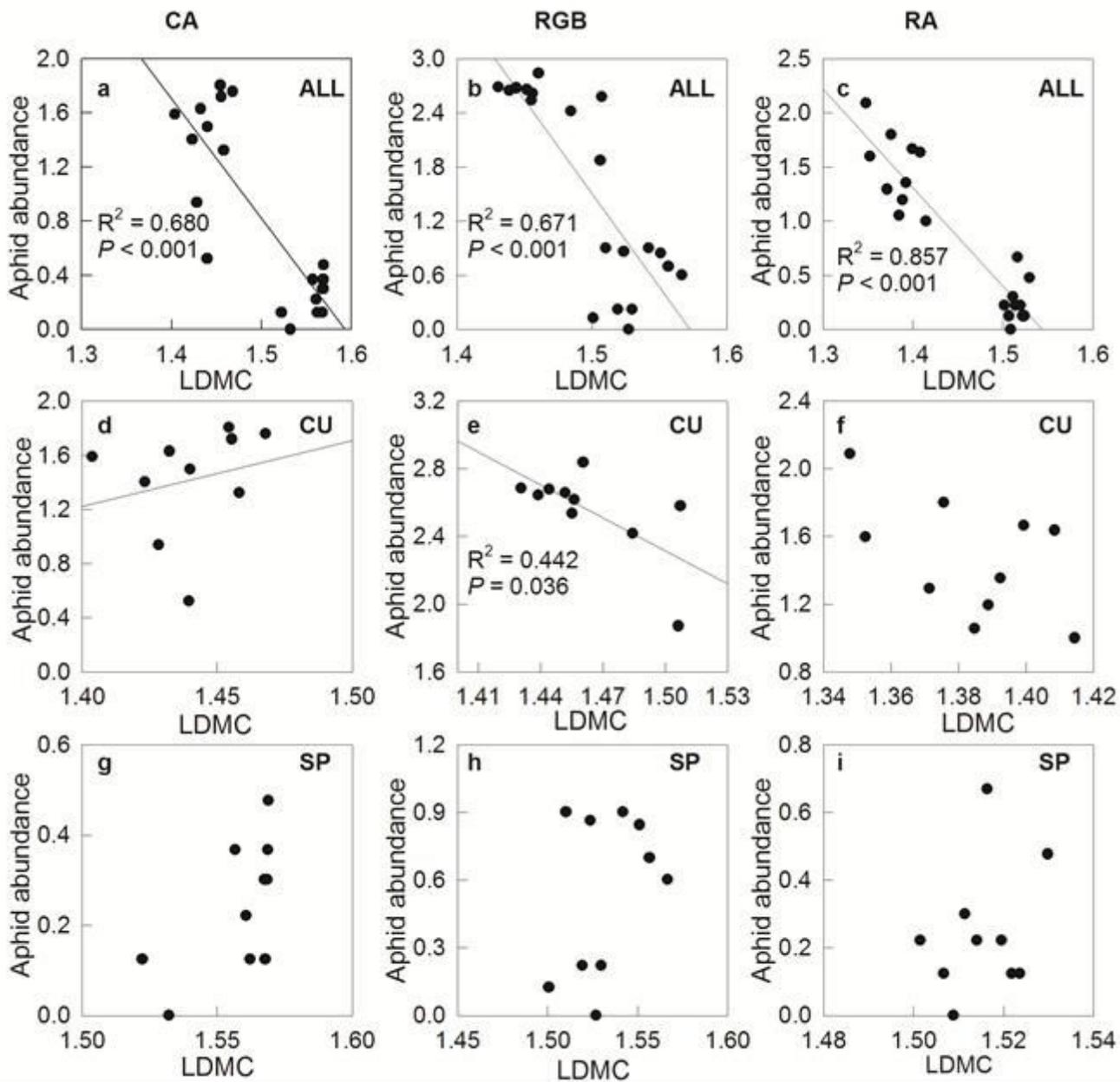


Figure 6

Linear regressions show the relationships between aphid abundance and leaf dry mass content (LDMC) across all samples (ALL, a, b, c) and samples of plants with cubic (CU, d, e, f) and spheroidal canopies (SP, g, h, i). The words above each column represent the different study sites; campus (CA), road green belt (RGB) and residential area (RA). All the data in this figure has been log-transformed.

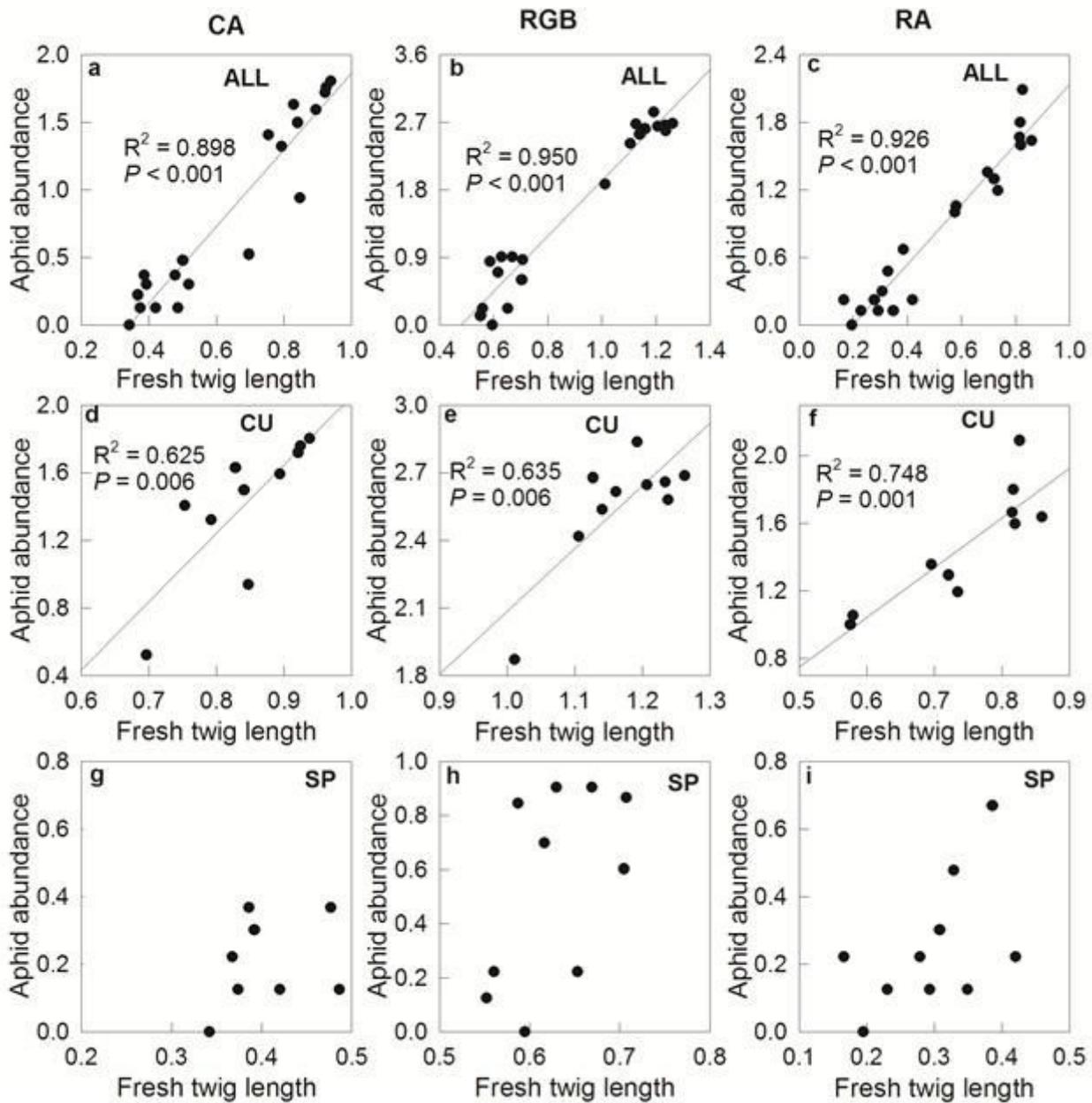


Figure 7

Linear regressions show the relationships between aphid abundance and fresh twig length across all samples (ALL, a, b, c) and samples of cubic (CU, d, e, f) and spheroidal canopies (SP, g, h, i). The words above each column represent the different study sites; campus (CA), road green belt (RGB) and residential area (RA). All the data in this figure has been log-transformed.

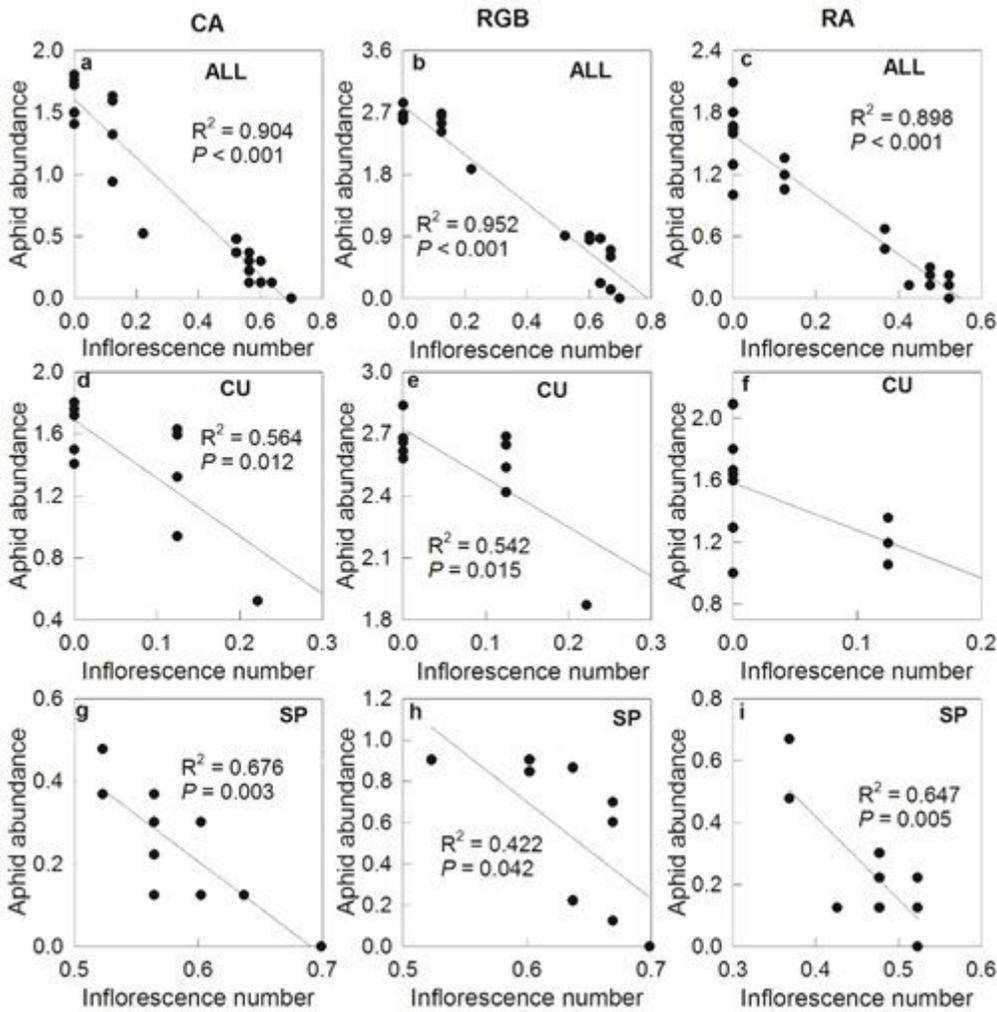


Figure 8

Linear regressions show the relationships between aphid abundance and inflorescence number across all samples (ALL, a, b, c) and samples of cubic (CU, d, e, f) and spheroidal canopies (SP, g, h, i). The words above each column represent the different study sites; campus (CA), road green belt (RGB) and residential area (RA). All the data in this figure has been log-transformed.

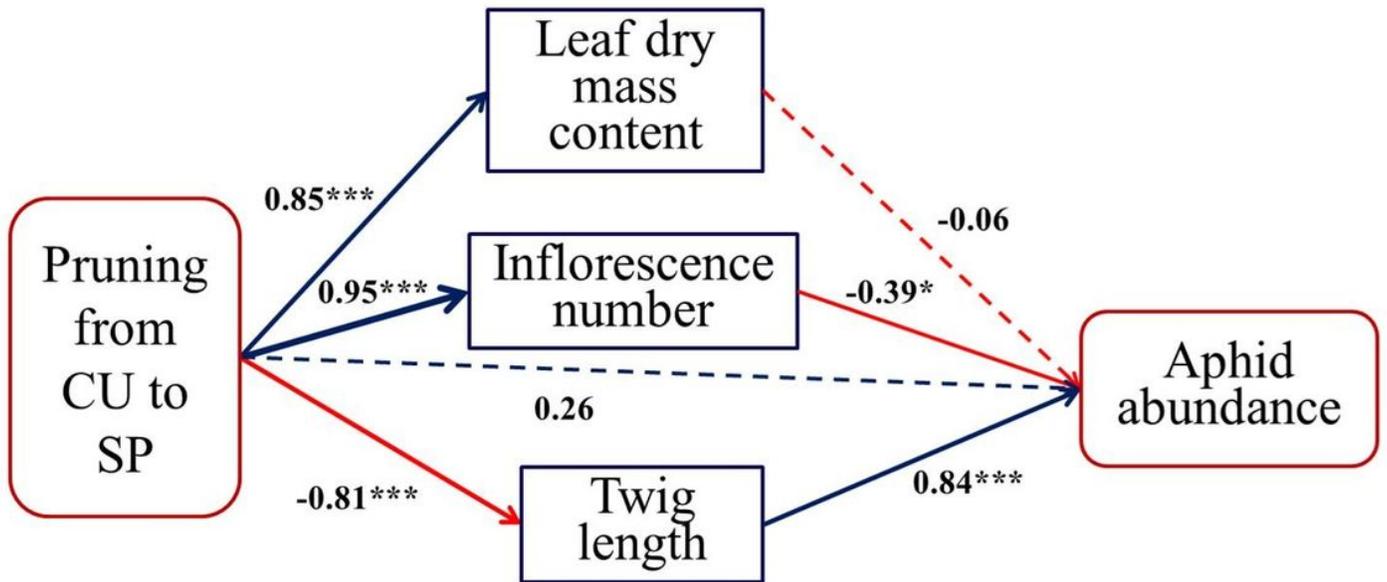


Figure 9

The results of the final structural equation model (SEM) showing the causal relationships from pruning, leaf dry mass content, inflorescence number and twig length to aphid abundance. The blue line represents a positive effect and the red line represents a negative effect. The numbers above the arrows indicate path coefficients (*P < 0.05, **P < 0.01, ***P < 0.001). The values above the variables represent the proportion of variance explained for each variable. Model fit summary: $\chi^2 = 2.797$, P = 0.094 > 0.05, df = 1, TLI = 0.959, CFI = 0.996, RMSEAR = 0.175.