

Light availability is improved for legume species grown in moderately N-fertilized mixtures with non-legume species

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Running title: Light availability is improved for legume species

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1 **Abstract**

2 Empirical evidence indicates a positive relationship between grassland phytodiversity and
3 yield. One cause may be species' complementary use of available resources. The aim of this
4 study was to investigate possible complementarities between grassland species with differing
5 spatial arrangements of leaves. Mixtures of *Trifolium pratense* L., *Phleum pratense* L.,
6 *Lolium perenne* L., and *Cichorium intybus* L. or *Medicago sativa* L. and pure stands of all
7 species were established in 2007 at Svalöv, Sweden, in a field experiment receiving a total
8 input of 100 kg N ha⁻¹. Community height, light transmission, yield, and species composition
9 as well as species' $\delta^{13}\text{C}$ signatures and N concentrations were measured on four mowing
10 occasions in 2009. Species' $\delta^{13}\text{C}$ signatures are directly affected by carbon assimilation and
11 stomatal conductivity for water, and indirectly by light, nitrogen and water availability as well
12 as community composition. Light transmission through the sward was greatest in pure stand
13 non-legumes; mixed communities intercepted more light than these, albeit not generally more
14 than pure legumes. Non-legume species had more depleted $\delta^{13}\text{C}$ signatures when grown in
15 mixtures than in pure stands, but the opposite was true for legumes. The $\delta^{13}\text{C}$ signatures
16 generally became enriched with increases in light transmission (grasses and legumes), but not
17 with increases in N concentration (grasses). Community composition affected the $\delta^{13}\text{C}$
18 signatures of all species except *C. intybus*. Our results suggest that mixing species of
19 contrasting leaf morphologies and biomass distribution contributed to (i) increased light
20 capture by mixtures over pure stand non-legumes, and (ii) better light availability in mixed
21 than in pure stand legumes.

22

23 **Zusammenfassung**

24 Empirische Untersuchungen zeigen einen positiven Zusammenhang zwischen pflanzlicher
25 Diversität im Grünland und dem Ertrag. Ein Grund dafür scheint die komplementäre Nutzung

26 von Ressourcen zu sein. Das Ziel dieser Studie war es, mögliche Komplementaritäten
27 zwischen Grünlandarten zu untersuchen, die sich im räumlichen Arrangement ihrer Blätter
28 unterscheiden. Mischungen aus *Trifolium pratense* L., *Phleum pratense* L., *Lolium perenne*
29 L., und *Cichorium intybus* L. oder *Medicago sativa* L. sowie Monokulturen aller Arten
30 wurden 2007 in einem Feldversuch in Svalöv, Schweden, angelegt. Die Bestandeshöhe,
31 Lichttransmission, Ertrag und botanische Zusammensetzung wurden an vier Erntezeitpunkten
32 2009 erhoben. Die $\delta^{13}\text{C}$ -Signaturen der Arten sowie die N-Konzentrationen der oberirdischen
33 Biomasse wurden analysiert. Die $\delta^{13}\text{C}$ -Signaturen werden direkt durch die
34 Kohlenstoffassimilation und stomatäre Wasserleitfähigkeit, sowie indirekt durch die
35 Verfügbarkeit von Licht, Stickstoff und Wasser sowie die botanische Zusammensetzung des
36 Bestandes beeinflusst. Das Experiment wurde insgesamt mit 100 kg N ha^{-1} gedüngt. Die
37 Lichttransmission durch den Grasbestand war in den Monokulturen der Nichtleguminosen am
38 höchsten. Mischkulturen absorbierten mehr Licht als letztere, aber generell nicht mehr als die
39 Leguminosen-Monokulturen. Nicht-Leguminosen in Mischungen waren angereicherter im
40 $\delta^{13}\text{C}$ als in Monokulturen, aber für Leguminosen galt das Gegenteil. Die $\delta^{13}\text{C}$ -Signaturen
41 wurden generell angereicherter mit höherer Lichttransmission (Gräser und Leguminosen) aber
42 nicht mit höherer N-Konzentration (Gräser). Die Artenzusammensetzung der Kulturen
43 beeinflusste die $\delta^{13}\text{C}$ -Signaturen aller Arten bis auf *C. intybus*. Unsere Ergebnisse deuten
44 darauf hin, dass das Mischen von Arten mit unterschiedlicher Blattmorphologie und
45 Biomasseverteilung (i) zu besserer Lichtabsorption von Mischungen als von Monokulturen
46 von Nicht-Leguminosen sowie (ii) zu besserer Lichtverfügbarkeit in Mischungen als in
47 Monokulturen von Leguminosen beiträgt.

48 **Keywords:** $\delta^{13}\text{C}$ signatures, forb, grass, legume, light transmission

49

50

51 **Introduction**

52 Empirical evidence indicates a positive relationship between grassland phytodiversity and
53 yield in both extensively (Weigelt, Weisser, Buchmann, & Scherer-Lorentzen 2009) and
54 intensively managed systems (Finn et al. 2013). One possible reason is complementary use of
55 available resources among species, such as resource partitioning by legumes and non-legumes
56 with respect to N acquisition (Temperton et al. 2007; Nyfeler, Huguenin-Elie, Suter, Frossard
57 & Lüscher 2011). Other complementarities may involve differences in the spatial arrangement
58 of leaves among species causing complementarity in light exploitation (Anten & Hirose 1999)
59 or in rooting depth causing complementarities in the use of water (Hoekstra, Finn, & Lüscher
60 2014) and mineral nutrients uptake (von Felten et al. 2009). The existing studies on
61 complementarities in light interception have shown that species diversity may increase light
62 capture (Spehn, Joshi, Schmid, Diemer, & Körner 2000; Jumpponen, Mulder, Huss-Danell, &
63 Högberg 2005; Roscher, Kutsch, & Schulze 2011a; Roscher, Schmid, Buchmann, Weigelt, &
64 Schulze 2011b; Gubsch et al. 2011).

65 Plant $\delta^{13}\text{C}$ signatures (i.e., the ratio of the stable isotopes of carbon, ^{13}C , and ^{12}C) in plant
66 leaves or shoots are affected by environmental conditions such as light availability with poor
67 light availability resulting in more depleted $\delta^{13}\text{C}$ signatures (Jumpponen, Mulder, Huss-
68 Danell, & Högberg 2005; Roscher, Kutsch, & Schulze 2011a; Roscher, Schmid, Buchmann,
69 Weigelt, & Schultze 2011b). Further, a poor nitrogen (N) nutrition will result in more
70 depleted $\delta^{13}\text{C}$ signatures (Bender & Berge 1979), since C assimilation is related to the N
71 concentration in the leaf (Evans, 1989). The $\delta^{13}\text{C}$ signatures are also related to water
72 availability via the influence of stomatal conductivity for H_2O on CO_2 assimilation (Farquhar
73 & Richards 1984). An overview of factors influencing $\delta^{13}\text{C}$ is shown in Fig. 1, highlighting
74 factors considered in the present study and their effects on $\delta^{13}\text{C}$. Water shortage was not a
75 major limiting factor during the study.

76 A growing body of evidence suggests that species identity or functional diversity has a larger
77 impact on ecosystem services than species richness per se (e.g. Emery & Gross 2007;
78 Mokany, Ash & Roxburgh 2008). The few studies that have so far systematically analysed the
79 effect of plant community diversity on $\delta^{13}\text{C}$ signature as a measure of light acquisition
80 (Jumpponen, Mulder, Huss-Danell, & Högberg 2005; Gubsch et al. 2011; Roscher, Kutsch, &
81 Schulze 2011a; Roscher, Schmid, Buchmann, Weigelt, & Schulze 2011b) show that the
82 plants' morphology, and their adaptive responses to increased competition for light had a
83 major influence on individual species' $\delta^{13}\text{C}$ signatures.

84 Here, we investigated the influence of community composition, light transmission through the
85 canopy, and N concentration on the $\delta^{13}\text{C}$ signatures of individual plant species. We used
86 potentially dominating species of contrasting morphologies: grasses (erect leaves), legumes
87 (horizontal leaves) and a forb with erect leaves. We hypothesized that (i) light transmission is
88 less in mixed communities than in pure stands, and (ii) legumes affect the non-legume
89 species' $\delta^{13}\text{C}$ signature through effects on N nutrition and light acquisition. The present study
90 differs from previous ones in establishing communities with constant species richness but
91 different proportions of the species sown, thus removing the sampling effect and enabling
92 assessment of the impact of functional diversity over a range of plant species compositions.
93 The potentially high-yielding species used were managed in a field experiment according to
94 common local agricultural practices.

95

96 **Material and methods**

97 *Study site and weather*

98 A field experiment was established at Svalöv, Sweden (55° 55'N, 13° 07'E, 55 m a.s.l.), in
99 June 2007. The climate is cool-temperate with an annual mean temperature of 7.7 °C and
100 annual mean precipitation of 700 mm. The soil at the site was a sandy loam with a pH of 5.8

101 containing 2.0% organic matter, 99 mg total phosphorus kg⁻¹, and 87 mg potassium (K) kg⁻¹.
102 The experimental plot received 42 kg phosphorus and 150 kg K ha⁻¹ at sowing (2007), and 45
103 kg K and 6 kg sulphur ha⁻¹ each harvest year. In the harvest years, 100 kg N ha⁻¹ yr⁻¹ was
104 applied in split dressings (i.e., 40 kg of N ha⁻¹ in early spring and 20 kg of N ha⁻¹ for each
105 summer regrowth in 2009). The plots were mowed three times in 2008 and four times in
106 2009. This paper uses data collected from the 2009 harvests (20 May, 24 June, 29 July, and 2
107 Sept.). Grasses were in vegetative stage in all harvests except the second, while the two
108 legumes and *C. intybus* exhibited reproductive structures in all harvests. The 2009 growing
109 season was slightly warmer and wetter than the average for the site (see Appendix A: Fig. 1).

110

111 ***Experimental treatments***

112 The species used were selected based on their contrasting functional traits and were combined
113 in two different four-species mixtures. All mixtures contained two grasses differing in their
114 rate of establishment and competitive ability, namely, *Lolium perenne* L. (cv. Birger, fast
115 establishment, competitive) and *Phleum pratense* L. (cv. Ragnar, slow establishment, non-
116 competitive), and one legume, namely, *Trifolium pratense* L. (cv. Vivi), which is a fast-
117 establishing, short-lived species (Frame 2005). These three species are moderate in height.
118 The fourth component was a tall forb, either *Cichorium intybus* L. (cv. Grasslands Puna) or
119 another slow-establishing legume, namely, *Medicago sativa* L. (cv. Pondus).

120 The experimental setup consisted of 48 communities (see Appendix A: Table 1). Thirty
121 communities followed a simplex design (Cornell 2002) with four pure stands of *P. pratense*,
122 *L. perenne*, *T. pratense* and *C. intybus*, and 11 mixtures of these four species all sown at two
123 densities (Mixture type 1). In addition, 18 communities followed a simplex design using *M.*
124 *sativa* instead of *C. intybus* (Mixture type 2), i.e. four pure stands of *P. pratense*, *L. perenne*,
125 *T. pratense* and *M. sativa*, and five mixtures of these four species all sown at two densities. In

126 total, 48 plots were arranged in a completely randomized design, with an individual plot size
127 of 17 m². As the plant species composition of the mixtures varied depending on the seeding
128 rates of each species and on the mowing occasion, we could evaluate the effect of plant
129 species composition on individual species' $\delta^{13}\text{C}$ signatures over four periods of the 2009
130 season. However, we also evaluated the $\delta^{13}\text{C}$ signature of each species by comparing the
131 species' values from mixed and pure stands.

132 Biomass harvested was normal for the site (<12 tons ha⁻¹ (Frankow-Lindberg & Dahlin
133 2013)). Mixtures always showed over-yielding (Frankow-Lindberg 2012). The two first
134 harvests were dominated by grasses, while the two legumes dominated the third and the
135 fourth harvests (for details on the plant species composition, see Frankow-Lindberg & Dahlin
136 (2013)). Both legumes were fixing N₂ from the atmosphere, and transfer of atmospherically
137 fixed N from the legumes to the grasses was observed (Frankow-Lindberg & Dahlin 2013).

138

139 *Measurements*

140 Community height was recorded by measuring the height (not extended) of the tallest plants
141 at five points along a transect through each plot before each mowing occasion (i.e. 18 May,
142 22 June, 27 July, and 17 Aug.). The light transmission through the canopy (i.e., percent of
143 incoming light (PAR)) of each plot was recorded on the same dates using a LiCor Quantum
144 sensor (1 m long, five readings per plot at each measurement occasion and covering
145 approximately half the length of each plot) connected to a Quantum meter (LI-189, LM 189;
146 Li-Cor, Lincoln, NE).

147

148 *Sampling*

149 Whole plots were cut to a stubble height of approx. 7 cm with a Haldrup plot harvester.
150 Samples for the analysis of dry matter (DM) yield and plant species composition (expressed

151 as species dry matter percent of the sampled biomass) were taken from the accumulated
152 biomass on each mowing occasion. The botanical samples were sorted into each sown and
153 unsown species, dried and weighed. Unsown species contributed less than 6% dry matter of
154 the harvested biomass. Most unsown species were annuals of a very small stature and were
155 therefore considered of minor importance with respect to light transmission. The sown
156 fractions from all harvests were ground per species to pass through a 1 mm screen, sub-
157 sampled by riffle splitting, ball milled, and finally analysed for ^{13}C abundance, i.e., ^{13}C
158 expressed in the standard notation ($\delta^{13}\text{C}$) in per mille relative to the international standard V-
159 PDB (Vienna PeeDee Belemnite) and N concentration using a PDZ Europa ANCA-GSL
160 interfaced to a PDZ Europa 20-20 isotope ratio spectrometer (Sercon Ltd., Cheshire, UK).
161 The $\delta^{13}\text{C}$ of the source air may affect plant $\delta^{13}\text{C}$ values to some extent, especially if canopy
162 density is high, but the major influence is photosynthetic carbon isotope discrimination
163 (Buchmann, Brooks, & Ehleringer 2002).

164

165 *Data analysis*

166 Community height and light transmission through the canopy were evaluated by completely
167 randomized repeated-measures ANOVAs according to the model $Y = \text{TYPE} + \text{DENS}$
168 $+ \varepsilon$, augmented with terms for interactions with mowing occasion. TYPE denotes the two
169 mixture types and the respective pure stands and DENS denotes the sowing density; both
170 were included as fixed factors. Light transmission data were log transformed before analysis
171 to reduce heteroscedasticity. Individual species' $\delta^{13}\text{C}$ signatures for each mowing occasion
172 were evaluated in two ways. In a first step, we used $\delta^{13}\text{C}$ as the dependent variable and
173 evaluated mixture versus pure stand effects. These were evaluated as completely randomized
174 repeated-measures ANOVAs according to the model: $Y = \text{MONOMIX} + \text{TYPE} + \text{DENS}$
175 $+ \varepsilon$, augmented with terms for interactions with mowing occasion. MONOMIX (a variable set

176 to 0 for pure stands and to 1 for mixtures), TYPE (as above), and DENS (as above) were all
177 included as fixed factors. In these analyses, data from individual species in all mixtures were
178 used as observations, resulting in a high number of observations ($n = 22$ for species in
179 Mixture type 1 and $n = 10$ for species in Mixture type 2). For the pure stands, though, there
180 were true replicates for all species. Because of strong correlations between legume proportion,
181 light transmission and N concentrations of the non-legume species, it was impossible to carry
182 out a meaningful multiple regression analysis involving all the measured variables, allowing
183 to identify the relative importance of the different variables on $\delta^{13}\text{C}$ signatures.

184 Therefore, in the next step, linear correlations were calculated: (i) between light transmission
185 as the dependent variable and functional group proportions of the sampled biomass as the
186 independent variables; (ii) between individual species' $\delta^{13}\text{C}$ signatures as the dependent
187 variable and light transmission through the canopy and functional group proportions of the
188 sampled biomass as the independent variables, respectively; and (iii) individual species' N
189 concentrations as the dependent variable (non-legume species only) and legume proportion of
190 the sampled biomass as the independent variable. These were performed as completely
191 randomized repeated-measures analyses with variables for sown density (DENS as above)
192 and mixture type (TYPE as above) included as fixed factors. Interactions between the
193 independent variables and the fixed factors and between the independent variables and
194 mowing occasion were also included. The analyses of variables for each of the two tall forbs
195 were carried out using data from each Mixture type separately, and then the factor TYPE and
196 all interactions with TYPE were omitted. Data from the pure stands were omitted from these
197 analyses.

198 All repeated-measures analyses were carried out using the MIXED procedure in SAS/STAT
199 software, Version 9.1 (SAS Institute Inc., Cary, NC). Based on the Akaike information
200 criterion, the most appropriate covariance structure (i.e., unstructured, compound symmetry,

201 autoregressive, or Toeplitz) for each response variable was used to describe the time
202 dependence among harvests. The significance of each variable was evaluated using Type III
203 *F*-tests.

204

205 **Results**

206 *Species height*

207 The forbs *M. sativa* and *C. intybus* were often the significantly tallest species, while *T.*
208 *pratense* was the shortest of all species at the beginning and end of the growing season (Table
209 1) and was always shorter than the average height of the mixed communities. There were no
210 significant differences in height between the two grass species before the two first mowing
211 occasions, but *L. perenne* was significantly shorter than *P. pratense* before the two last
212 mowing occasions ($P < 0.001$). On these occasions, the former was also significantly shorter
213 than the average height of the mixed communities, while this was never the case for *P.*
214 *pratense*.

215

216 *Individual species $\delta^{13}\text{C}$ signatures in mixed and pure stands*

217 The $\delta^{13}\text{C}$ signatures were always more depleted in *P. pratense* ($P < 0.05$) grown in mixtures
218 than in pure stands (Fig. 2). For *L. perenne*, this effect was significant on the third mowing
219 occasion ($P < 0.05$). The $\delta^{13}\text{C}$ signatures of *C. intybus* were not significantly different
220 between mixtures and pure stands. In *T. pratense*, on the other hand, the $\delta^{13}\text{C}$ signatures were
221 often more depleted in plants grown in pure stands than in mixtures, significantly so on the
222 second mowing occasion ($P < 0.05$). This effect was also observed in *M. sativa*, but was not
223 significant. The $\delta^{13}\text{C}$ signatures of all species but *P. pratense* differed significantly between

224

225 mowing occasions, the most depleted signatures being observed on the third mowing occasion
($P < 0.001$). The identity of the tall forb did not significantly affect the $\delta^{13}\text{C}$ signatures of *P.*
226 *pratense*, *L. perenne*, or *T. pratense*. Sown density never significantly affected the $\delta^{13}\text{C}$
227 signatures.

228

229 ***Light transmission through the canopy***

230 The legumes, *M. sativa* in particular, formed closed canopies that resulted in very small
231 amounts of light reaching the soil surface before each harvest (Table 2). In contrast, *C.*
232 *intybus*, and – except on the first mowing occasion – the grasses formed quite open swards
233 where considerable light fell on the soil surface. Light transmission through the mixed
234 communities was generally small.

235 Light transmission through the sward was negatively correlated with legume proportion
236 ($P < 0.001$) and positively correlated with grass and *C. intybus* proportions at some mowing
237 occasions (see Appendix A: Table 2, Fig. 3).

238

239 ***Linear correlations with species' $\delta^{13}\text{C}$ signatures***

240 Increasing light transmission through the canopy was positively correlated with the $\delta^{13}\text{C}$
241 signatures of all species ($P < 0.05$) except *C. intybus* (see Appendix A: Table 2, Fig. 4). For
242 the grasses, the $\delta^{13}\text{C}$ signature was significantly smaller with *M. sativa* than with *C. intybus* as
243 the tall forb ($P < 0.01$).

244 There was a significant negative correlation between the $\delta^{13}\text{C}$ signatures of *P. pratense* (P
245 < 0.01) and *M. sativa* ($P < 0.05$) and the legume proportion in the sampled biomass, and
246 between the $\delta^{13}\text{C}$ signatures of *L. perenne* and *T. pratense* and the legume proportion in the
247 sampled biomass on some of the four mowing occasions, but no such correlations with the

248

249 signature of *C. intybus* (see Appendix A, Table 2, Fig. 5). Furthermore, there was a significant
positive correlation between the $\delta^{13}\text{C}$ signatures of all species except *C. intybus* and the grass
250 proportion in the sampled biomass ($P < 0.05$), while no such correlation existed between
251 species' $\delta^{13}\text{C}$ signatures and the *C. intybus* proportion in the sampled biomass (see Appendix
252 A: Table 2).

253
The correlation between the $\delta^{13}\text{C}$ signatures of the two grasses and their respective N
254 concentrations was strongly negative ($P < 0.001$), while no such effect was observed for *C.*
255 *intybus* (see Appendix A: Table 2, Fig. 6).

256

257 *Species N concentrations*

258 The N concentrations of the grasses were positively correlated with legume proportion in the
259 sampled biomass on all harvest occasions ($P < 0.001$). Furthermore, there was a positive
260 correlation between the N concentration of *C. intybus* and legume proportion on the second
261 mowing occasion ($P < 0.01$, see Appendix A: Table 2, Fig. 7).

262

263 **Discussion**

264 *Community composition, light transmission and $\delta^{13}\text{C}$ signatures*

265 The forbs *C. intybus* and *M. sativa* generally grew taller than the other species. Nevertheless,
266 the results suggest that height was not the major factor affecting light transmission through
267 the sward, since these two species had opposing effects on light transmission. This is in
268 contrast to results from more extensively managed swards with few yearly harvests, where
269 taller species had a strong negative impact on light capture and the performance of species
270 with a small stature (Anten & Hirose 1999; Jumpponen, Mulder, Huss-Danell, & Högberg
271 2005; Roscher, Kutsch, & Schulze 2011a). Instead, the contrasting morphologies of legumes
272 and non-legumes were the most important factor affecting light transmission through the

273 sward, and light transmission was negatively correlated with increasing legume proportion, a
274 result corroborated by Spehn, Joshi, Schmid and Körner (2000) and Roscher, Kutsch and
275 Schulze (2011a). Of the non-legume species, increasing proportions of both grasses and *C.*
276 *intybus* contributed to improved light transmission through the sward, despite differences in
277 realized heights. It is pertinent to note here that the heights of individual species were only
278 measured in the pure stands, and that height adjustments certainly happened in the mixed
279 stands (Lorentzen, Roscher, Schumacher, Schulze, & Schmid 2008; Roscher, Schmid,
280 Buchmann, Weigelt, & Schulze 2011b).

281
The $\delta^{13}\text{C}$ signatures were positively correlated with light transmission for all species
282 except *C. intybus*. Since light transmission was negatively correlated with legume proportion,
283 it is unsurprising that the $\delta^{13}\text{C}$ signatures of all species except *C. intybus* were also negatively
284 correlated with legume proportion, in line with Gubsch et al. (2011). The positive correlation
285 between $\delta^{13}\text{C}$ signatures and light transmission was strongest for the two legumes, suggesting
286 that despite their height differences their more horizontal leaf arrangement was a disadvantage
287 in the competition for light. In fact, the more enriched $\delta^{13}\text{C}$ signatures of both legumes in the
288 mixed versus the pure stand communities suggest that these species gained in light acquisition
289 in the mixed communities, even though at least *T. pratense* probably had to spend part of its
290 gain on growing longer internodes (Roscher, Schmid, Buchmann, Weigelt, & Schulze 2011b).
291 It is often noted that the N_2 fixation of legumes increases when grown in mixtures rather than
292 pure stands (Carlsson & Huss-Danell 2003), and this was also observed in the present
293 experiment (Frankow-Lindberg & Dahlin 2013). Part of this increase is likely due to the
294 uptake of soil N by non-legume species, forcing legumes to increase N_2 fixation (Nyfeler,
295 Huguenin-Elie, Suter, Frossard & Lüscher 2011), but the improvement in light conditions for
296 legumes in mixtures may also make more energy available for this energy-demanding
297 process. Both these possible sources of improved legume growth resulted in a slight legume

298 yield increase in the mixed communities (Frankow-Lindberg & Dahlin 2013) and more
299 enriched $\delta^{13}\text{C}$ signatures. However, in more heavily N-fertilized swards, light conditions
300 would be expected to be poorer, leading in turn to poorer legume performance (Nyfeler,
301 Huguenin-Elie, Suter, Frossard & Lüscher 2011).

302

303 *N nutrition and $\delta^{13}\text{C}$ signatures*

304 Unexpectedly, we found a negative correlation between the $\delta^{13}\text{C}$ signatures and N
305 concentrations of *P. pratense* and *L. perenne* (but not of *C. intybus*). Normally, an increasing
306 N concentration is expected to improve photosynthetic enzyme availability and thus CO_2
307 assimilation. However, results have varied depending on the species studied. Thus, for grasses
308 Gubsch et al. (2011) and Roscher, Kutsch and Schulze (2011a) found no correlation, while for
309 legumes Roscher, Schmid, Buchmann, Weigelt and Schulze (2011b) found a negative
310 relationship caused by morphological changes of the legumes with increasing diversity. In
311 our study, the results indicate that light transmission exerted a confounding effect, and that
312 shading by the legumes more strongly affected the non-legumes' $\delta^{13}\text{C}$ signatures than their
313 effect on N concentrations.

314

315 In conclusion, our results suggest that mixing species of contrasting leaf morphologies and
316 biomass distribution contributed to (i) increased light capture by mixtures over pure stand
317 non-legumes, and (ii) a better light availability for legumes in mixtures than in pure stands. In
318 turn, this may have contributed to the over-yielding recorded. A putative positive effect of
319 legumes on non-legume N nutrition and hence C assimilation could not be detected here
320 because of the legumes' strong and confounding effect on light transmission through the
321 canopy.

322

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327

328 Appendix A. Supplementary data

329 Supplementary data associated with this article can be found, in the on-line version, at

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331

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Figure legends

Fig. 1 Overview of factors influencing the stable carbon isotope composition of plant tissue ($\delta^{13}\text{C}$). Pluses indicate positive interactions, minuses negative ones (in the case of $\delta^{13}\text{C}$: enrichment (+) or depletion (-)) Factors highlighted were considered in the present study. Please note that only interactions of interest for isotopic composition are shown rather than all possible interactions among factors.

Fig. 2 Shoot $\delta^{13}\text{C}$ signatures of the species grown in pure stands (◆) and mixed communities (■ = mixtures with *C. intybus* and ▲ = mixtures with *M. sativa*) on each mowing occasion

Fig. 3 Light transmission through mixed swards was negatively correlated with legume (A) proportion and positively correlated with grass (B) and *C. intybus* (C, data from Mixture type 1 only) proportions of the sampled biomass before each mowing occasion. Light measurements were made two days before the harvest in all cases except the last, when they were made two weeks before the harvest. Significant correlations are denoted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For the full statistical analyses see Appendix A: Table 2

Fig. 4 With the exception of *C. intybus*, species' shoot $\delta^{13}\text{C}$ signatures were positively correlated with increasing light transmission through mixed swards. Data on *M. sativa* are from Mixture type 2 only. First (A), second (B), third (C), and fourth (D) mowing occasions. Light measurements were made two days before the harvest in all cases except the last, when they were made two weeks before the harvest. Significant correlations are denoted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For the full statistical analyses see Appendix A: Table 2

Fig. 5 With the exception of *C. intybus*, species' shoot $\delta^{13}\text{C}$ signatures were negatively correlated with legume proportion of the sampled biomass in mixed communities. Data on *M. sativa* are from Mixture type 2 only. First (A), second (B), third (C), and fourth (D) mowing occasions. Significant correlations are denoted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For the full statistical analyses see Appendix A: Table 2



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Fig. 6 Non-legume species' shoot $\delta^{13}\text{C}$ signatures were negatively correlated with shoot N concentrations. First (A), second (B), third (C), and fourth (D) mowing occasions. Significant correlations are denoted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For the full statistical analyses see Appendix A: Table 2

Fig. 7 Non-legume species' shoot N concentrations were positively correlated with legume proportion of the sampled biomass in mixed communities. First (A), second (B), third (C), and fourth (D) mowing occasions. Data on *C. intybus* are from Mixture type 1 only. Significant correlations are denoted * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For the full statistical analyses see Appendix A: Table 2

Table 1. Height (cm) of pure and mixed stands. Means and standard deviations are shown. Superscript letters indicate statistical differences within harvests. H1, H2, H3, and H4 denote the four harvest occasions

Crop	H1	H2	H3	H4
<i>P. pratense</i>	34 ± 2.2 ^b	36 ± 2.5 ^b	32 ± 4.2 ^b	25 ± 1.3 ^b
<i>L. perenne</i>	30 ± 2.2 ^b	39 ± 2.5 ^b	19 ± 4.2 ^c	16 ± 1.3 ^d
<i>T. pratense</i>	22 ± 2.2 ^c	40 ± 2.5 ^b	32 ± 4.2 ^b	17 ± 1.3 ^d
<i>C. intybus</i>	32 ± 3.1 ^b	65 ± 3.5 ^a	66 ± 6.0 ^a	30 ± 1.9 ^a
<i>M. sativa</i>	68 ± 3.1 ^a	39 ± 3.5 ^b	70 ± 6.0 ^a	32 ± 1.9 ^a
Mixture type 1	32 ± 0.9 ^b	41 ± 1.1 ^b	39 ± 1.8 ^b	19 ± 0.6 ^c
Mixture type 2	35 ± 1.4 ^b	36 ± 1.6 ^b	41 ± 2.7 ^b	22 ± 0.8 ^b

Table 2. Light transmission (% of incoming PAR light) through the canopy of pure and mixed stands; back-transformed values. Biomass production, and species proportions in the mixtures, varied between harvests which means that the light climate differed between harvests. Means and standard deviations are shown. Superscript letters indicate statistical differences within harvests. H1, H2, H3, and H4 denote the four harvest occasions

Crop	H1	H2	H3	H4
<i>P. pratense</i>	11.3 ± 3.63 ^b	35.3 ± 14.67 ^a	27.2 ± 9.59 ^a	24.8 ± 3.24 ^a
<i>L. perenne</i>	11.1 ± 3.57 ^b	21.5 ± 8.94 ^a	30.0 ± 10.58 ^a	26.0 ± 3.39 ^a
<i>T. pratense</i>	6.3 ± 2.02 ^{bc}	1.0 ± 0.42 ^c	1.9 ± 0.67 ^e	17.9 ± 2.33 ^b
<i>C. intybus</i>	55.5 ± 25.21 ^a	40.1 ± 23.57 ^a	34.3 ± 17.11 ^a	29.3 ± 5.41 ^a
<i>M. sativa</i>	1.8 ± 0.82 ^d	3.7 ± 2.17 ^c	1.9 ± 0.95 ^c	7.9 ± 1.46 ^c
Mixture type 1	6.1 ± 0.84 ^b	7.0 ± 1.24 ^{bc}	6.0 ± 0.90 ^b	18.0 ± 1.00 ^b
Mixture type 2	3.4 ± 0.69 ^{cd}	4.6 ± 1.21 ^{bc}	3.7 ± 0.83 ^{bc}	16.0 ± 1.32 ^b

Figure 1

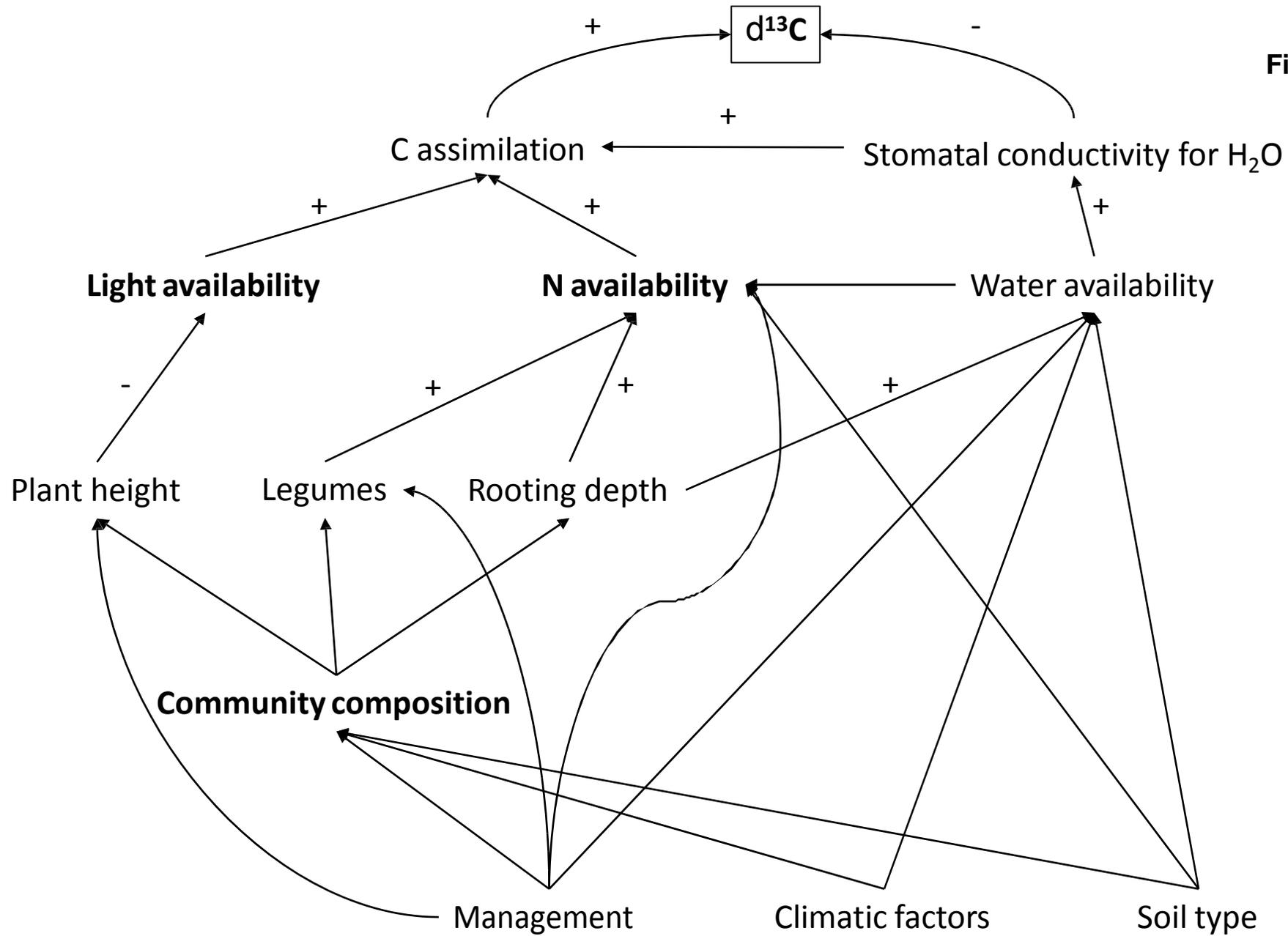
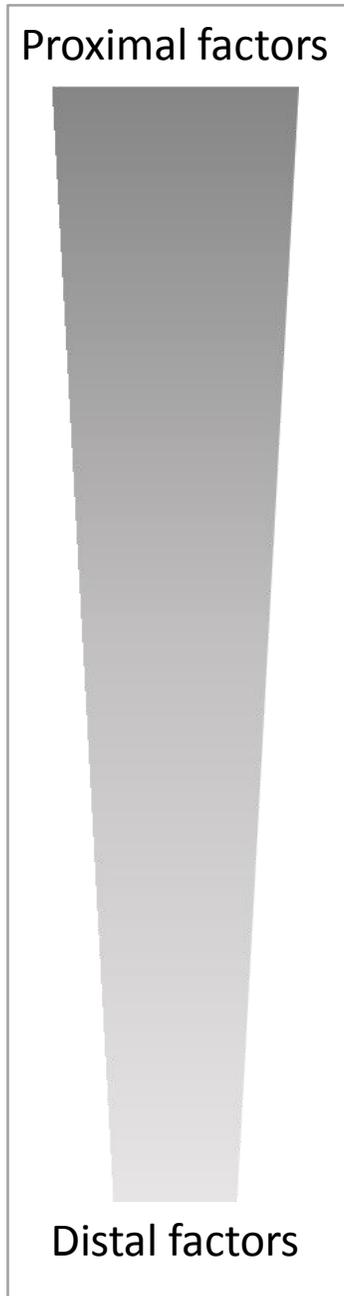
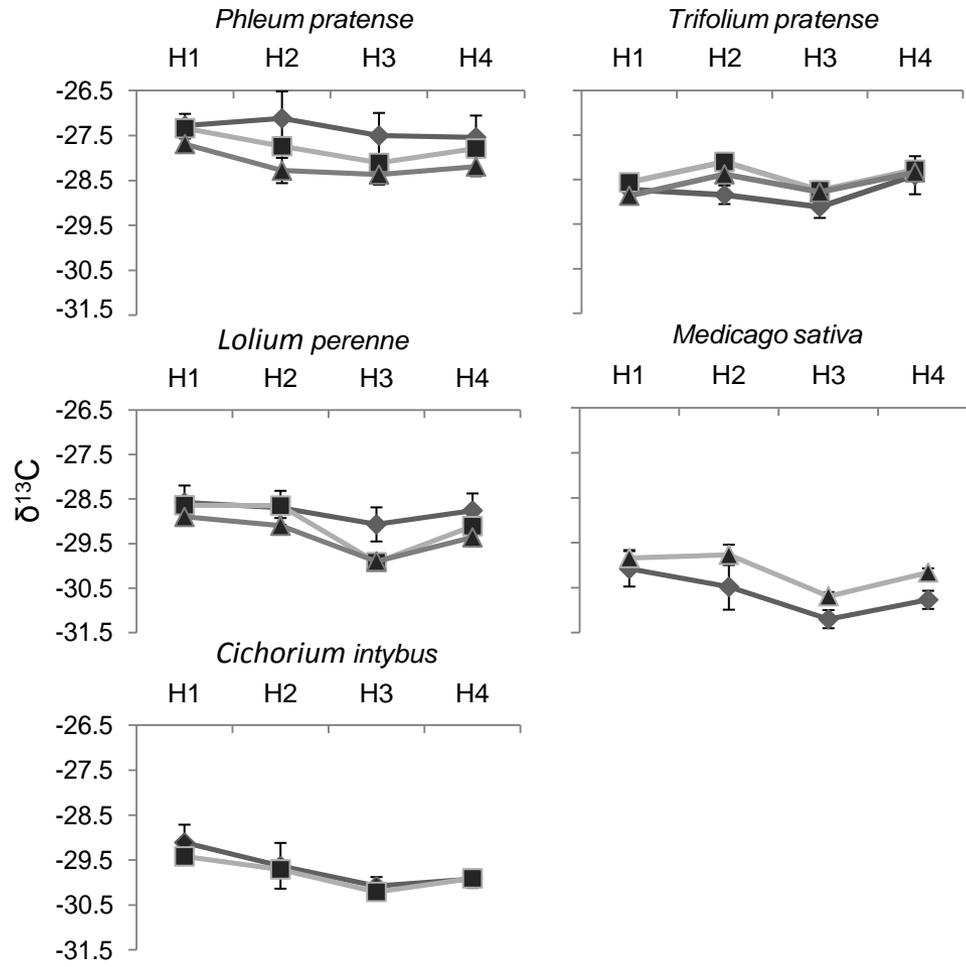


Fig. 1

Figure 2

Fig. 2



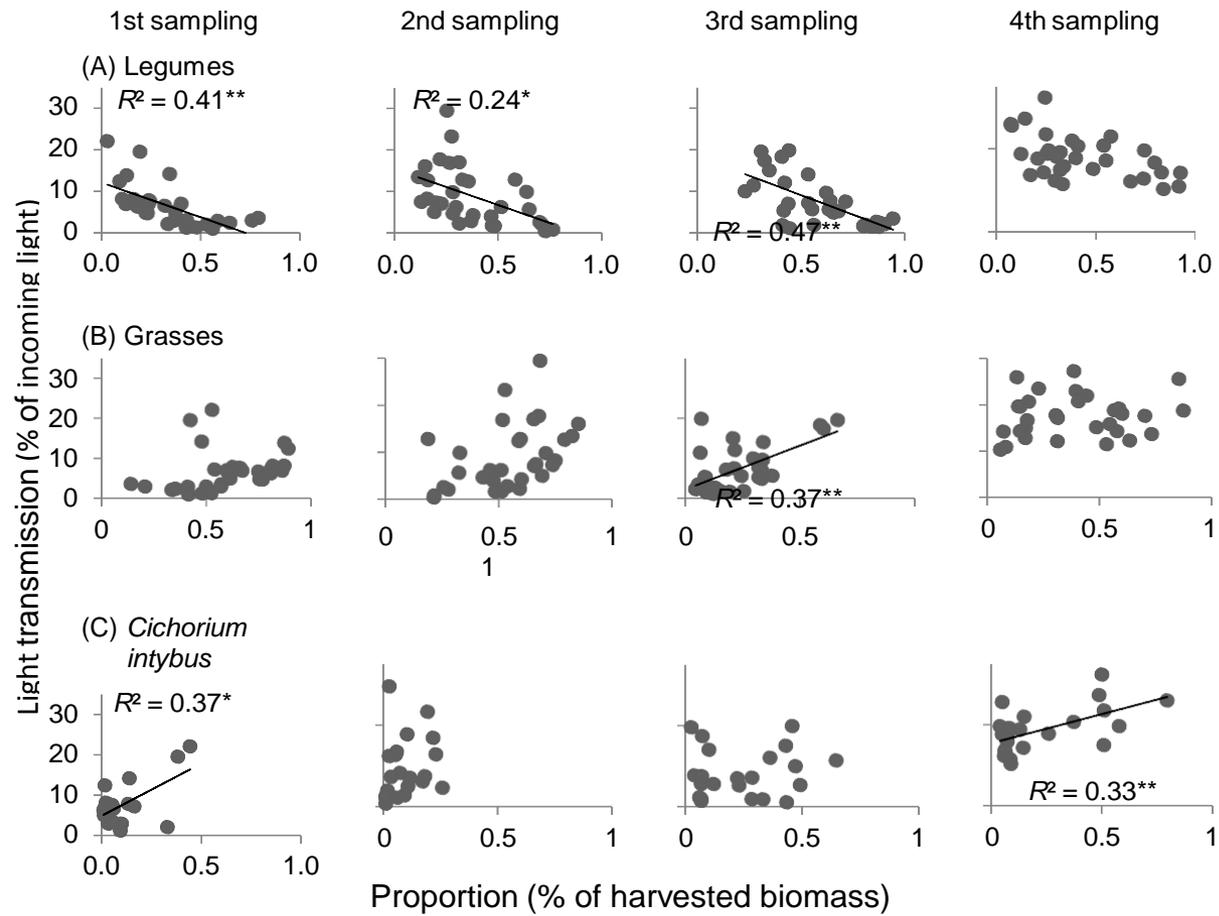
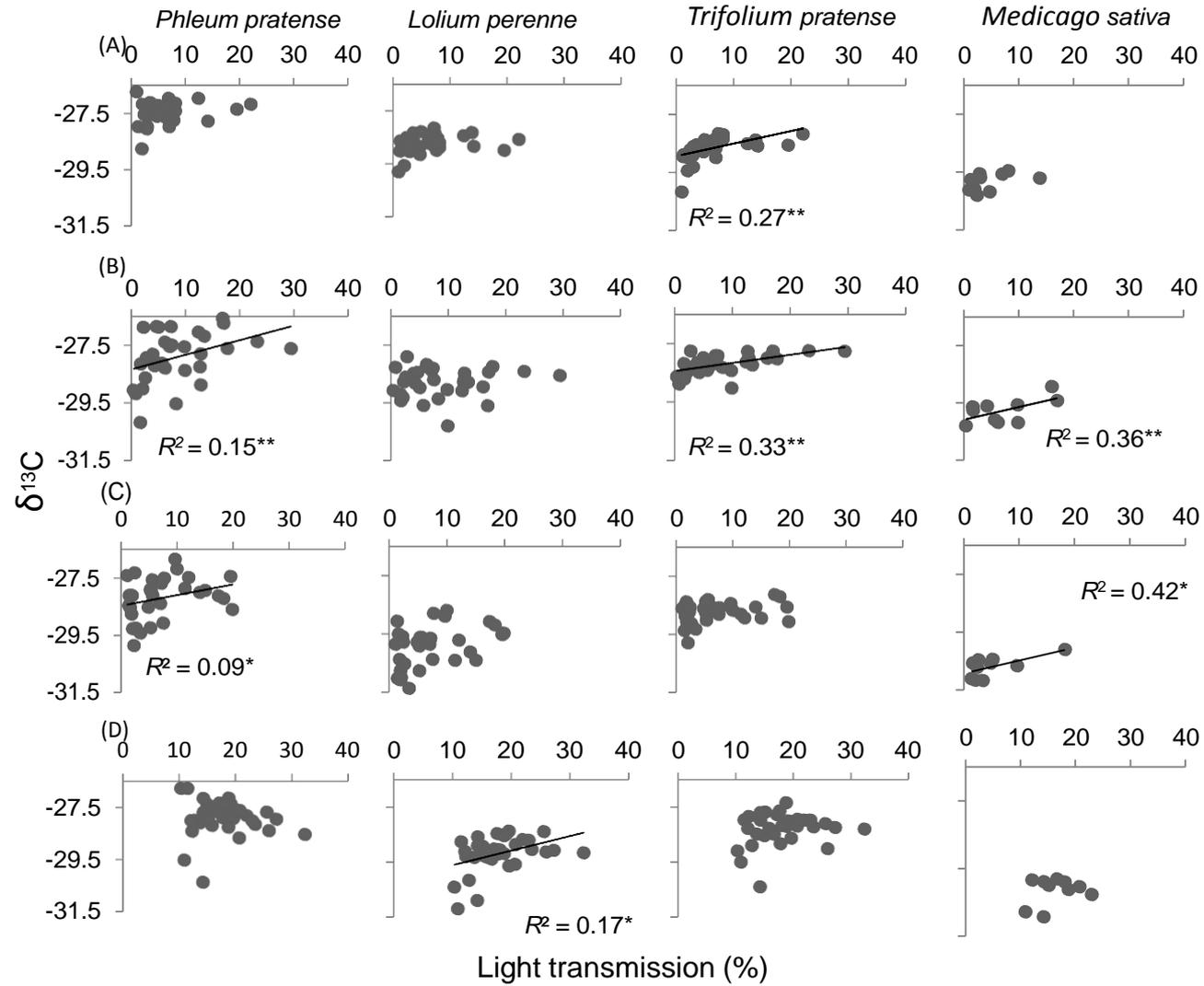


Figure 4

Fig. 4



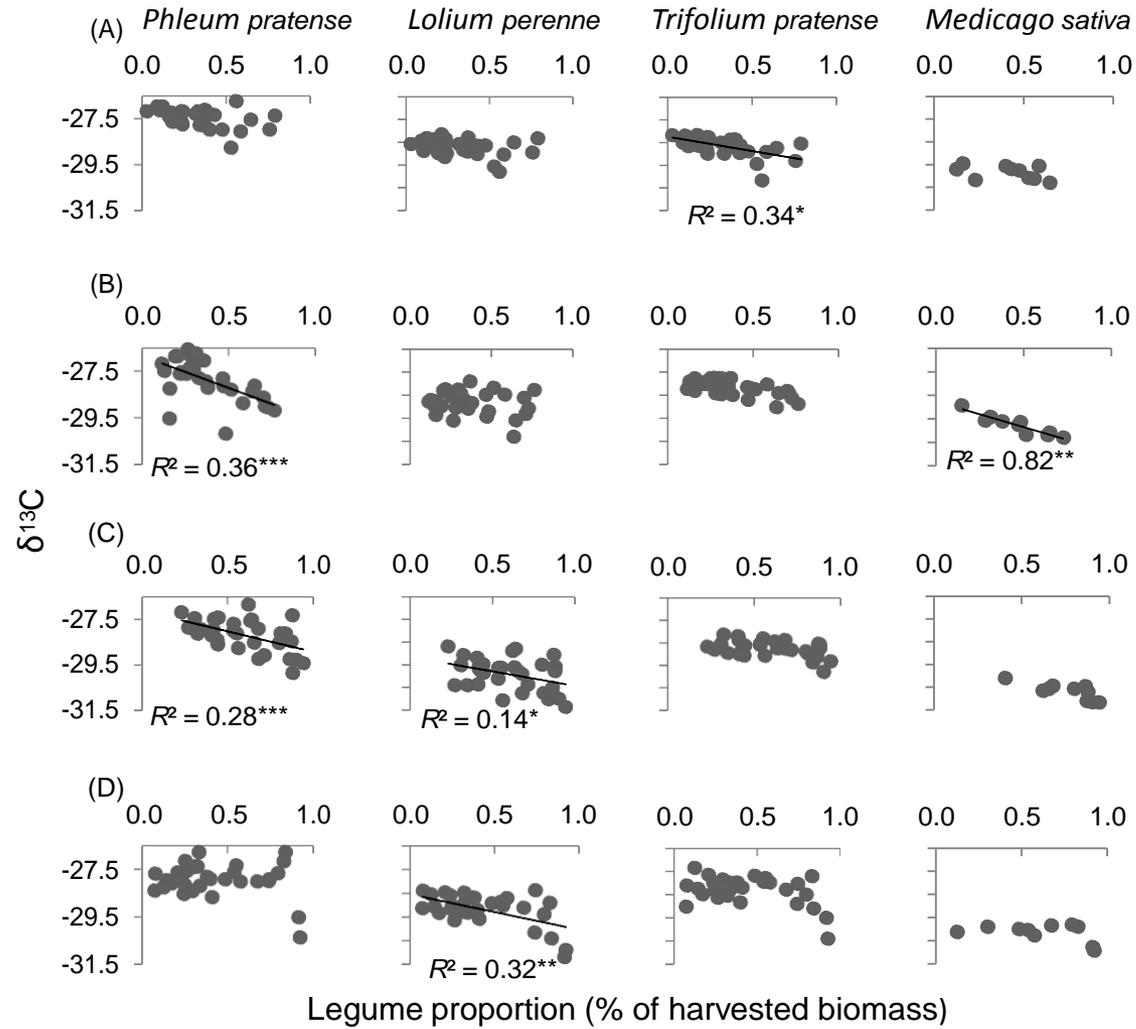


Figure 6

Fig. 6

