

**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<sup>-1</sup>. 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  $100 \text{ kg N ha}^{-1}$  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,  $^{13}\text{C}$ , and  $^{12}\text{C}$ ) 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  $\text{H}_2\text{O}$  on  $\text{CO}_2$  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<sup>-1</sup>, and 87 mg potassium (K) kg<sup>-1</sup>.  
102 The experimental plot received 42 kg phosphorus and 150 kg K ha<sup>-1</sup> at sowing (2007), and 45  
103 kg K and 6 kg sulphur ha<sup>-1</sup> each harvest year. In the harvest years, 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> was  
104 applied in split dressings (i.e., 40 kg of N ha<sup>-1</sup> in early spring and 20 kg of N ha<sup>-1</sup> 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 m<sup>2</sup>. 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<sup>-1</sup> (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 N<sub>2</sub> 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  $^{13}\text{C}$  abundance, i.e.,  $^{13}\text{C}$   
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  $\text{N}_2$  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  $\text{N}_2$  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  $\text{CO}_2$   
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

323 **Acknowledgements**

324 We thank SW Seed for access to their fields and for the excellent help provided by their field  
325 staff. This work was funded by the Swedish Research Council for Environment, Agricultural  
326 Sciences and Spatial Planning, contract 2005-3470-4745-69, and by the Behms Fund.

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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## 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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Basic and applied Ecology.

This paper has been peer-reviewed but may not include the final publisher  
proof-corrections or pagination.

Citation for the published paper:

Bodil E. Frankow-Lindberg & Nicole Wrage-Mönnig. (2015) Light  
availability is improved for legume species grown in moderately N-fertilized  
mixtures with non-legume species. *Basic and applied ecology*. Volume: 16,  
Number: 5, pp 403-412.

<http://dx.doi.org/10.1016/j.baae.2015.04.007>.

Access to the published version may require journal subscription.

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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

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

**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

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

Figure 1

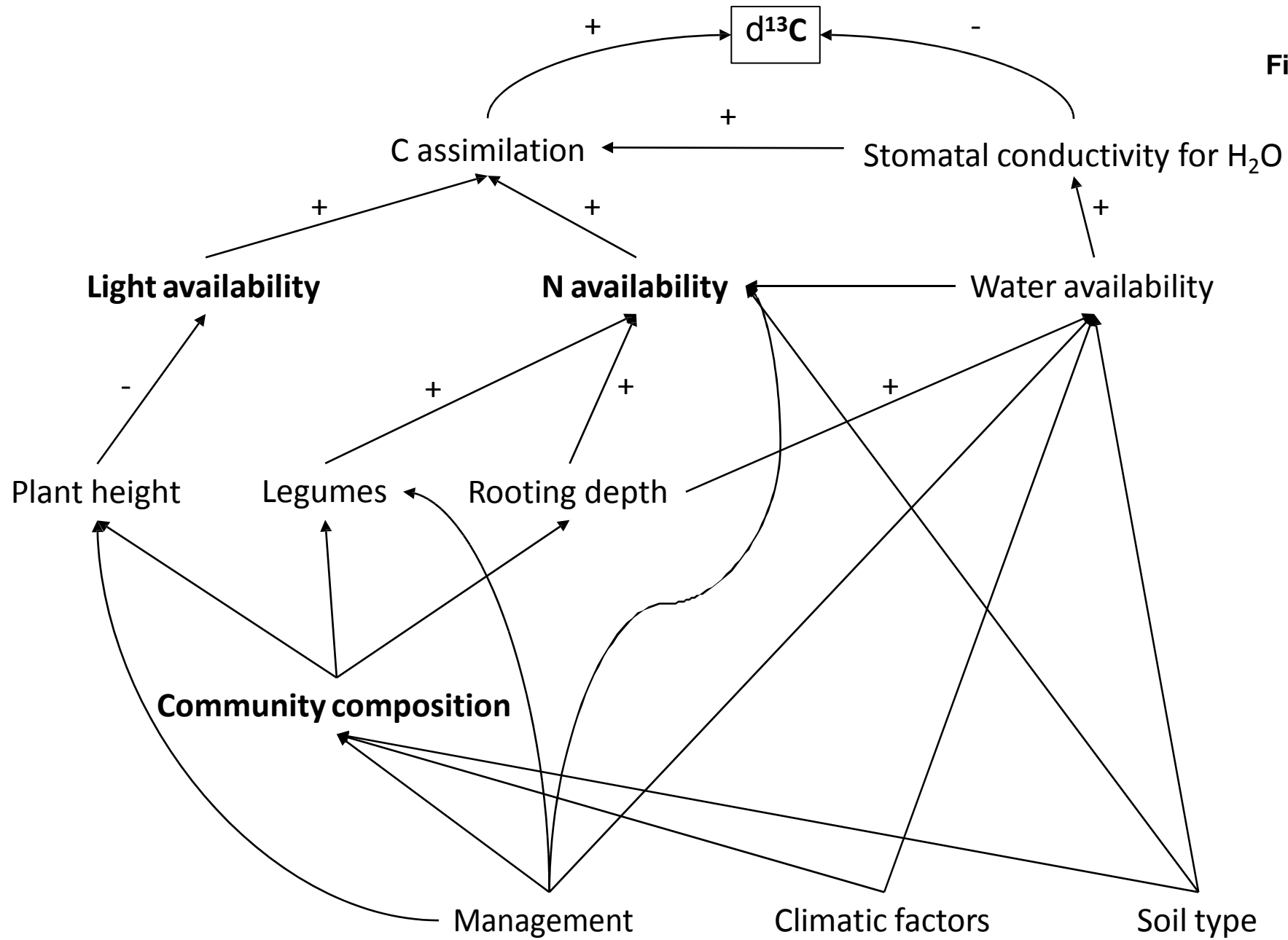
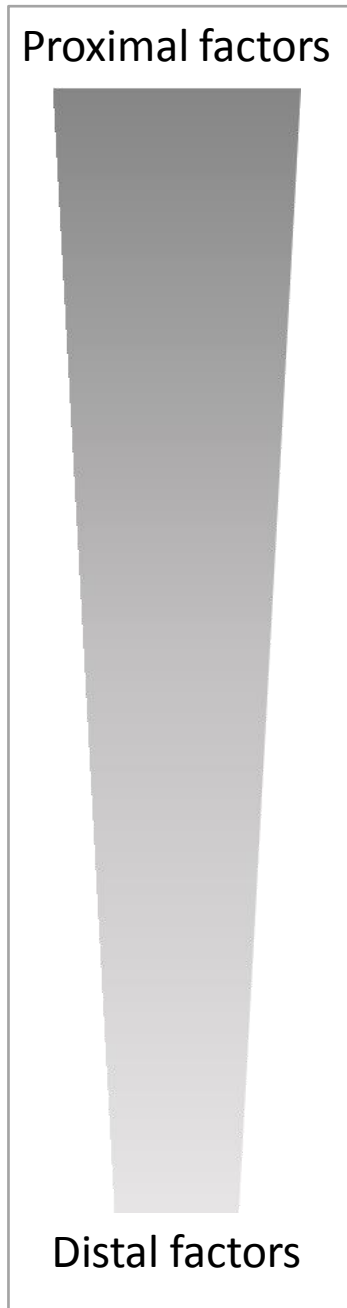
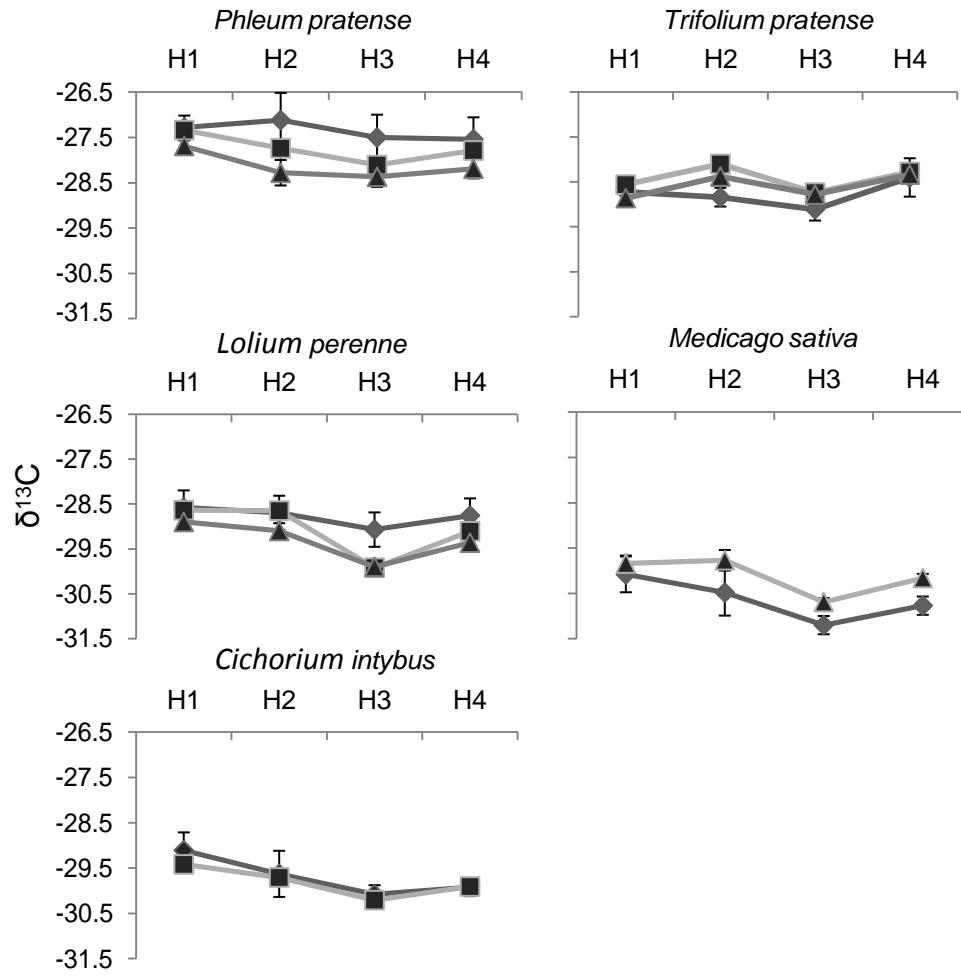


Fig. 1



Figure 2

Fig. 2



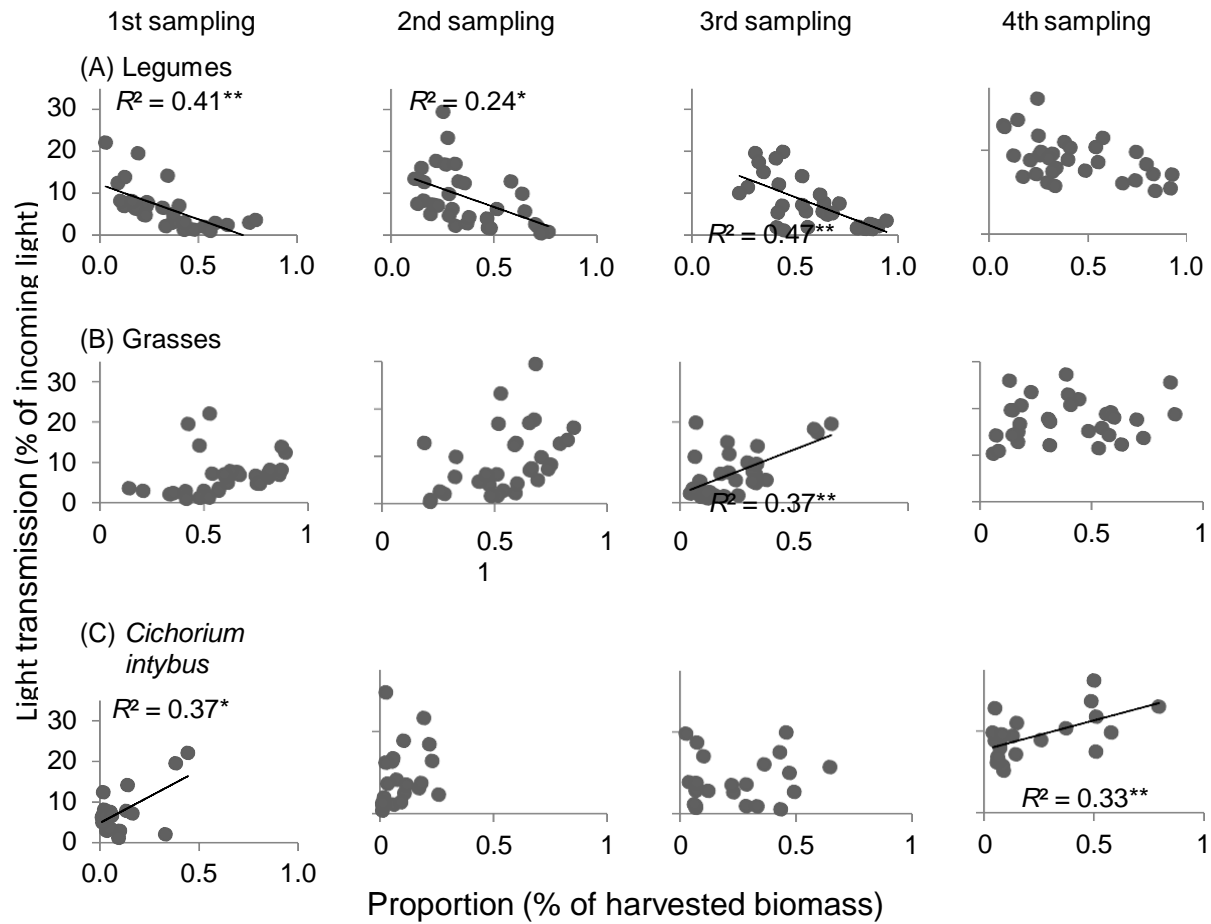
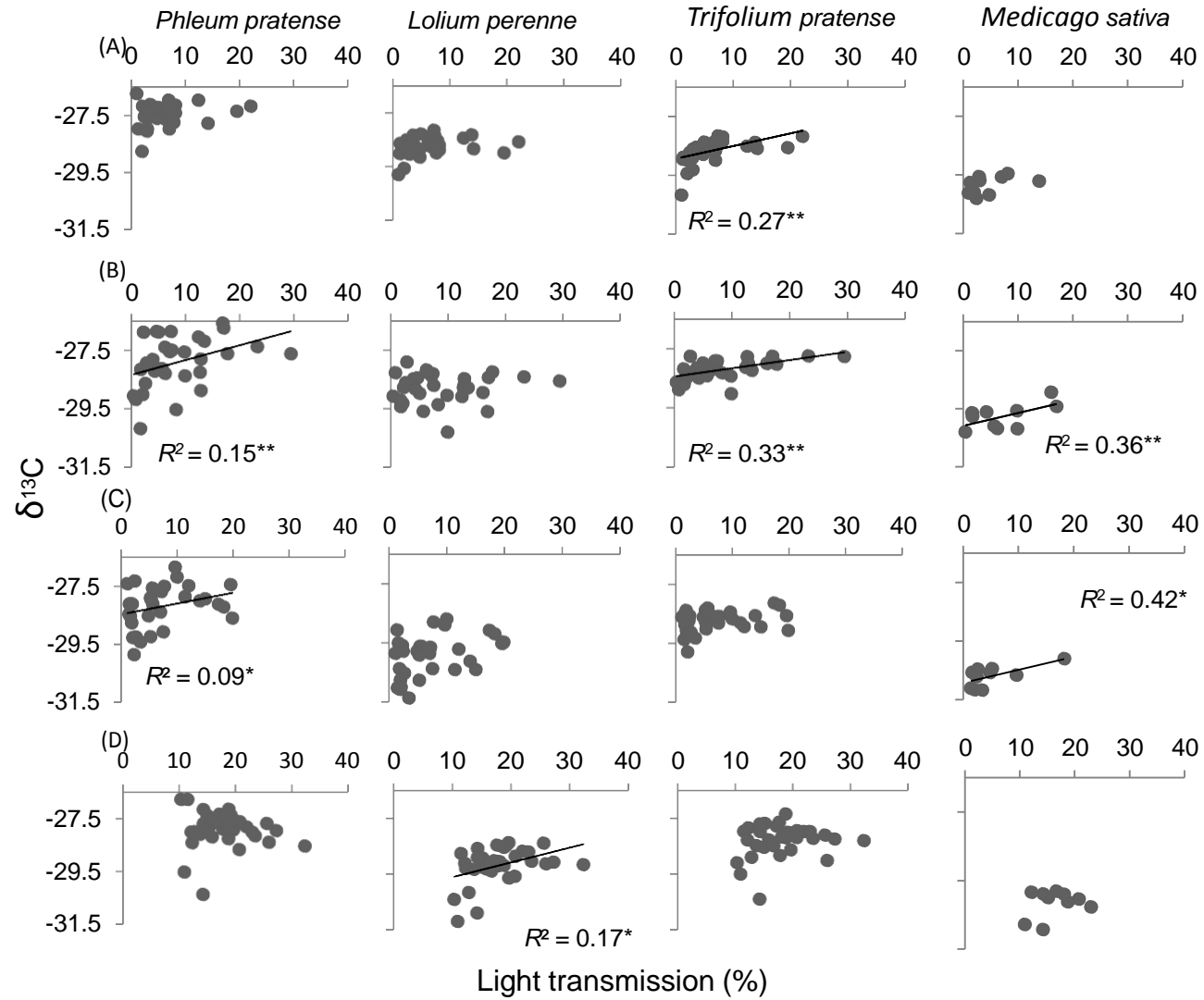


Figure 4

Fig. 4



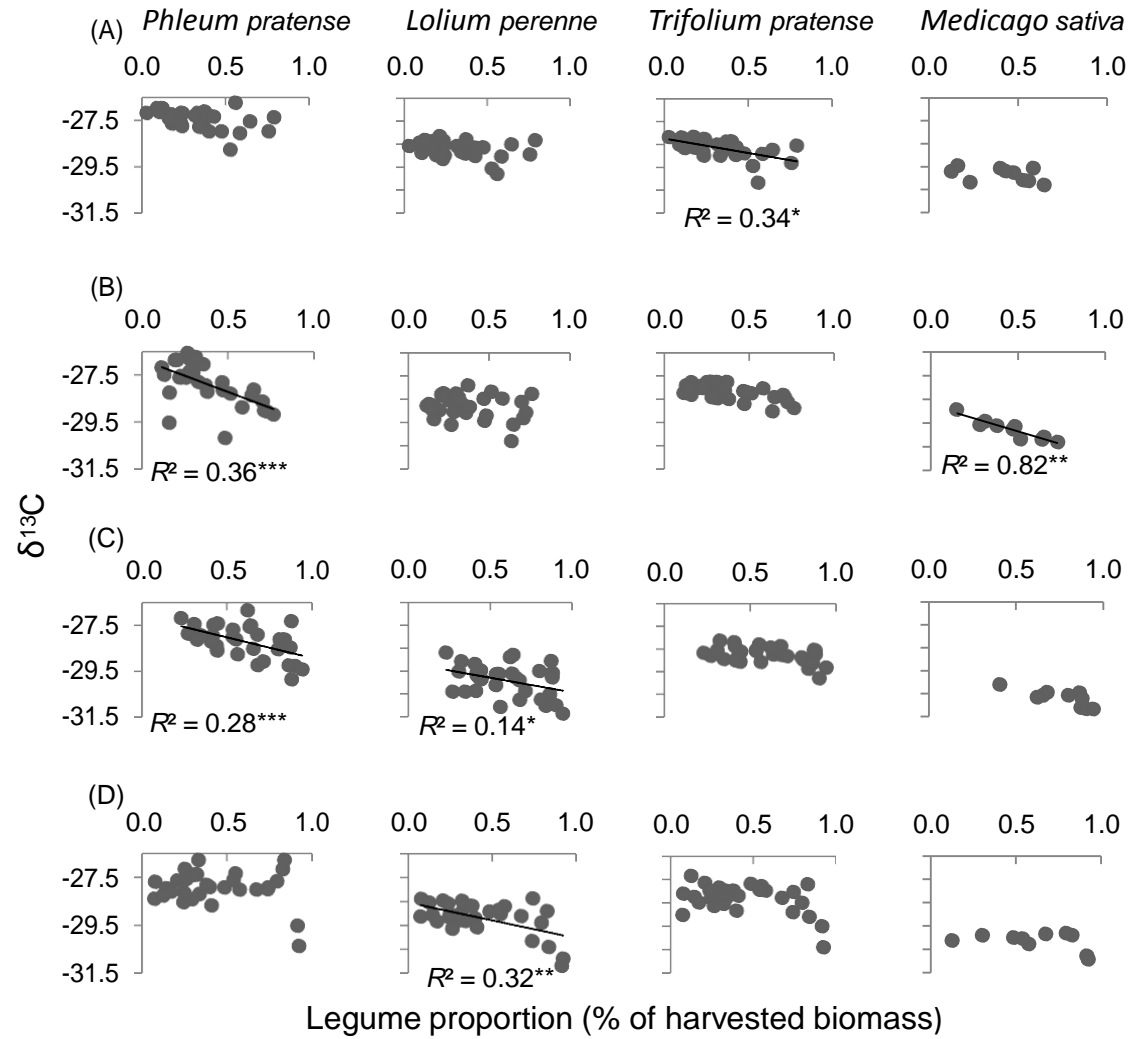


Figure 6

Fig. 6

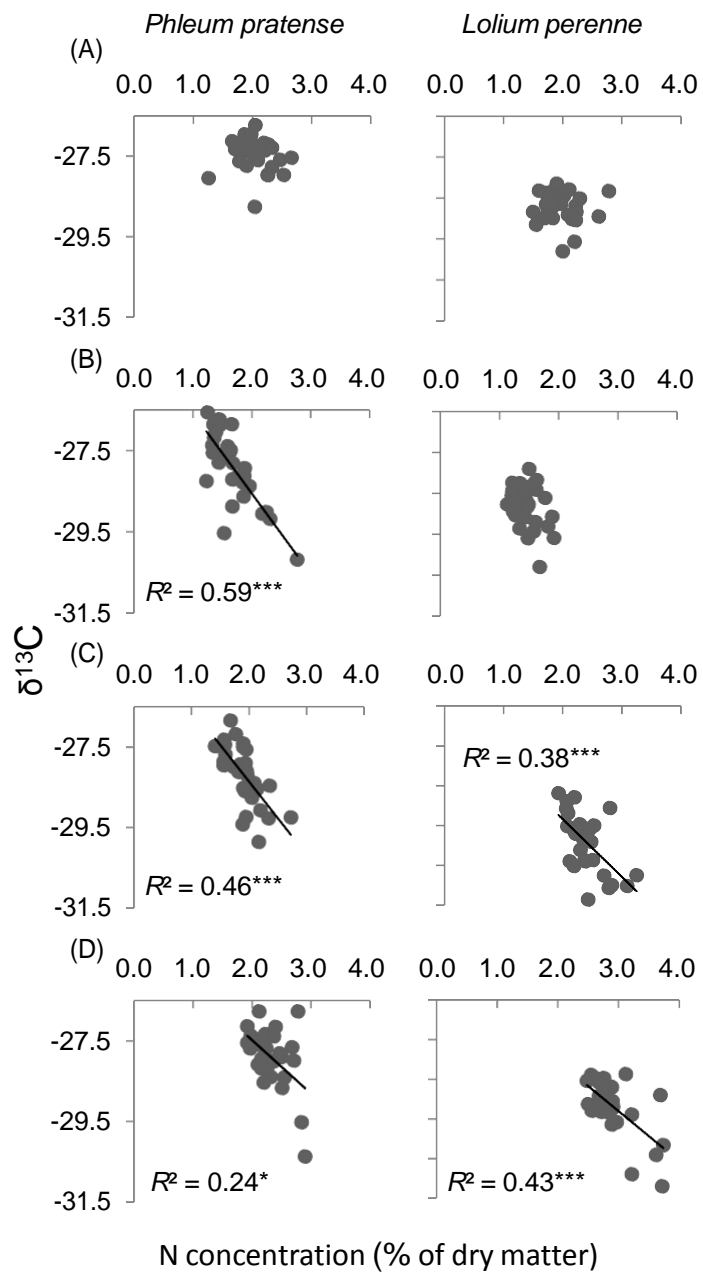


Figure 7

Fig. 7

