Effects of shading on the growth and leaf photosynthetic characteristics of three forages in an apple orchard on the Loess Plateau of eastern Gansu, China

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Background. Inclusion of forage into the orchard is of great help to promote the use efficiency of resources, while shading from trees restricts forage growth and production in the Loess Plateau of China. This study was aimed to investigate how tree shading affected leaf trait, photosynthetic gas exchange and chlorophyll feature of forages under the tree in the orchard-forage system.

Methods. The shading treatments were set as partially cutting branches (reduced shading), normal fruit tree shading (normal shading) and normal tree shading plus sun-shading net (enhanced shading) in an apple orchard. Leaf trait, photosynthesis, chlorophyll component and fluorescence related parameters were measured with lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and cocksfoot (*Dactylis glomerata*) which were sown under apple trees.

Results. Shading imposed significant impacts on the growth and leaf photosynthetic characteristics, while there were differences among species. Enhanced shading decreased leaf thickness, leaf dry matter content (LDMC) and leaf mass per unit area (LMA). Biomass accumulation decreased with enhanced shading in cocksfoot, but did not change in white clover and lucerne which had much lower biomass accumulation than cocksfoot. Enhanced shading reduced net photosynthetic rate (Pn) of white clover and lucerne, but rarely affected cocksfoot, while it decreased instantaneous water use efficiency (WUEi) of cocksfoot but had few effects on the other forages. Enhanced shading reduced leaf dark respiration rate (Rd), light compensation point (LCP) and maximum assimilation rate. The Rd and LCP of cocksfoot were much lower than those of white clover and lucerne. Chlorophyll contents and chlorophyll a/b changed little with shading. Cocksfoot had the highest contents but lowest ratio. Maximum photochemical rate of photosystem II increased and non-photochemical quenching decreased with enhanced shading in cocksfoot, while did not change in the other forages.

Discussion. Leaf trait, photosynthetic gas exchange and chlorophyll feature were variously affected by species, shading and their interaction. Cocksfoot was more efficient than the other two forages in use of weakened light and more tolerant to tree shading. In the apple orchard, we recommend that reducing the density of apple tree or partially cutting branches together with selecting some shading-tolerant forages, i.e. cocksfoot, would be a practical option for the orchard-forage system in the Loess Plateau of China.

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15 Abstract

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40   affected by species, shading and their interaction. Cocksfoot was more efficient than the other
41   two forages in use of weakened light and more tolerant to tree shading. In the apple orchard, we
42   recommend that reducing the density of apple tree or partially cutting branches together with
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44   orchard-forage system in the Loess Plateau of China.
45
46   ## Introduction
47   Traditional orchard performance with bare ground or simple tillage to the soil has led to serious
48   soil erosion and low use efficiency of resources, such as light, soil water and nutrients (*Shui et al.*,
49   *2008*). Inclusion of grass (and/or forage) into the orchard is an advanced management mode for
50   orchard soil (*Skroch & Shribbs*, *1986*), offering a solution to deal with such issues, and has been
51   widely used as an efficient conservation tillage in the orchard (*Neves et al.*, *2010*). However, the
52   possible competition on soil water and nutrient between tree and grass under the tree has lit up
53   some worries that sowing grass in the orchard might result in loss of fruit yield and quality,
54   especially in some areas where water deficit and soil infertility happen a lot (*Monteiro & Lopes*,
55   *2007*; *Teravest et al.*, *2010*), an example being the Loess Plateau of China. So how the grass
56   roles in such an integrative system have attracted wide attention.
57   The inclusion of grass into the orchard can modulate soil features like water and fertility.
58   Sowing grass may potentially adjust the enrichment and paucity of soil water content to keep it
59   relatively stable (*Liu et al.*, *2013*). It can reduce surface runoff and enhance infiltration,
60   alleviating soil erosion (*Fourie et al.*, *2007*). Moreover, with the increase of grass age, soil
61   infiltration and water holding capacity will be greatly improved *(Palese et al.*, *2014)*. There is
62   competition for water between grass and tree, which varies with plant species and the amount of
63   rainfall. The competition can be weak under suitable species combination and system
64   management. Inclusion of grass can also be beneficial to improve the contents of organic matter
65   (*Sánchez et al.*, *2007*), nitrogen (N), phosphorus (P) and potassium in soils (*Shui et al.*, *2008*).
66   For instance, some legume species may potentially improve soil N availability as they have
67   strong capacity to biologically fix atmospheric N (*Yang et al.*, *2011*). Additionally, soil microbial
68   diversity and activity also increase in the orchard after sowing grass (*Whitelaw-Weckert et al.*,
69   *2007*), which may be helpful for the decomposition of soil organism humus (*Wardle et al.*, *2001*).
70   Therefore under this system the competition for soil nutrient is relatively subtle due to the
71   improvement of soil fertility by grasses and artificial fertilization. In addition, as the pattern of
72   orchard-grass performance is continuously improved, transforming from firstly a single mode
73   (ground cover) to the complex three–dimensional mode (combination of cover, farming and
74   animal husbandry), the role of grass in the system is diversified. The grasses sown in the orchard
75   may also be used as forages for feeding animals with countable amount of biomass accumulation
76   in certain areas. So inclusion of grass into the orchard shows advantages both at ecological and
77   economic scales.

78   The inclusion of grass has broken the water and heat exchange in soil – fruit tree – air
79   continuum and has transformed into soil – fruit tree + grass – air continuum (*Bing et al., 2002*).
80   In this way, water and heat can be more fully utilized in the system, which requires a balance
81   between the growths of tree and grass to maximum their functions in the system. However, tree
82   shading may be a problem for the growth of grass under the tree as insufficient light causes
83   adverse effects on grass growth and production. Generally, leaf net photosynthetic rate (Pn) may
84   fall under shading and rapid stomatal closure occurs (*Kim et al.*, *2016*), while appropriate
85   shading can improve water use efficiency (WUE) of plants, which varies with plant species.
86   *Delucia et al. (1998)* found that the plants usually increased photosynthetic efficiency to improve
87   light utilization by increasing leaf area under shading. With shading, leaf chlorophyll content
88   increases and chlorophyll a/b value decreases to improve plant photosynthetic activity (*Abrams*,
89   *1987*; *Lambers & Poorter*, *1992*). *Singhakumara et al. (2003)* found that the shade-tolerant
90   plants generally had larger leaf area, higher chlorophyll content and lower leaf mass per unit area
91   (LMA) than the shade-sensitive ones. These aforementioned traits are important measures in
92   plant adaptation to adverse light environments (*Grassi & Bagnaresi*, *2001*) and thus may be
93   helpful in selecting suitable grasses for the orchard. However, little knowledge has been
94   achieved on how these traits of grass under the tree respond to shading in the orchard.
95   On the Loess Plateau of eastern Gausu, China, apple orchard is widely established as a
96   profitable option in this arid and infertile area. There was approximately 1.02×10^5 ha apple
97   orchard established in this area with apple yield of 6.7×10^8 kg per year. The existing orchards are
98   mostly lightly tilled, which is unfavorable for controlling soil and water loss (*Wang et al.*, *2015*).
99   Some traditional thoughts, i.e. grass and tree fight for water and nutrient in soils, and thus sowing
100 grass may increase the costs of money and labour, have retarded the performance of grass
101 sowing in the orchards of this area. Appropriate grass species are essential for the establishment
102 of a sustainable orchard-grass system (*Wang et al.*, *2015*) but there was still rare species suitable
103 for the system in this region. The lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and
104 cocksfoot (*Dactylis glomerata*) are common forage crops widely sown and used to feed domestic
105 animals in this region. However, it was not clear how they can be better used in the orchard.
106 We proposed a hypothesis that tree shading would impose heavy impacts on grass species
107 included in the orchard in some species-specific way. In this study, biomass, leaf trait,
108 photosynthetic gas exchange and chlorophyll feature of three forages (grasses) were measured
109 under three shading treatments in an apple orchard. The objectives were to find out: 1) how tree
110 shading affects biomass accumulation, leaf trait, photosynthetic gas exchange and chlorophyll
111 feature of the forages? 2) Which of the three species is more tolerant to shading in the orchard?
112

Materials & Methods

Plant material and experimental design

115 The experiment was conducted in a 7 year–old apple orchard at Qingyang Loess Plateau Pastoral
116 Agriculture Station of Lanzhou University (35°40′ N, 107°51′ E), which locates in Qingyang,
117 eastern Gansu of China with a typical continental climate. The mean annual precipitation is 543

118 mm and 70% of this total usually falls in July to September. The mean annual temperature is
119 9.3℃ with the lowest in January (-21.3℃) and the highest in July (40℃). The annual frost-free
120 duration is 255 d in average. The soil is Heilu soil with 70% silt and 23% clay, representing the
121 main cropping soil in this area.
122   In the intervals between tree lines (4 m wide), three forages were broadcast sown, which are
123 lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and cocksfoot (*Dactylis glomerata*)
124 in July 4, 2014. The sowing rates were 22.5, 15.0 and 15.0 kg ha^{-1} for lucerne, white clover and
125 cocksfoot respectively. For this test, 6 m long (×4 m wide) plots were chosen and for each
126 treatment, four replicates were set. All plots were broadcast applied with 300 kg ha^{-1} N fertilizer
127 in the form of urea before sowing. The forage was supposed to be cut and the shoot was removed
128 out so that great amount of nutrient (especially N) would be lost from the system, so N fertilizer
129 was applied, even to legume species. Soil P in the orchard was excessive due to long term P
130 fertilization and slow release of soil P source. And the inclusion of forages would benefit the
131 release of soil residual P in the orchard. So P fertilizer was not applied. No irrigation and
132 pesticide spraying were performed. All the forages were cut to feed domestic animals after plant
133 samples taken. Notably, no treatment and measurement were conducted in the first year in order
134 to favour the establishment of grasslands under the trees.
135   The shading treatments were started in April 12 before the forages were reviving in the
136 second year. Three shading treatments were set as reduced shading (partially cutting branches),
137 normal shading (normal tree shading) and enhanced shading (normal tree shading plus sun-
138 shading net), and these treatments made the light intensity equal to about 70%–80%, 40%–50%
139 and 10%–20% photosynthetically active radiation (PAR) above the canopy, which we measured
140 every 2 weeks on sunny days using a portable photosynthesis system (LI–6400, Li–Cor, USA).
141 All measurements were conducted at about two months later after shading treatment when
142 lucerne and white clover were at early flowering stage and cocksfoot was at late heading stage.
143 Due to budgetary limit, all measurement was only conducted in this year. Considering all three
144 species are perennials and the second year is very close to the stabilized ages of artificial
145 grassland in this area, the data we obtained should show the characteristics of the second year's
146 forages.
147
Measurements and calculations
149 At least 20 youngest fully expanded leaves were sampled for each treatment. The sampled leaves
150 were then brought back to laboratory as soon as possible for further measurements. Leaf biomass
151 at saturated moisture content and dry weight were measured to determine leaf dry matter content
152 (LDMC) (*Garnier et al., 2001*) using the equation: LDMC (mg g^{-1}) = leaf dry weight / leaf
153 saturated fresh biomass. Fresh leaf area (cm^2) was scanned with Win FOLIA (LA2400, Canada)
154 and the LMA was determined using the equation: LMA (g m^{-2}) = leaf dry weight / leaf area. In
155 addition, leaf thickness (LT) was measured with a vernier caliper. Biomass was measured with
156 drying method. After sampling with quadrat frame of 1m×1m, the samples were dried at 80℃
157 until constant weight and measured the biomass on the ground.

158   Constant photosynthetic gas exchange was measured with a portable photosynthesis system
159   (LI–6400, Li–Cor, USA) at 9:30 – 11:30 am on a clear sunny day during leaf sampling. The CO_2
160   concentration was maintained at 400 μL L^{-1} using CO_2 supplying cartridge. The Pn (μmol m^{-2} s^{-1}
161    $^{-1}$) and transpiration rate (E, mmol m^{-2} s^{-1}) were recorded and instantaneous WUE (WUEi, μmol
162    $mmol^{-1}$) was calculated as Pn/E. In each replicate, three plants were selected randomly and at
163   least three healthy and fully expanded leaves were measured. The Pn response to light gradient
164   was measured at 09:00–11:00 on a clear sunny day using the red and blue light source equipped
165   with LI–6400. During the measurements, CO_2 concentration was maintained at 400 μL L^{-1} using
166   CO_2 supplying cartridge and light intensity was set according to the Equipment Instruction. The
167   curve was then fit with the classic Farquhar model (*Farquhar et al., 2001*) to obtain light
168   compensation point (LCP), light saturation point (LSP), dark respiration rate (Rd), maximum
169   assimilation rate (Amax) and apparent quantum efficiency (Qapp).
170   Chlorophyll a and b was extracted by mixture of propanone and anhydrous ethyl alcohol,
171   and then the contents were determined by spectrophotometer method of *Arnon (1949)*. The
172   contents of chlorophyll a, b and a+b were calculated using the following equations:
173   Chlorophyll a (mg g^{-1}) = $[(12.7 \times A663 - 2.59 \times A645)V/W]$,
174   Chlorophyll b (mg g^{-1}) = $[(22.9 \times A645 - 4.67 \times A663)V/W]$,
175   Chlorophyll a+b (mg g^{-1}) = $[(20.3 \times A645 + 8.04 \times A663)V/W]$,
176   Where A is absorbance at specific wavelengths; V is final volume of chlorophyll extract; W is
177   fresh weight of leaf extracted; 12.7, 2.59, 22.9, 4.67, 20.3 and 8.04 are the constants. In the
178   present experiment, the volume (V) and weight (W) were 100 ml and 0.1 g, respectively.
179   Chlorophyll fluorescence was measured at 09:00–11:00 on a clear sunny day to obtain actual
180   photochemical efficiency of photosystem II (ϕPS II), photochemical quenching coefficient (qP)
181   and non-photochemical quenching (NPQ) using fluorescent leaf chamber of LI–6400 with
182   controlled light intensity of 1500 μmol m^{-2} s^{-1}. Prior to these measurements, marked leaves were
183   measured in dark to determine maximum photochemical rate (Fv/Fm) at 01:00 deep night. In
184   each replicate, three plants were selected randomly and at least three healthy and fully expanded
185   leaves were measured.
186
Data analysis
187   The effects of shading treatment, forage species and their interaction on leaf trait, gas exchange,
188   chlorophyll component and fluorescence were analyzed using factor analysis. The differences in
189   leaf traits, gas exchange parameters and chlorophyll features among forages or shading
190   treatments were analyzed using Two–Way ANOVA. The Pn–PAR curve was fit with the classic
191   Farquhar model to gain related parameters. Correlations among the growth and leaf
192   photosynthetic characteristics of three forages under shading were analyzed using Spearman's
193   rank correlation analysis. The SPSS 17.0 was used for statistical analysis.
194
195

Results
196
Leaf traits and biomass growth under shading
197

198   The LDMC and LMA of three forages were affected by shading, species and their interaction,
199   while the LT was only affected by shading and the biomass was only affected by species (Table
200   1). The LT of cocksfoot did not change under all treatments but it tended to decrease with the
201   enhancement of shading. The LT of white clover decreased with the enhancement of shading
202   (Fig. 1). For lucerne, LT was reduced by enhanced shading and did not change under reduced
203   shading. The LDMC of cocksfoot and lucerne decreased with the enhancement of shading (Fig.
204   1). For white clover, LDMC was reduced by enhanced shading and was not changed by reduced
205   shading. The LMA of all three forages decreased with the enhancement of shading (Fig. 1). In
206   response to the enhancement of shading, LT of cocksfoot did not change, but LDMC and LMA
207   decreased, and its LMA was the lowest among all forages under enhanced shading. The biomass
208   of cocksfoot was much higher than the other forages, which tended to decrease with enhanced
209   shading. The biomass of white clover and lucerne were not changed by shading (Fig. 1).
210
211   **Leaf photosynthetic gas exchange under shading**
212   The Pn and WUEi were affected by shading, species and their interaction (Table 1). The Pn of
213   cocksfoot was reduced by enhanced shading and did not change under reduced shading (Fig. 2).
214   The Pn of white clover and lucerne decreased with the enhancement of shading. The WUEi of
215   cocksfoot decreased with the enhancement of shading (Fig. 2). For whiter clover and lucerne,
216   WUEi was elevated by enhanced and reduced shading. In response to the enhancement of
217   shading, the Pn of cocksfoot changed little and both Pn and WUEi were not different from those
218   of other two forages under enhanced shading.
219   The shading imposed various impacts on Pn–PAR curves of different forages (Table 2).The
220   Rd of cocksfoot was elevated by reduced shading but was not impacted by enhanced shading.
221   The Amax and LCP of cocksfoot tended to decrease with the enhancement of shading, while
222   LSP was reduced by enhanced and reduced shading but Qapp was elevated. The Rd of white
223   clover was reduced by enhanced and reduced shading. The Amax, LCP and LSP of white clover
224   tended to decrease with the enhancement of shading. Its Qapp tended to decrease under enhanced
225   and reduced shading. For lucerne, the Rd, Amax and LCP tended to decrease with the
226   enhancement of shading. The LSP tended to decrease under enhanced and reduced shading but
227   the Qapp was elevated. The Rd and LCP of cocksfoot were lower than other forages but the
228   Amax was not different and the Qapp was even higher under enhanced shading.
229
230   **Leaf chlorophyll component and fluorescence under shading**
231   The content and proportion of chlorophyll component were affected by species, but seldom by
232   shading and their interaction (Table 1). For cocksfoot, the contents of all chlorophyll components
233   and chlorophyll a/b were not impacted by enhanced and reduced shading (Fig. 3). For white
234   clover, the contents of chlorophyll a, b and a+b increased with the enhancement of shading,
235   while chlorophyll a/b decreased. For lucerne, the contents of all chlorophyll components and
236   chlorophyll a/b were not changed by enhanced and reduced shading. The contents of chlorophyll

237   a, b and a+b were highest in cocksfoot, while for chlorophyll a/b, it appeared as white clover >
238   lucerne> cocksfoot.
239   Chlorophyll fluorescence was affected by species and their interaction (Table 1). The Fv/Fm
240   increased with the enhancement of shading in cocksfoot and was not changed in white clover,
241   while in lucerne, Fv/Fm was elevated by enhanced and reduced shading, and it was higher under
242   reduced shading than enhanced shading (Fig. 4). The ϕPS II and qP of cocksfoot were lowered
243   by enhanced and reduced shading and in white clover they were not impacted, while in lucerne,
244   the ϕPS II and qP decreased with the enhancement of shading (Fig. 4). In cocksfoot, the ϕPS II
245   and qP were higher under enhanced shading than reduced shading. The NPQ of cocksfoot
246   decreased with the enhancement of shading (Fig. 4). For white clover, NPQ was lowered by
247   enhanced and reduced shading, while in lucerne, NPQ was elevated. In response to the
248   enhancement of shading, the Fv/Fm increased in cocksfoot and its NPQ was far lower than those
249   in other two forages.
250

251 **Correlations among the growth and leaf photosynthetic characteristics of three**
252 **forages under shading**
253   The biomass was positively correlated with WUEi, chlorophyll a, b and a+b contents, but
254   negatively correlated with chlorophyll a/b, Fv/Fm, ϕPS II, qP and NPQ (Table 3). The Pn was
255   positively correlated with LDMC, LMA, chlorophyll a/b, Fv/Fm, ϕPS II and qP, but negatively
256   correlated with chlorophyll a+b content. The WUEi was positively correlated with LDMC, LMA,
257   chlorophyll a, b and a+b contents, but negatively correlated with chlorophyll a/b, Fv/Fm, ϕPS II,
258   qP and NPQ.
259

260 **Discussion**
261 **Effects of shading on leaf traits of three forages**
262   Leaf trait is partly the consequence that a plant responds to the external environments at leaf
263   scale (*Vendramini et al., 2002*) and its change is one of the most important strategies that the
264   plant has developed to cope with adverse environments. This study showed that species, shading
265   and their interaction imposed significant impacts on leaf traits. Shading reduced LT, LDMC and
266   LMA. Thus, shading may reduce assimilates accumulation but enhance the allocation for
267   potential enhancement of photosynthetic photon capture because lower LT, LDMC and LMA
268   generally indicate more input into photosynthetic area (*Modrzy et al., 2015*).These changes
269   would result in enhanced photosynthesis. In addition, shading may help to maintain soil water
270   status and improve air humidity under the tree, which potentially ameliorate the possible water
271   stress that the forages are encountering, especially in this semi-arid and rainfed region. The LT
272   wasn't affected by species, but it tended to be higher in cocksfoot than other species and did not
273   change with shading, suggesting that cocksfoot may be more tolerant to shading. Much quicker
274   decline in LMA of cocksfoot also proved that this species is more adaptive to shading, as lower
275   LMA shows stronger potential to use weak light under the tree.
276

Effects of shading on chlorophyll contents and fluorescence of three forages

Generally, a plant with high chlorophyll content and low chlorophyll a/b has stronger resistance to shading (*Boardman*, *1977*). In this study, species and its interaction with shading significantly affected chlorophyll a, b, a+b contents and a/b, while shading showed rare effect. Only in white clover, the contents reduced and chlorophyll a/b increased along with the reduction in shading, suggesting that chlorophyll content and ratio weren't influenced by shading in cocksfoot and lucerne. Intriguingly, the contents of chlorophyll component in cocksfoot were highest, but chlorophyll a/b was lowest, indicating that cocksfoot is more efficient in use of weak light because high chlorophyll b content and proportion promise a plant to do so (*Abrams*, *1987*; *Threlfall*, *1981*).

Chlorophyll fluorescence reflects the actual and maximum photosynthesis, the function of reaction center and the heat dissipation of a plant (*Govindjee, 2002*). In this study, all fluorescence was significantly affected only by species. The NPQ tended to be lowest in cocksfoot, and the Fv/Fm increased with the enhancement of shading, suggesting that this species is more tolerant to shading. The increased Fv/Fm reflects the enhancement of potential PSII photochemical efficiency of leaves after a fully dark adaptation (*Demmig & Björkman*, *1987*). The lower NPQ shows less light energy consumption as heat dissipation (*Genty et al.*, *1989*). Compared to lucerne and white clover, cocksfoot showed normal light conversing efficiency and light trapping efficiency under shading, but much lower light energy loss, helping to adapt to weakened light environments.

Effects of shading on Pn, WUEi and biomass of three forages

In this study, constant Pn and WUEi were significantly affected by species, shading and their interaction. The Pn and WUEi tended to decrease with the enhancement of shading. These may suggest that under tree shading, weakened light led to Pn decrease because generally, Pn and light intensity are positively correlated with suitable water supply under natural light. However, improved water status in soils and relative air humidity under the trees would have kept stomata open, consequently leading to great transpiration (rate). Thus, the WUEi would decrease with shading as it was calculated with Pn/E. From another viewpoint, it also proved that shading may improve water supply around the forage and tree. Changes in Pn with shading among species may be due to variations in leaf traits and chlorophyll features as there were positive correlations of Pn with LDMC, LMA and chlorophyll a/b, ϕPS II and qP, and negative correlation with chlorophyll a+b content. As for WUEi, there were contrasting roles played by chlorophyll features as the WUEi was positively correlated with chlorophyll a, b and a+b contents, but negatively correlated with chlorophyll a/b, Fv/Fm, ϕPS II, qP and NPQ. Compared with other forages, the Pn of cocksfoot changed little with shading, and both Pn and WUEi were not different from other forages, suggesting that this species was more tolerant to shading.

The Pn–PAR curve provides very useful parameters to address photosynthetic responses of a plant to adverse environment, while eliminating much interference, i.e. insufficient light radiation (*Lewis et al.*, *2000*). In this study, the LCP and Amax decreased with the enhancement

317   of shading, suggesting that all species are acclimating to shading, while the Rd, Qapp and LSP
318   changed in a species-specific way. Compared to white clover and lucerne, the Rd and LCP of
319   cocksfoot were lower, but the Amax was similar, and the Qapp was even higher under shading,
320   indicating that cocksfoot may be more tolerant. The lower LCP indicates that the plant can
321   survive in weakened light environments (*Taiz & Zeiger*, *2010*), i.e. tree shading, and generally,
322   shading-tolerant plants have lower Rd (*Lewis et al.*, *2000*). The greater Qapp reflects stronger
323   photosynthesis to use weak light. Thus, it suggested that cocksfoot could make better use of
324   weak light and adapt to shading, compared to other species.
325   The biomass of cocksfoot was much higher than the other forages, which decreased little
326   under enhanced shading compared to normal shading. Cocksfoot has good adaptability to various
327   environmental conditions, such as drought and restricted light conditions, with good regrowth
328   characteristics (*Sanada et al.*, *2010*). Change in biomass accumulation with shading among
329   species was more correlated with WUEi, but not Pn in the orchard environment. Therefore,
330   cocksfoot might be more beneficial to provide biomass under tree shading.
331   As known, plant biomass accumulation was not only impacted by light, but also by soil
332   carbon and nutrients. Soil nutrients (such as N and P) can indirectly affect the utilization of light
333   radiation by regulating photosynthesis apparatus (*Arain et al.*, *2002*; *Palmroth et al.*, *2014*). In
334   this study, there were similar basic soil feature and relative enough nutrient supply to soils.
335   Therefore, the difference in effects of soil nutrients on the plant might be negligible. However, it
336   is obliged to admit the fact soil nutrient availability would change after longer time forage
337   growth and this would affect the response of forage to light radiation, so further studies would be
338   required in the future.
339

Conclusions

341   Shading imposed significant impacts on the growth and leaf photosynthetic characteristics, while
342   there were differences among species. Shading affected chlorophyll content and fluorescence,
343   LDMC and LMA, which finally changed biomass accumulation. Cocksfoot was more efficient
344   than the other two forages in use of weak light and more tolerant to tree shading. In the apple
345   orchard, we recommend that selecting some shading-tolerant grasses, i.e. cocksfoot, or widening
346   the distance between individual apple trees, would be practical options for the orchard-forage
347   system in the Loess Plateau of China.
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Table 1(on next page)

Effects of species, shading and their interaction on leaf trait, photosynthetic gas exchange and chlorophyll feature in the orchard-forage system.

NS indicates non-significant; * indicates significance at $P \leq 0.05$; ** indicates significance at $P \leq 0.01$; *** indicates significance at $P \leq 0.001$. LT, leaf thickness; LDMC, leaf dry matter content; LMA, leaf mass per unit area; Pn, net photosynthetic rate; WUE_i, instantaneous water use efficiency; Fv/Fm, maximum photochemical rate; φPS II, actual photochemical efficiency of PS II; qP, photochemical quenching coefficient; NPQ, non-photochemical quenching.

Factor	Biomass	LT	LDMC	LMA	Pn	WUE_i	Chlorophyll a	Chlorophyll b	Chlorophyll $a+b$	Chlorophyll a/b	Fv/Fm	ϕPS II	qP	NPQ
Species	***	NS	***	**	**	***	***	***	***	***	***	***	***	***
Shading	NS	*	***	***	***	***	NS	NS	NS	*	NS	NS	NS	NS
Species×Shading	NS	NS	*	***	***	***	*	NS	*	NS	**	**	**	**

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Table 2(on next page)

Optimized parameters of the exponential rise to max function from Pn-PAR curves of three forages under shading.

Rd, dark respiration rate; Qapp, apparent quantum efficiency; LCP, light compensation point; LSP, light saturation point. Amax, maximum assimilation rate.

	Cocksfoot			White clover			Lucerne		
	Enhanced shading	Normal shading	Reduced shading	Enhanced shading	Normal shading	Reduced shading	Enhanced shading	Normal shading	Reduced shading
Rd (μmol m^{-2} s^{-1})	0.35	0.33	1.54	1.22	1.60	1.29	1.24	2.21	3.28
Qapp (μmol mol^{-1})	0.07	0.04	0.10	0.06	0.07	0.05	0.06	0.05	0.06
LCP (μmol m^{-2} s^{-1})	5.1	8.3	15.9	21.3	24.1	26.7	21.4	46.8	58.7
LSP (μmol m^{-2} s^{-1})	722	1757	707	524	544	1079	814	880	819
Amax (μmol m^{-2} s^{-1})	13.8	17.7	25.3	13.2	16.4	19.8	18.2	20.0	24.5

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Table 3(on next page)

Correlations among the biomass, leaf thickness (LT), leaf dry matter content (LDMC), leaf mass per unit area (LMA), net photosynthetic rate (Pn), instantaneous water use efficiency (WUEi), chlorophyll content and fluorescence of three forages under trees.

Spearman's correlation coefficients (n=108) are shown. Significance are shown with *** ($P \leq$ 0.001), ** ($P \leq 0.01$) and * ($P \leq 0.05$). Fv/Fm, maximum photochemical rate; ϕPS II, actual photochemical efficiency of PS II; qP, photochemical quenching coefficient; NPQ, non-photochemical quenching.

	LT	LDMC	LMA	Pn	WUEi	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a+b*	Chlorophyll *a/b*	Fv/Fm	ϕPS II	qP	NPQ
Biomass	0.06	0.14	0.04	-0.15	0.49***	0.54***	0.79***	0.73***	-0.79***	-0.52***	-0.60***	-0.63***	-0.49***
Pn	0.11	0.30**	0.31**	1	0.30**	-0.17	-0.19	-0.19*	0.24*	0.25**	0.29**	0.32***	-0.05
WUE$_i$	0.04	0.22*	0.38***	0.30**	1	0.23*	0.39***	0.34***	-0.39***	-0.29**	-0.32***	-0.33***	-0.31**

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Figure 1(on next page)

Leaf thickness (LT), leaf dry matter content (LDMC), leaf mass per unit area (LMA) and biomass of three forages under shading.

Different capital letters denote significant differences among species under the same shading treatment ($P \leq 0.05$). Different lowercase letters denote significant differences among shading treatments for the same species ($P \leq 0.05$). Bars show standard deviation.
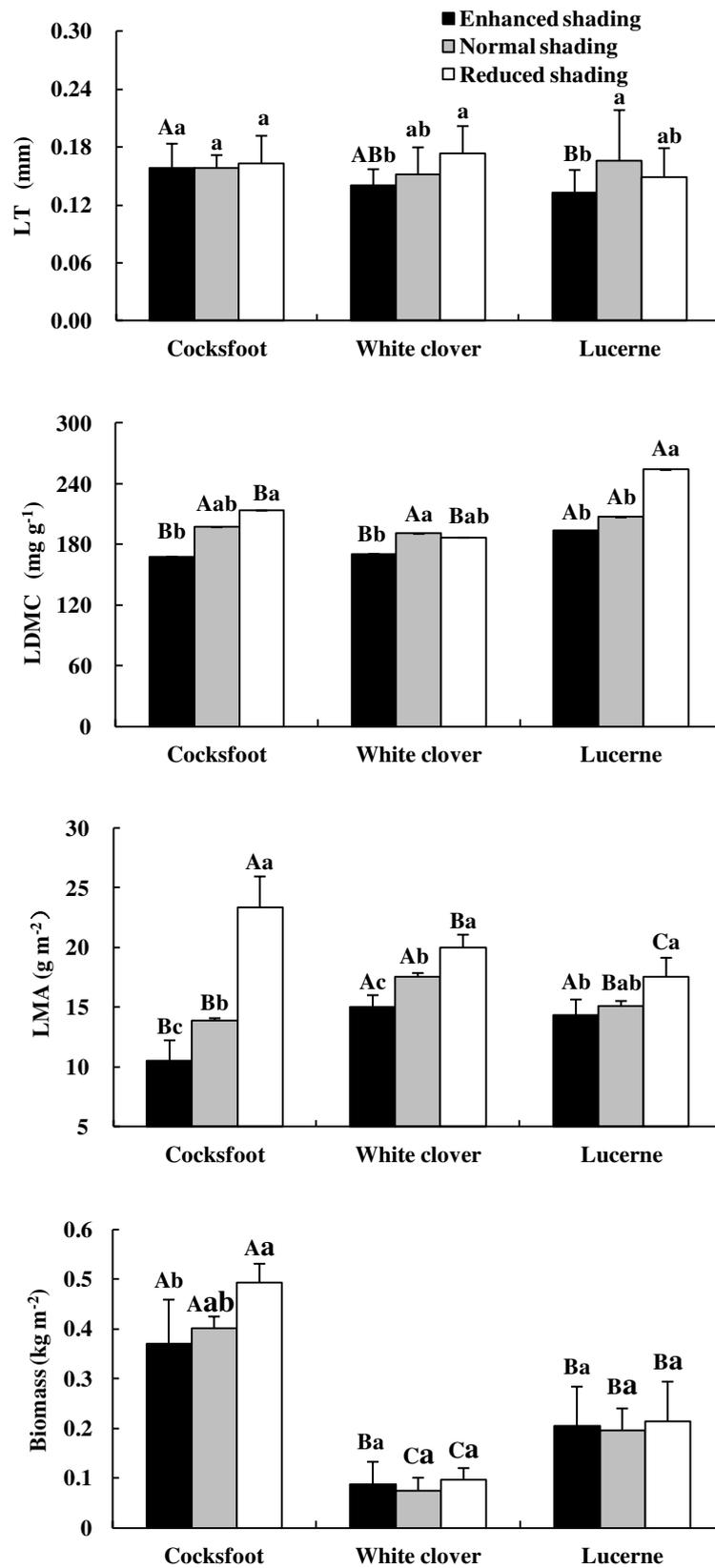
Figure 2(on next page)

Net photosynthetic rate (Pn) and instantaneous water use efficiency (WUEi) of three forages under shading.

Different capital letters denote significant differences among species under the same shading treatment $(P \leq 0.05)$. Different lowercase letters denote significant differences among shading treatments for the same species $(P \leq 0.05)$. Bars show standard deviation.
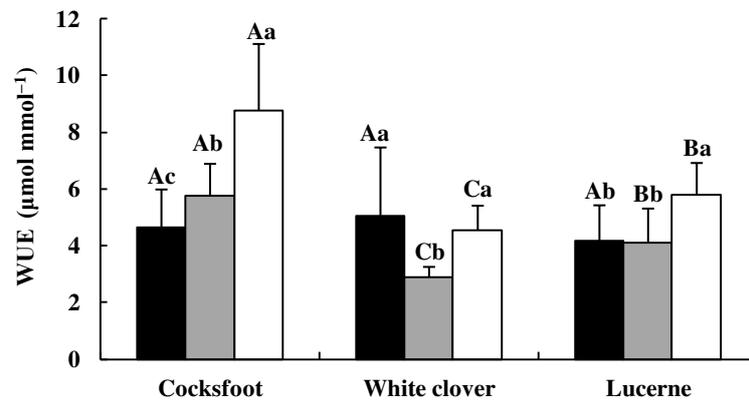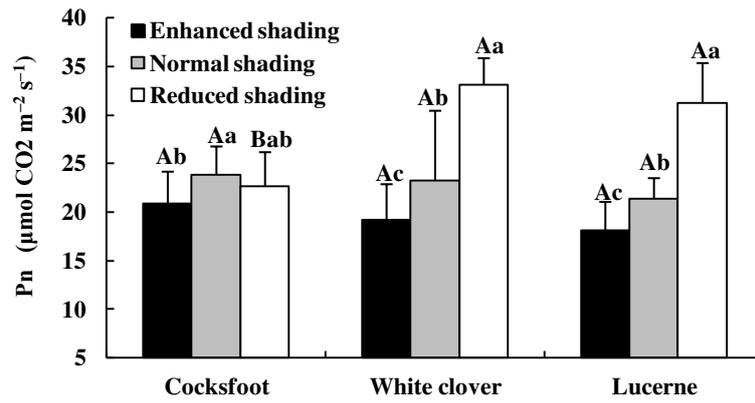
Figure 3(on next page)

Chlorophyll component and content in leaves of three forages under shading.

Different capital letters denote significant differences among species under the same shading treatment ($P \leq 0.05$). Different lowercase letters denote significant differences among shading treatments for the same species ($P \leq 0.05$). Bars show standard deviation.
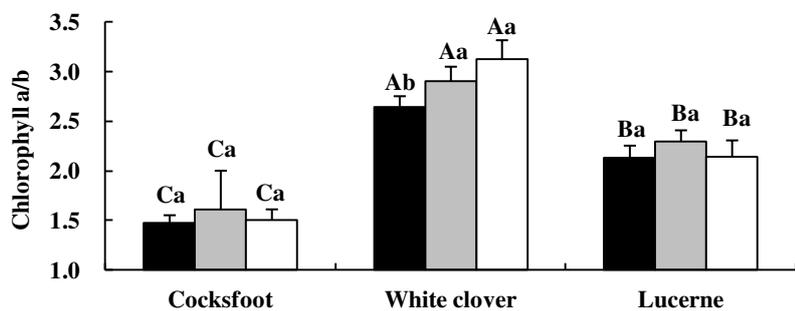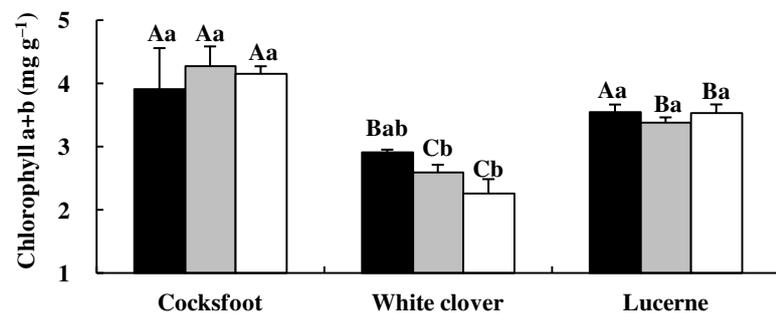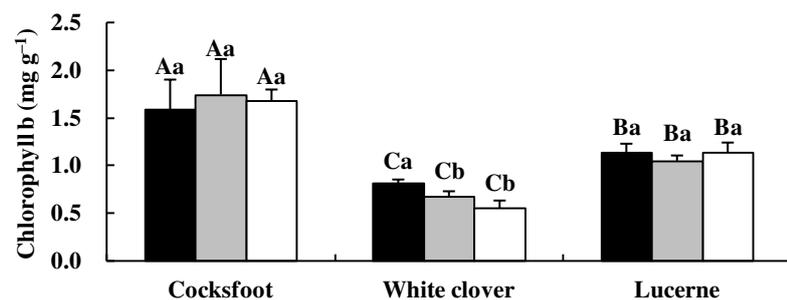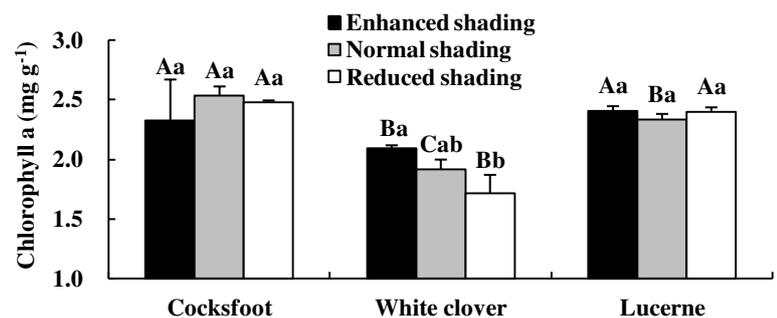
Figure 4 (on next page)

Chlorophyll fluorescence in leaves of three forages under shading.

Different capital letters denote significant differences among species under the same shading treatment ($P \leq 0.05$). Different lowercase letters denote significant differences among shading treatments for the same species ($P \leq 0.05$). Bars show standard deviation.