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1 **Morphological response of weed and crop species to nitrogen stress in**
2 **interaction with shading**

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24

25 **Abstract**

26

27 Reducing herbicide use may result in a residual weed flora in arable fields, increasing crop-
28 weed competition for resources. In temperate cropping systems, light and nitrogen are the main
29 resources for which plants compete. As weed competition increases, soil mineral nitrogen might
30 become less abundant as the use of mineral fertilizer is reduced for environmental reasons
31 (pollutions, biodiversity loss). Better understanding crops and weeds competitive abilities for
32 nitrogen and light might help manage weeds and promote weed biological regulation by sowing
33 more competitive crops. Our objectives were to (1) quantify the diversity in species
34 morphological response to nitrogen stress, in interaction with shading, and (2) investigate
35 relationships between species responses and species features (clade, crop vs weed, Ellenberg
36 indexes). Response of three aboveground variables, important for light competition, (specific
37 leaf area SLA, leaf to aboveground biomass ratio LBR and height per unit aboveground biomass
38 HBR) to nitrogen stress was measured on four crops and four weeds grown in greenhouse under
39 various nitrogen treatments and two light levels. Increasing SLA, LBR and HBR in response to
40 nitrogen stress improve plant light competitiveness in situation of nitrogen deficiency. SLA
41 decreased in response to nitrogen stress for all species, with a higher decrease for nitrophilic
42 species. LBR response to nitrogen stress varied between crops and weeds, weeds decreasing
43 LBR while crops tended to increase it. HBR was independent of nitrogen stress for most
44 species. Shading had a small to no effect on the morphological response to nitrogen stress.
45 These results suggest that the investigated parameters characterize species intrinsic response of
46 morphological variables to nitrogen stress. In the next future, integrating these species
47 properties into a mechanistic model and performing simulations will allow drawing generic
48 conclusions on species competitiveness in various situations.

49

50 **Keywords**

51 Morphology, plasticity, specific leaf area, leaf biomass ratio, height biomass ratio, competition,
52 light

53

54 1. Introduction

55

56 Weeds are the most harmful pest in arable fields (Oerke, 2006), because they compete with the
57 crops for resources (light, nutrients, water). Herbicides are the most effective curative weed
58 management technique, but their use needs to be reduced because of the harm it may do to
59 human and environmental health (Waggoner et al., 2013, Haarstad and Ludvigsen, 2007).
60 Furthermore, frequent usage of herbicide can lead to increased herbicide resistance and thus to
61 technical lock-ins (Davis and Frisvold, 2017). Reducing herbicide use may result in an altered
62 residual weed flora, thus increasing competition between weeds and crops. Choosing crop
63 species/varieties that are competitive for resources might be an option for managing weeds
64 while reducing herbicide use.

65 In intensive temperate cropping systems, light is generally the main resource for which plants
66 compete (Wilson and Tilman, 1993; Perry *et al.*, 2003). However, reducing mineral fertilizers
67 to decrease their environmental impacts (Sutton *et al.*, 2011) may limit soil-nitrogen
68 availability, and result in periods of nitrogen stress for plants. Thus, it is crucial to identify crop
69 plants that are competitive for both light and nitrogen. To address this issue, it is necessary to
70 better understand the diversity of species morphological response to nitrogen in interaction with
71 light. Experiments have shown that interactions between competition for light and nitrogen can
72 occur (DiTomaso, 1995). These interactions vary with resource supply (Casper and Jackson,
73 1997; Cahill, 1999) and are difficult to predict (McPhee, 2001; Mariotti *et al.*, 2009, Little *et*
74 *al.*, 2021).

75 Specific leaf area, leaf biomass ratio or height per unit aboveground biomass are three key
76 morphological variables that respond to shading and are thus crucial to determine plant success
77 in crop-weed competition for light. Specific plant height, or plant height per unit of
78 aboveground biomass (HBR, $\text{cm}\cdot\text{g}^{-1}$) illustrates the plant's effort in occupying vertical space.
79 The leaf biomass ratio (LBR in $\text{g}\cdot\text{g}^{-1}$, plant leaf biomass / total aboveground plant biomass)
80 reflects how much effort the plant invests into light-intercepting organs. Finally, the specific
81 leaf area (SLA in $\text{cm}^2\cdot\text{g}^{-1}$, ratio of plant leaf area to plant leaf biomass) illustrates how efficiently
82 the plant translates this effort into light-intercepting area. Studies have characterised the
83 diversity of species morphological response to shading (Colbach *et al.*, 2020b) which is useful
84 to determine traits that improve crop competitiveness against weeds (Colbach *et al.*, 2019).

85 Previous studies have investigated the effect of soil-nitrogen or -nutrient gradients on these
86 three morphological variables (Vanderwerf *et al.*, 1993; Poorter *et al.*, 1995; VanArendonk *et*
87 *al.*, 1997; Knops and Reinhart, 2000; Evans *et al.*, 2003; Singh *et al.*, 2017; Berger *et al.*, 2007)
88 sometimes in interaction with shading (Meziane and Shipley, 1999; de Groot *et al.*, 2002;
89 Shipley and Almeida-Cortez, 2003; An and Shangguan, 2008). Yet, the nitrogen actually
90 available to plants varies with environmental factors (soil characteristics, water and light,
91 cropping techniques). To date, no studies have documented, at the plant scale, the effect of plant
92 nitrogen stress on aboveground morphology in interaction with shading.

93 The objective of this study was to characterize the response of three aboveground
94 morphological variables (specific leaf area, leaf biomass ratio and height per unit biomass),
95 crucial regarding plant-plant competition for light, to plant nitrogen stress in interaction with
96 shading on a range of crop and weed species. We (1) quantified the interspecies diversity in
97 response of plant morphological variables to nitrogen stress, (2) investigated to which extent
98 these responses varied with shading, and (3) investigated the effects of clade, species type and
99 habitat requirements to explain the interspecific variability.

100

101 **2. Materials and methods**

102

103 *2.1. Principle*

104

105 To characterize the response of aboveground morphology to nitrogen stress, we used the
106 calculation approach derived from (Munier-Jolain *et al.*, 2014) and (Colbach *et al.*, 2020b)
107 based on experimental measurements on individual plants. In our study, individual plants of
108 different crop and weed species were grown in optimal and stressed conditions regarding light
109 and nitrogen (details in section 2.2). We investigated the impact of nitrogen stress on three
110 morphological variables which were chosen for their importance in plant-plant competition for
111 light: specific leaf area (SLA in $\text{cm}^2\cdot\text{g}^{-1}$, ratio of plant leaf area to plant leaf biomass), leaf
112 biomass ratio (LBR in $\text{g}\cdot\text{g}^{-1}$, plant leaf biomass / total aboveground plant biomass) and height
113 per unit of aboveground biomass (HBR, $\text{cm}\cdot\text{g}^{-1}$) (details in section 2.3 and 2.4). Equations
114 linking morphological variables to stress indices were established (details in section 2.5).
115 Finally, the species morphological response to nitrogen stress was linked to species features
116 such as clade, species type and habitat requirements (details in section 2.6).

117 2.2. *Greenhouse experiments*

118

119 Greenhouse experiments were conducted in Dijon (France), growing four crop and four weed
120 species (Table 1) common in French arable fields. They included monocotyledonous and
121 dicotyledonous species, and covered the range of the N-number of Ellenberg (1974). This index
122 ranks species from 1 (oligotrophic species mostly found in nitrogen-poor soils) to 9 (nitrophilic
123 species mostly found in nitrogen-rich soils).

124 Plants were grown under different concentrations of nitrogen solution and two contrasted light
125 treatments (except *F. arundinacea* whose plants did not survive in shaded conditions) (Table
126 1). The set-up of experiments 1, 2 and 3 of Table 1 is fully described in (Perthame *et al.*, 2020).
127 Additional experiments 4 and 5 were conducted similarly. Briefly, in all experiments, plants
128 were grown in individual 2-L pots filled with a solid, inert and draining substrate (60%
129 attapulgitite and 40% expanded clay). To obtain a gradient of plant nitrogen nutrition from
130 suboptimal to supraoptimal, plants were grown under four or five nutrient solutions, varying in
131 their nitrate concentration from 0.2 to 14 mM depending on species requirements. The different
132 nutrient solutions were applied under two contrasted light treatments: 100% (unshaded) and
133 40% or 10% (shaded with a shading net) of incident light. These light treatments were chosen
134 because weed species can experience shading up to 90% during their growth (Munier-Jolain *et*
135 *al.*, 2014). Each nutrient solution was provided by automatic watering several times a day at a
136 frequency allowing the pots to drain, avoiding the accumulation of ions in the substrate and
137 ensuring non-limiting irrigation. Twenty-four plants were grown per species × nitrogen
138 treatment × light treatment combination, except for *T. aestivum* and *P. aviculare* in the shaded
139 treatment at the highest nitrogen concentration of the solution where twenty plants were grown.

140

141 2.3. *Plant measurements*

142

143 For each species × nitrogen treatment × light treatment combination, four to six plants were
144 sampled randomly at four dates (from 5 to 18-weeks after sowing). Samplings were carried out
145 during the vegetative stage for all species, except for *M. sativa* (for two dates) and *P. aviculare*
146 (for all dates) which were at reproductive stage. For each plant and at each sampling, plant
147 height was measured (except for *B. napus*, *G. molle* and *A. myosuroides* in the shaded treatment
148 due to human error). Plant height was measured with a ruler as “the shortest distance between

149 the upper boundary of the main photosynthetic tissues on a plant and the ground level” (i.e. the
150 substrate level in our experiments) as described in Cornelissen *et al.* (2003). In addition, plant
151 leaf area was determined with a leaf area meter (LI-3100 Area Meter; Li-Cor, Lincoln, NE,
152 USA), and leaf and stem biomass were measured after drying for 48h at 80°C. Finally, the
153 nitrogen concentration in the aboveground plant part (leaves and stems) was measured using
154 Dumas procedure after plant grinding (ThermoScientific™ FLASH 2000 CHNS/O Analyzer).

155

156 2.4. Nitrogen stress index

157

158 To estimate the nitrogen nutrition level of a plant independently of its light environment, the
159 linear relationship between aboveground nitrogen amount (Nsh in g) and leaf biomass (B_L in g)
160 was used, as demonstrated in Perthame *et al.*, 2020. The relationship between aboveground
161 nitrogen amount and leaf biomass at optimal nitrogen nutrition was calculated for a range of
162 crop and weed species (Table S1 section A online). For a given leaf biomass, the aboveground
163 nitrogen amount measured can be compared to the optimal aboveground nitrogen amount
164 indicated by the relationship. At the plant scale, the ratio between the measured aboveground
165 nitrogen amount and the optimal aboveground nitrogen amount given by the relationship
166 corresponds to the nitrogen nutrition status of a plant. In the present study, this ratio was
167 calculated for each plant to estimate the nitrogen nutrition stress of the plant.

168 The nitrogen stress index (Nstress in g·g⁻¹) was calculated as the mean of the four to six plants
169 of each species × light treatment × nitrogen treatment × sampling date combination as:

$$170 \quad [1] \quad N_{\text{stress}} = 1 - N_{\text{sh}}_{\text{measured}} / N_{\text{sh}}_{\text{at optimal nitrogen nutrition}}$$

171 This index varies (i.e. increases or decreases) with the nitrogen nutrition status of a plant. When
172 the nitrogen stress index is equal to 0, the plant is at optimal nitrogen nutrition level. The more
173 the nitrogen stress index exceeds 0, the more nitrogen-deficient the plant is. Conversely, the
174 more negative the index is, the more supraoptimal the plant nitrogen level is.

175

176 2.5. The species parameters of response to nitrogen stress

177

178 The principle to determine the species parameters for all three morphological variables was
 179 derived from (Munier-Jolain *et al.*, 2014) and (Colbach *et al.*, 2020b). It consists in fitting an
 180 exponential equation to the morphological variable vs the nitrogen stress index values for each
 181 species. This equation was fitted to the mean of the four to six plants of each species \times light
 182 treatment \times nitrogen treatment \times sampling date combination. The equation contains two
 183 parameters. One represents the value of the morphological variable in the absence of nitrogen
 184 stress: it quantifies potential morphology (i.e. SLA_{0sl} in equation [2]). The other quantifies the
 185 species response to nitrogen stress (i.e. SLA_muN_{sl} in equation [2]). To account for the effect
 186 of light treatment on potential morphology and response to nitrogen stress, two further
 187 parameters were included (equation [3]).

188 Applied to Specific Leaf Area (SLA, $cm^2 \cdot g^{-1}$), one parameter assesses the species potential SLA
 189 at optimal nitrogen nutrition (SLA_{0s} , $cm^2 \cdot g^{-1}$) and the other the species SLA response to
 190 nitrogen stress (SLA_muN_s , no unit). Negative values of the latter parameter indicate that
 191 nitrogen-deficient plants decrease their specific leaf area. The parameters were estimated by
 192 fitting the following equation for each species and light treatment (schematic representation in
 193 Figure S2 section B online):

$$194 \quad [2] \quad SLA_{psl} = SLA_{0sl} \cdot \exp(SLA_muN_{sl} \cdot N_{stress_{psl}}) + error_{psl}$$

195 which was $\log_{natural}$ -transformed into:

$$196 \quad [3] \quad \log_n(SLA_{psl}) = \log_n(SLA_{0s}) + L_SLA_{0l} + SLA_muN_s \cdot N_{stress_{psl}} + L_SLA_muN_l \cdot$$

$$197 \quad N_{stress_{psl}} + error_{psl}$$

198 where SLA_{psl} ($cm^2 \cdot g^{-1}$) and $N_{stress_{psl}}$ ($g \cdot g^{-1}$) are respectively the specific leaf area and nitrogen
 199 stress index measured on plant p of species s in light condition l. L_SLA_{0l} ($cm^2 \cdot g^{-1}$) and
 200 $L_SLA_muN_l$ (no unit) are the effect of light treatment on respectively SLA at optimal nitrogen
 201 nutrition and SLA response to nitrogen stress. Equation [3] was fitted with the lm function of
 202 R and weighting sum of squares by the inverse of the relative standard deviation of SLA for
 203 each species \times light treatment \times nitrogen treatment \times sampling date (R Stats package,
 204 RCoreTeam, 2019). Only factors significant at $p \leq 0.05$ were kept. To quantify the relative effect
 205 of each significant factor (light treatment, Nstress and interaction between light treatment and
 206 Nstress) on the variation of SLA, partial R^2 were calculated (Anova function of car package,
 207 type III).

208 The same approach was used for the Leaf Biomass Ratio (LBR, $\text{g}\cdot\text{g}^{-1}$) to estimate the species
 209 potential LBR (LBR0_s , $\text{g}\cdot\text{g}^{-1}$) and the species LBR response to nitrogen stress (LBR_muN_s , no
 210 unit) as well as the shading effect (L_LBR0_l and L_LBR_muN_l). Negative values of
 211 LBR_muN_s indicate that nitrogen-deficient plants decrease their leaf biomass ratio.

212 The approach for the Height Biomass Ratio (HBR, $\text{cm}\cdot\text{g}^{-1}$) included a third parameter
 213 (b_HBR_s) to illustrate the sensitivity of plant height to plant aboveground biomass (Bsh in g)
 214 (Munier-Jolain *et al.*, 2014):

$$215 \quad [4] \quad H_{\text{psl}} = \text{HBR0}_{\text{sl}} \cdot \text{Bsh}_{\text{psl}}^{\text{b_HBR}_s} \exp(\text{HBR_muN}_{\text{sl}} \cdot \text{Nstress}_{\text{psl}}) + \text{error}_{\text{psl}}$$

216 Which was \log_n -transformed into:

$$217 \quad [5] \quad \log_n(H_{\text{psl}}) = \log_n(\text{HBR0}_s) + \text{L_HBR0}_l + \text{b_HBR}_s \cdot \log_n(\text{Bsh}_{\text{psl}}) + \text{HBR_muN}_s \cdot \\ 218 \quad \text{Nstress}_{\text{psl}} + \text{L_HBR_muN}_l \cdot \text{Nstress}_{\text{psl}} + \text{error}_{\text{psl}}$$

219 where H_{psl} (cm) is the height measured on plant p of species s in light condition l. Similarly to
 220 SLA and LBR, parameter HBR0_s ($\text{cm}\cdot\text{g}^{-1}$) assesses species potential HBR at optimal nitrogen
 221 nutrition, HBR_muN_s (no unit) the species HBR response to nitrogen stress, L_HBR0_l ($\text{cm}\cdot\text{g}^{-1}$)
 222 and L_HBR_muN_l (no unit) the light effect on respectively potential HBR and HBR
 223 response to nitrogen stress. For *B. napus*, *A. myosuroides* and *G. molle*, L_HBR0_l and
 224 L_HBR_muN_l were removed from the model as height was measured only in unshaded
 225 treatment (see 2.3). Negative HBR_muN_s values indicate that nitrogen-deficient plants
 226 decrease their height per unit biomass. The third parameter b_HBR_s (no unit) estimates whether
 227 heavy and light plants at optimal nitrogen nutrition level of a given species produce the same
 228 height for a given biomass ($\text{b_HBR}_s = 1$) or whether light plants produce more height for a
 229 given biomass than heavy plants at optimal nitrogen nutrition ($\text{b_HBR}_s < 1$). As before,
 230 equation [5] was fitted with the lm function of R and weighting observations by the inverse of
 231 the relative standard deviation of height for each species \times light treatment \times nitrogen treatment
 232 \times sampling date, and partial R^2 were calculated.

233 Here, for each morphological variable, we focused on the two parameters characterizing
 234 response to nitrogen stress and the effect of light treatment (for example, applied to SLA, it
 235 corresponds to SLA_muN_s and L_SLA_muN_l). Indeed, response to shading in unstressed
 236 nitrogen conditions was extensively studied in Colbach *et al.* (2020b) and was analysed in
 237 supplementary material section C online.

238

2.6. Linking nitrogen-stress response to species features

239
240

241 The determinants underlying the interspecific differences in morphological responses to
242 nitrogen stress (i.e. parameters **SLA_muN_s**, **LBR_muN_s**, and **HBR_muN_s**) were analysed. To
243 achieve this, we studied the effects of clade (monocotyledonous versus dicotyledonous species),
244 species types (crop versus weed species) and habitat requirement (for nitrogen and light with
245 respectively the N-number and the L-number of Ellenberg; Table 1), in interaction with
246 shading. The effect of these species features on parameter values was analysed with linear
247 models (lm function of R). Only factors significant at $p \leq 0.1$ were kept.

248

249 3. Results

250

251 3.1. Response of plant morphology to nitrogen stress

252

253 For all eight species, the effect of nitrogen stress on specific leaf area (SLA) was significant
254 (Table 2): plants produced smaller thicker leaves (i.e. SLA decreased) when nitrogen deficiency
255 increased (**SLA_muN_s** < 0) (Figure 1). When looking at unshaded treatment only, the response
256 to nitrogen stress varied among species, with the slope decreasing by a three-fold factor between
257 *A. myosuroides*, the least plastic species, and *P. aviculare*, the most plastic species.

258 For four out of eight species, the leaf biomass ratio (LBR) was independent of nitrogen stress
259 index (Table 3). For the others, the response of leaf biomass to nitrogen stress varied from
260 species investing relatively more biomass in stems than leaves (i.e. LBR decreased) when
261 nitrogen deficiency increased (**LBR_muN_s** < 0 for *T. aestivum* in unshaded treatment, *P.*
262 *aviculare*, and *A. myosuroides*), to species increasing their leaf biomass ratio in case of nitrogen
263 deficiency (**LBR_muN_s** > 0 for *M. sativa*) (Figure 2).

264 Finally, height was independent of nitrogen stress index (Table 4), except for two species.
265 Plants of *T. aestivum* decreased their height for a given biomass in situation of nitrogen
266 deficiency (**HBR_muN_s** = -0.17) while plants of *F. arundinacea* etiolated (i.e. increased their
267 height for a given biomass) in situation of nitrogen deficiency (**HBR_muN_s** = 0.13).

268

269

270 *3.2. Did the response of plant morphology to nitrogen stress vary with shading?*

271

272 The response of specific leaf area to nitrogen stress depended on light treatment for only two
273 out of seven species (Table 2). For *G. aparine*, the decrease of specific leaf area when nitrogen
274 stress increased was stronger in shaded treatment than in unshaded treatment (Figure 1G) (p-
275 value = 0.0028). It was the opposite for *P. aviculare* (Figure 1F) (p-value = 0.0007). It should
276 be noted that, for these two species, light treatment in interaction with nitrogen stress index
277 explained little variability of specific leaf area, particularly compared to nitrogen stress index
278 and light treatment as a single factor (Table 2).

279 Among the four species for which leaf biomass ratio varied with nitrogen stress index, only the
280 response of *T. aestivum* depended on light treatment (Table 3, p-value = 0.0006). For this
281 species, unshaded plants were leafier when nitrogen nutrition was supraoptimal (**LBR_muN_s**
282 < 0), whereas for shaded plants leaf biomass ratio was independent of nitrogen stress index
283 (Figure 2D).

284 Finally, height response to nitrogen stress was independent of light treatment for all species
285 (Table 4).

286

287 *3.3. Correlations between species nitrogen responses and species features*

288

289 Ellenberg-N number as a sole factor was the only significant factor at $p \leq 0.1$ explaining the
290 response of specific leaf area to nitrogen stress (**SLA_muN_s**). The decrease of specific leaf area
291 with increasing nitrogen stress tended to be stronger for nitrophilic species (high Ellenberg-N
292 values) (Table 5).

293 Similarly, the species type (crop/weed) as a sole factor was the only significant factor at $p \leq 0.1$
294 explaining the response of leaf biomass ratio to nitrogen stress (**LBR_muN_s**). Weed species
295 tended to invest more biomass into stems than leaves when nitrogen deficiency increased
296 (**LBR_muN_s** < 0) while it was the opposite for crop species (**LBR_muN_s** > 0) (Table 5).

297 We did not investigate the effect of species type, clade or habitat requirement on height response
298 to nitrogen stress as height was independent of nitrogen stress for most species.

300 4. Discussion

301

302 4.1. An original approach to quantify intrinsic species response to nitrogen stress

303

304 The present study was the first to quantify the response of three aboveground morphological
305 variables, crucial for light competition, to plant nitrogen stress in interaction with shading for
306 both crop and weed species, at the plant scale. The originality of the approach consisted in using
307 a plant nitrogen stress index (Perthame *et al.*, 2020) independent of light environment as
308 explanatory variable. In contrast, previous studies investigated the effect of environmental
309 variables such as nitrogen input rate (in field conditions) (Poorter *et al.*, 1995; Knops and
310 Reinhart, 2000; Evans *et al.*, 2003; Singh *et al.*, 2017) or nitrogen solution concentration (in
311 controlled conditions) (VanArendonk *et al.*, 1997; Meziane and Shipley, 1999; de Groot *et al.*,
312 2002; Shipley and Almeida-Cortez, 2003; Berger *et al.*, 2007; An and Shangguan, 2008) on
313 morphological variables which might explain the contrasted results of these previous studies.
314 Indeed, in field conditions, a nitrogen input rate provided in different fields does not necessarily
315 result in similar nitrogen nutrition for plants, depending on cropping techniques and biophysical
316 processes.

317 For example, Ravier *et al.* (2017) computed wheat nitrogen nutrition index from a large range
318 of experiments on different sites and cropping systems. The distribution of nitrogen nutrition
319 index at various growth stages was different from the distribution of nitrogen rate for the
320 experimental treatments. Similarly, in controlled conditions experiments, a nitrogen solution
321 concentration can result in different plant nitrogen nutrition depending on other environmental
322 variables (such as pot size, substrate, watering).

323 The present study allowed separating the nitrogen availability in the environment from the plant
324 nitrogen nutrition. We showed that shading only had a small to no effect on the response of the
325 three morphological variables to nitrogen stress. Consequently, our results are more generic,
326 valid in every plant growth environment, thus reflecting species intrinsic properties regarding
327 their response to nitrogen stress.

328

329 4.2. Are our results consistent with literature?
330

331 Due to the originality of our approach (section 4.1), it was difficult to directly compare our
332 results with previous studies. We thus focused on confronting the direction of variation of
333 morphological variables, by considering that plant nitrogen stress decreases with increasing
334 nitrogen inputs (either in field conditions or controlled conditions).

335 It is well known that specific leaf area (SLA) decreases with lower nitrogen supply (thus higher
336 nitrogen stress) (Poorter *et al.*, 1995; VanArendonk *et al.*, 1997; Knops and Reinhart, 2000;
337 Freschet *et al.*, 2015; Singh *et al.*, 2017), as we found in the present study. Leaf biomass ratio
338 (LBR) was independent of nitrogen supply in Berger *et al.* (2007) or increased with lower
339 nitrogen supply in Freschet *et al.* (2015). In contrast, in our study, LBR decreased with
340 increasing nitrogen stress for three species (it was the opposite for *M. sativa*). Also, Berger *et al.*
341 (2007) found that plant height per unit biomass increased with higher nitrogen supply while
342 we did not observe such changes with nitrogen stress. These morphological changes all affect
343 plant light capture, and thus carbon nutrition. In situations of non-limiting nitrogen nutrition,
344 carbon nutrition becomes limiting. In such situations, increasing leaf area per unit biomass and
345 proportion of leaf biomass results in higher light capture and thus higher plant carbon nutrition.
346 Our results suggest, for a few species, that carbon nutrition is mostly driven by specific leaf
347 area and leaf biomass ratio and less by height per unit biomass.

348 An interaction between nitrogen supply and light availability on SLA was often reported, SLA
349 decreasing more importantly with lower nitrogen supply in low light environments (Meziane
350 and Shipley, 1999; de Groot *et al.*, 2002; Shipley and Almeida-Cortez, 2003; Freschet *et al.*,
351 2015). In contrast, in our results, interaction between light treatment and nitrogen stress on SLA
352 had a small significant effect only for two out of seven species and the direction of the
353 interaction depended on the species. Similarly, a significant interaction between nitrogen and
354 light availability was reported, LBR increasing more in response to lower nitrogen supply in
355 low light environments (Freschet *et al.*, 2015), while there was no significant interaction
356 between light treatment and nitrogen stress on LBR in our study. These contrasted results can
357 be explained by the use, in our study, of the plant nitrogen stress index independent of plant
358 light growth environment, the latter strongly affecting plant nitrogen nutrition (Seginer, 2004),
359 instead of an environmental variable (i.e. nitrogen supply). The plant variable allows
360 disentangling the effect of nitrogen availability from the effect of light availability on plant
361 morphology.

362 We observed interspecific variations of plasticity. In particular, our approach allowed
363 explaining ecological integrative features (such as Ellenberg-N number) that could be useful to
364 characterise the plasticity of a large number of species without dedicated experiments.
365 Nitrophilic species tended to be more plastic than oligotrophic species. In particular, contrary
366 to Freschet *et al.* (2015), nitrophilic species showed a stronger decrease in their efficiency to
367 produce leaf area per unit leaf biomass in response to increasing nitrogen stress. This result
368 should be confirmed with more species as we tested only eight species in the present study and
369 used a rather high significance threshold ($p \leq 0.1$). This finding lines up knowledge on
370 nitrophilic species growth (mostly found in nitrogen-rich soils) which strongly decreases in
371 response to lower nitrogen supply because they cannot fulfil their nitrogen requirements
372 (Moreau *et al.*, 2013; Moreau *et al.*, 2014).

373 Our results showed that the response of leaf biomass ratio to nitrogen stress was highly plastic
374 and depended on the species. We were able to detect a novel trend, i.e. crop species tended to
375 increase their leaf biomass ratio in response to increasing nitrogen stress while it was the
376 opposite for weed species, which should be confirmed with more species.

377 It is important to note that our results were seen on a few number of species, thus more species
378 should be investigated to support our conclusions. Also, our results were obtained regardless of
379 the plant growth stages. In previous studies, the interaction of plant stages (or harvest dates)
380 with light and/or nitrogen treatments on morphological variables were significant (Shipley and
381 Almeida-Cortez, 2003; Colbach *et al.*, 2020b). It means that the response of morphological
382 variables to nitrogen stress might vary between plant stages. This aspect should be investigated
383 via experiments with sampling dates made at key growth stages.

384

385 *4.3. Practical application: impact on field competition*

386

387 Specific leaf area (SLA) and leaf biomass ratio (LBR) are crucial for light competition and the
388 differential species responses of these morphological variables to nitrogen stress might affect
389 crop-weed competition. For example, *P. aviculare* was the species that the most decreased its
390 SLA (in unshaded conditions) and its LBR in response to nitrogen stress. In the case of nitrogen
391 stress, this species might grow less than other species, as it decreased the area and proportion
392 of its photosynthetically active tissues the most as well as its capacity to shade neighbouring

393 plants. At the opposite, *A. myosuroides*, which decreased its SLA and LBR the least, might be
394 more competitive for light than the other species in the case of nitrogen stress.

395 Height is also important for light competition and for the establishment of plant hierarchies
396 (Nagashima, 1999; Colbach *et al.*, 2019), so differential responses of plant height per unit
397 biomass to plant nitrogen stress could affect the outcome of crop-weed competition. For
398 example, at a given biomass, *T. aestivum* growth could be more disfavoured by nitrogen stress
399 than weed growth because weed height per unit biomass remains unchanged (for the species
400 experimented here) whereas *T. aestivum* decreases height per biomass. Conversely, *F.*
401 *arundinacea* (whose height per unit biomass increases with nitrogen stress) would be
402 advantaged relatively to weeds in the case of nitrogen stress.

403 However, the outcome of crop-weed competition regarding light and nitrogen depends on many
404 other factors. For instance, the competitive advantage of a species depends on its efficiency to
405 produce specific leaf area, leaf biomass ratio or height per unit biomass at optimal nitrogen
406 nutrition (parameters **SLA0**, **LBR0** and **HBR0**). These parameters values change with light
407 environment of the plants. For example, shaded plants are more efficient to produce leaf area
408 per unit leaf biomass at optimal nitrogen nutrition than unshaded plants (Colbach *et al.*, 2020b).

409 Many other processes can affect the outcome of crop-weed competition, such as plant
410 germination and growth dynamics, phenology, access to water, pedoclimate and cropping
411 techniques, such as fertilisation (which affects timing of nutrient availability), soil management,
412 crop cultivar, sowing date and density or herbicide use (Little *et al.*, 2021). Mechanistic crop
413 models (i.e. based on biological or physical processes) allow synthesizing existing knowledge,
414 exploring diverse prospective scenarios and designing cropping systems (Renton and Chauhan,
415 2017; Gaudio *et al.*, 2019). Thus, integrating the parameters outlined in the present study into
416 a mechanistic model simulating weed dynamics and crop yield over the years from cropping
417 system, pedoclimate and species traits such as FLORSYS (Colbach *et al.*, 2020a; Moreau *et al.*,
418 2021) and performing simulations help to identify the key traits that influence plant-plant
419 competition and how they impact it (Colbach *et al.*, 2019). Global sensitivity analysis analyse
420 the variability of model outputs in response to the simultaneous variation of model inputs (such
421 as species parameters) throughout their entire range of variation, taking into account the
422 interactions between inputs (Saltelli *et al.*, 2008). Such analyses would allow to identify and
423 rank parameters in function of their influence on plant-plant competition, thus providing
424 guidelines on competitive crop species/cultivars to sow.

425

426 **5. Conclusions**

427

428 By using a plant nitrogen stress indicator instead of an environmental variable (i.e. nutrient
429 supply), we could characterize species morphological intrinsic response to nitrogen stress in
430 every plant growth environment. Specific leaf area (SLA) decreased in response to nitrogen
431 stress for all species, while leaf biomass ratio (LBR) was more plastic, weed species increasing
432 more their stem biomass than leaf biomass in response to higher nitrogen stress, in contrast to
433 crop species. Height per unit biomass (HBR) was mostly independent of nitrogen stress. This
434 novel plant indicator has the benefits of disentangling the effect of light from nitrogen
435 availability. Morphological response to nitrogen stress was independent from shading for a
436 majority of species, the light effect observed for a few rare species was negligible compared to
437 their response to nitrogen stress. Our approach also allowed us to partially explain species
438 response to habitat (characterised by ecological integrative indices). These results help to better
439 understand the behaviour of a given species in situations of competition for light and nitrogen,
440 an increase of SLA, LBR and HBR in response to nitrogen stress increasing species light
441 competitiveness in situation of nitrogen deficiency. As species competitiveness depends on
442 many factors, the parameters developed in this study can be integrated in mechanistic crop
443 models. Simulations with such models will allow ranking parameters depending on their
444 influence and effect on plant-plant competition.

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446

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456

457 **Conflict of interest**

458 The authors declare that they have no conflict of interest.

459

460 **CRedit authorship contribution statement**

461 Laurène Perthame: Formal analysis, Investigation, Writing - original draft, Writing - review &
462 editing, Visualization, Project administration.

463 Nathalie Colbach: Formal analysis, Writing - review & editing, Supervision, Funding
464 acquisition, Project administration.

465 Hugues Busset: Methodology, Investigation.

466 Annick Matejcek: Methodology, Investigation.

467 Delphine Moreau: Conceptualization, Methodology, Investigation, Writing - review &
468 editing, Supervision, Project administration, Funding acquisition.

469

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471

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571 disturbance and fertilization. *Ecology*, **74**: 599-611.

573 **Supporting Information**

574 Additional Supporting Information may be found in the online version of this article.

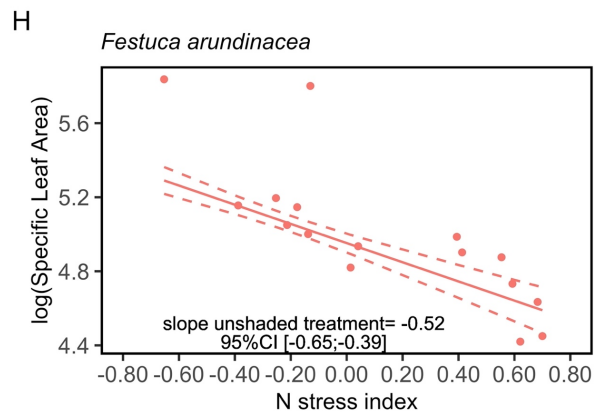
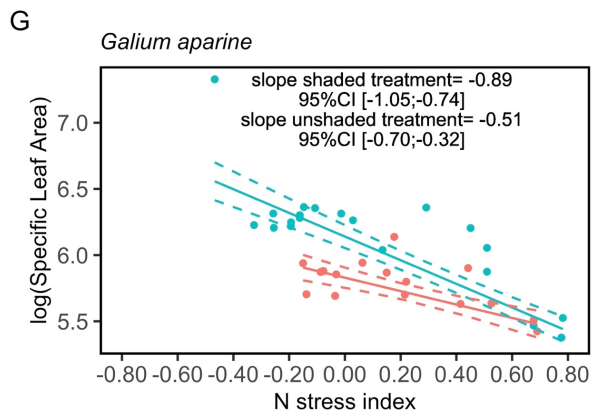
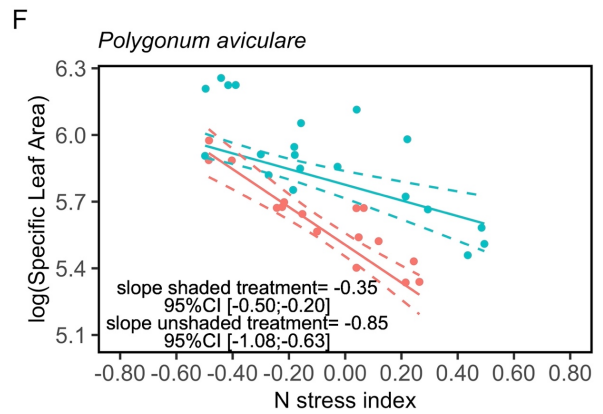
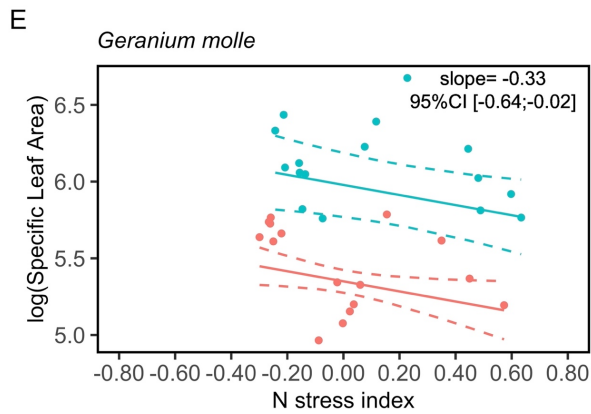
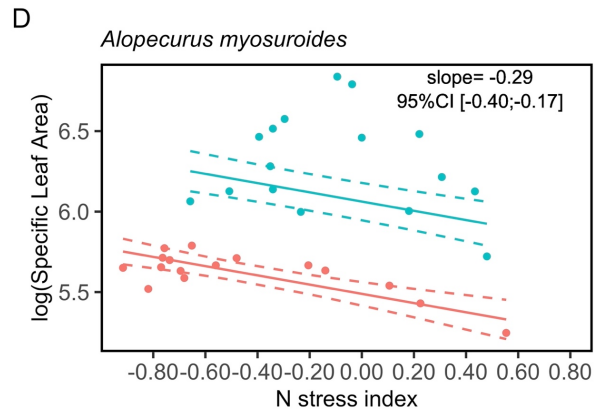
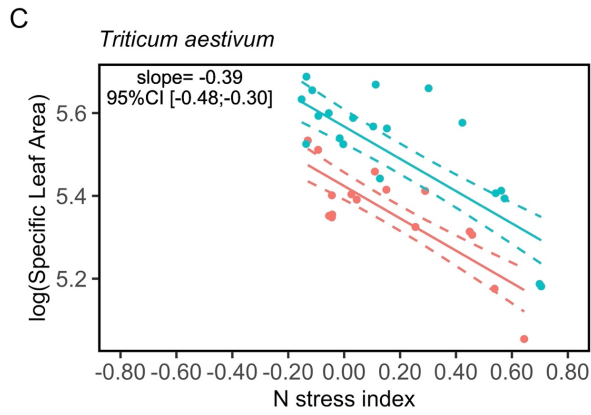
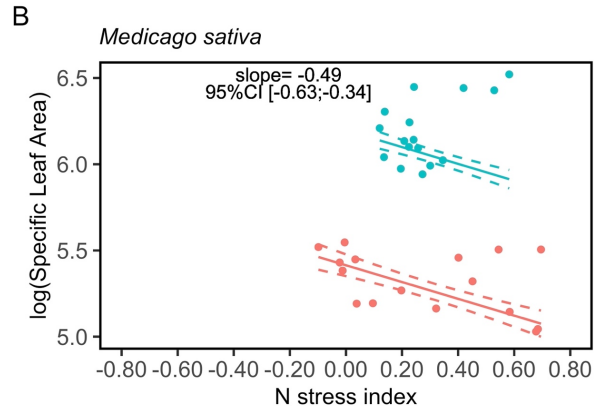
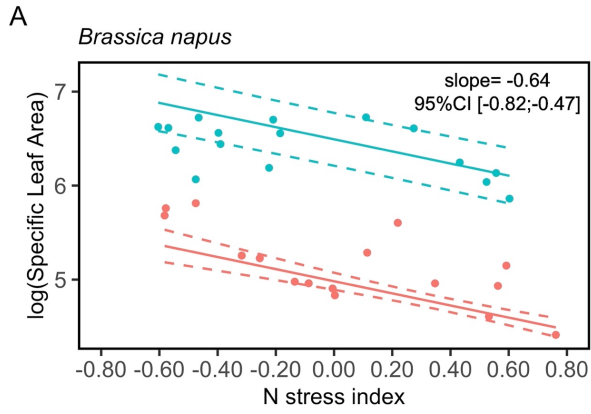


Figure 1: Relationship between \log_n -transformed values of specific leaf area and nitrogen stress index for different species grown in unshaded (red) and shaded treatment (blue). Points correspond to the mean of the four to six plants for a species \times light treatment \times nitrogen treatment \times sampling date. Lines result from fitting to our data linear model **Erreur ! Source du renvoi introuvable.** without the interaction between nitrogen stress index and light treatment (A-E), complete linear model **Erreur ! Source du renvoi introuvable.** (F-G) and linear model **Erreur ! Source du renvoi introuvable.** without light treatment effect (H) (choices depending on **Erreur ! Source du renvoi introuvable.**). Dashed lines indicate 95% confidence interval of the regression. Slopes correspond to SLA_{muN} (with 95% confidence interval).

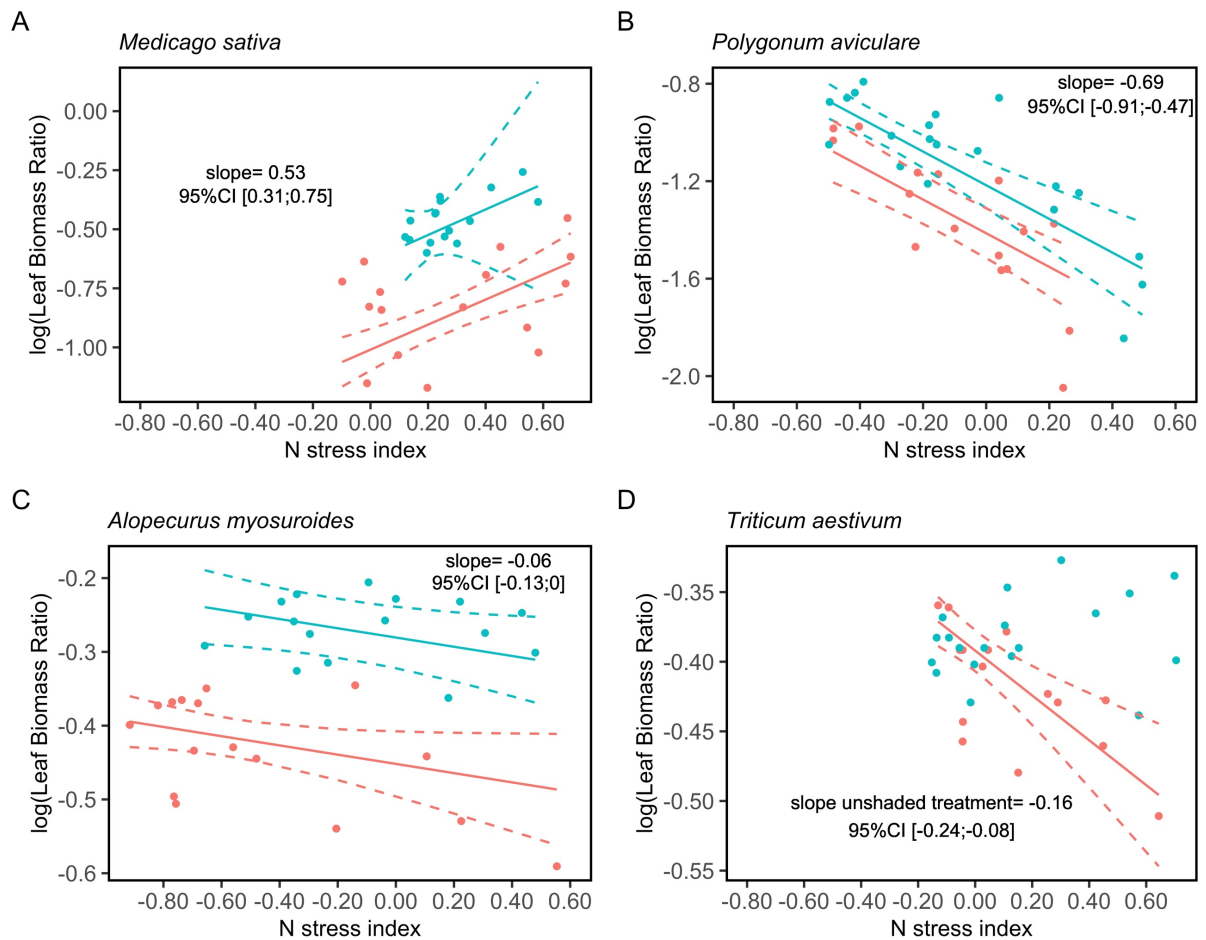


Figure 2: Relationship between \log_n -transformed values of leaf biomass ratio and nitrogen stress index for different species grown in unshaded (red) and shaded treatment (blue). Points correspond to the mean of the four to six plants for a species \times light treatment \times nitrogen treatment \times sampling date. Lines result from fitting to our data linear model **Erreur ! Source du renvoi introuvable.** (applied to leaf biomass ratio instead of specific leaf area) without the interaction between nitrogen stress index and light treatment (A-C) and complete linear model **Erreur ! Source du renvoi introuvable.** (D) (choices depending on **Erreur ! Source du renvoi introuvable.**). Dashed lines indicate 95% confidence interval of the regression. Slopes correspond to LBR_{μN} (with 95% confidence interval).

- 1 **Table 1: Details of the experiments performed in greenhouse in Dijon (France).** Plants were grown under four or five nitrogen treatments and
 2 two light treatments: unshaded (100% of incident light available to plants) or shaded.

Experiment	Species (cultivar for crops)	Mono- or dicotyledonous species	Ellenberg N-number ^b	Ellenberg L-number ^b	Nitrogen treatments (mM)		Percentage of incident light available to plants in shaded treatment
					Unshaded treatment	Shaded treatment	
#1	<i>Medicago sativa</i> (Agathe NF ^a)	Dicotyledon	NA	8	0.4, 1, 5 and 10	0.2, 0.4, 1 and 5	10%
	<i>Festuca arundinacea</i> (Soni)	Monocotyledon	5	8		Only unshaded treatment	Only unshaded treatment
#2	<i>Brassica napus</i> (Kadore)	Dicotyledon	12.4	NA	1, 5, 10 and 14	0.4, 1, 5 and 10	10%
	<i>Geranium molle</i>	Dicotyledon	4	7			
#3	<i>Triticum aestivum</i> (Caphorn)	Monocotyledon	4.4	NA	1, 5, 10 and 14	0.4, 1, 5, 10 and 14	40%
	<i>Polygonum aviculare</i>	Dicotyledon	6	7			
#4	<i>Alopecurus myosuroides</i>	Monocotyledon	6	6	1, 5, 10 and 14	0.4, 1, 5 and 10	10%
#5	<i>Galium aparine</i>	Dicotyledon	8	7	1, 5, 10 and 14	0.4, 1, 5, 10 and 14	40%

3 ^a Non-N₂-fixing

4 ^b Ellenberg N- and Ellenberg L-number estimated from Ellenberg (1974) for all species except the N-number of *B. napus* and *T. aestivum* (Moreau
 5 et al., 2013). NA for non-available data.

6 **Table 2: Partial R² values** (calculated from the type-III sum of squares of ANOVA) **from**
7 **model** Erreur ! Source du renvoi introuvable. **to explore the proportion of variance of log_e-**
8 **transformed values of specific leaf area explained by nitrogen stress index and light**
9 **treatment**, except for *F. arundinacea* analysed only in unshaded treatment. Only factors
10 significant at p≤0.05 were kept, NS indicate non-significant factors that were removed from the
11 model. P-values are indicated in parenthesis.

Factor	Partial R ² (p-value)							
	Crop species				Weed species			
	<i>Brassica napus</i>	<i>Festuca arundinacea</i>	<i>Medicago sativa</i>	<i>Triticum aestivum</i>	<i>Alopecurus myosuroides</i>	<i>Galium aparine</i>	<i>Geranium molle</i>	<i>Polygonum aviculare</i>
Light Treatment	0.571 (<0.0001)	Only unshaded treatment	0.904 (<0.0001)	0.246 (<0.0001)	0.577 (<0.0001)	0.361 (<0.0001)	0.479 (<0.0001)	0.279 (<0.0001)
Nstress	0.305 (<0.0001)	0.843 (<0.0001)	0.058 (<0.0001)	0.520 (<0.0001)	0.190 (<0.0001)	0.361 (<0.0001)	0.066 (0.0215)	0.486 (<0.0001)
Nstress × Light Treatment	NS	Only unshaded treatment	NS	NS	NS	0.125 (0.0028)	NS	0.084 (0.0007)
Total variance explained	0.876	0.843	0.962	0.766	0.767	0.847	0.545	0.849

13 **Table 3: Partial R² values** (calculated from the type-III sum of squares of ANOVA) **from**
 14 **model** Erreur ! Source du renvoi introuvable. **applied to leaf biomass ratio to explore the**
 15 **proportion of variance of log_n-transformed values of leaf biomass ratio explained by**
 16 **nitrogen stress index and light treatment**, except for *F. arundinacea* analysed only in
 17 unshaded treatment. Only factors significant at p≤0.05 were kept, NS indicate non-significant
 18 factors that were removed from the model. P-values are indicated in parenthesis.

Factor	Partial R ² (p-value)							
	Crop species				Weed species			
	<i>Brassica napus</i>	<i>Festuca arundinacea</i>	<i>Medicago sativa</i>	<i>Triticum aestivum</i>	<i>Alopecurus myosuroides</i>	<i>Galium aparine</i>	<i>Geranium molle</i>	<i>Polygonum aviculare</i>
Light Treatment	0.670	Only unshaded treatment	0.437	0.000	0.552	0.330	0.690	0.142
	(<0.0001)		(0.0113)	(0.993) ^a	(<0.0001)	(0.0002)	(<0.0001)	(0.0019)
Nstress	NS	NS	0.248	0.307	0.052	NS	NS	0.559
			(<0.0001)	(<0.0001)	(0.0089)			(<0.0001)
Nstress × Light Treatment	NS	Only unshaded treatment	NS	0.181	NS	NS	NS	NS
				(0.0006)				
Total variance explained	0.670	NS	0.685	0.488	0.604	0.330	0.690	0.701

19 ^a Non-significant factor kept in the model because of significant interaction with nitrogen stress
 20 index.

21 **Table 4: Partial R² values** (calculated from the type-II sum of squares of ANOVA) **from**
 22 **model** Erreur ! Source du renvoi introuvable. **to explore the proportion of variance of log_n-**
 23 **transformed values of height explained by nitrogen stress index, light treatment and log_n-**
 24 **transformed values of aboveground biomass (Bsh in g)** (Munier-Jolain *et al.*, 2014). Only
 25 factors significant at p≤0.05 were kept, NS indicate non-significant factors that were removed
 26 from the model. P-values are indicated in parenthesis.

Factor	Partial R ² (p-value)							
	Crop species				Weed species			
	<i>Brassica napus</i>	<i>Festuca arundinacea</i>	<i>Medicago sativa</i>	<i>Triticum aestivum</i>	<i>Alopecurus myosuroides</i>	<i>Galium aparine</i>	<i>Geranium molle</i>	<i>Polygonum aviculare</i>
Light Treatment	Only unshaded treatment	Only unshaded treatment	0.269 (0.012)	0.144 (0.0009)	Only unshaded treatment	0.044 (0.0032)	Only unshaded treatment	NS
Nstress	NS	0.343 (0.0392)	NS	0.103 (0.0031)	NS	NS	NS	NS
Log _n (Bsh)	0.986 (<0.0001)	NS	0.662 (<0.0001)	0.572 (<0.0001)	0.936 (<0.0001)	0.875 (<0.0001)	NS	0.599 (0.0001)
Nstress × Light Treatment	Only unshaded treatment	Only unshaded treatment	NS	NS	Only unshaded treatment	NS	Only unshaded treatment	NS
Total variance explained	0.986	0.343	0.931	0.819	0.936	0.919	NS	0.599

28 **Table 5: Response of two parameters (response of specific leaf area to nitrogen stress and**
 29 **response of leaf biomass to nitrogen stress) to species features (Ellenberg-N number and**
 30 **species type).**

Factor		SLA_muN	LBR_muN
Ellenberg-N number	Slope	-0.037	Non-significant at $p \leq 0.1$
	Intercept	-0.246	
	R ²	0.269	
	p-value	0.07	
Species type	Weed	Non-significant at $p \leq 0.1$	-0.375
	Crop		0.233
	R ²		0.490
	p-value		0.05

31

32