Barley shoot biomass responds strongly to N:P stoichiometry and intraspecific competition, whereas roots only alter their foraging

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18 **Abstract**

19 *Aims* Root system responses to the limitation of either nitrogen (N) or phosphorus (P) are well documented, but how

20 the early root system responds to (co-) limitation of one (N or P) or both in a stoichiometric framework is not well-

21 known. In addition, how intra-specific competition alters plant responses to N:P stoichiometry is understudied.

22 Therefore, we aimed to investigate the effects of N:P stoichiometry and competition on root system responses and

23 overall plant performance.

24 *Methods* Plants (*Hordeum vulgare* L.) were grown in rhizoboxes for 24 days in the presence or absence of competition

25 (three vs. one plant per rhizobox), and fertilized with different combinations of N:P (low N+low P, low N+high P,

26 high N+low P, and high N+high P).

27 *Results* Shoot biomass was highest when both N and P were provided in high amounts. In competition, shoot biomass

28 decreased on average by 22%. Total root biomass (per plant) was not affected by N:P stoichiometry and competition

29 but differences were observed in specific root length and root biomass allocation across soil depths. Specific root

30 length depended on the identity of limiting nutrient (N or P) and competition. Plants had higher proportion of root

31 biomass in deeper soil layers under N limitation, while a greater proportion of root biomass was found at the top soil

32 layers under P limitation.

33 *Conclusions* With low N and P availability during early growth, higher investments in root system development can

34 significantly trade off with aboveground productivity, and strong intra-specific competition can further strengthen

35 such effects.

36 **Keywords:** Root system responses, vertical root distribution, specific root length, nutrient stoichiometry, intraspecific

37 competition

Introduction

Nutrient foraging capacity of roots determines plant performance under both heterogeneous soil nutrient availability and belowground competition with neighbors (Stibbe and Märländer, 2002; Soleymani et al. 2011; Bennett et al. 2016; Reiss and Drinkwater, 2018). Given that nutrient foraging by roots is an active process (Zhang et al. 2019), it is very likely that plant biomass allocation and root system responses will be driven by the nutrient which is limiting plant growth the most (Poorter et al. 2012). It has previously been shown for many crops how eco-physiological (Gastal and Lemaire, 2002), morphological (Fransen et al. 1998), architectural (Williamson et al. 2001; Postma and Lynch, 2012; Lynch, 2013), and anatomical (Wahl et al. 2001; Postma and Lynch, 2011) root traits respond to nitrogen (N) and phosphorus (P) availability in soil. For instance, Wang et al. (2015) showed contrasting root morphological and physiological trait responses of canola, barley, and potato in relation to low P availability. In order to increase P uptake, canola exuded more citric acid and developed longer roots, barley increased exudation of malic acid and reduced its root surface area and total root length, whereas potato reduced the exudation of organic acids but increased the number of root tips. Overall, it is clear that root systems respond in a species-specific way to nutrient stimuli by modifying their size and architecture (Kembel et al. 2008; Wang et al. 2015; McNickle et al. 2016). Further, Kembel and Cahill (2005) showed for more than 100 plant species that such nutrient foraging responses can be taxonomically and phylogenetically conserved. Thus, it is important to understand how plants respond to the balance between the availability of two major growth-limiting macronutrients (N and P) with consequences on cell metabolism and overall growth and development. Addition of either N or P to soil increases their uptake and consequently plant growth. This suggests widespread N and P co-limitation (Elser et al. 2011; Capek et al. 2018). Leaf N:P stoichiometry has previously been studied to establish their relationship with plant growth (Cornelissen et al. 1997; He et al. 2008). For instance, for optimal plant physiology, the elemental N and P ratio in plant biomass should be relatively stable (Güsewell, 2004). Nitrogen is an integral part of most of the enzymatic machinery, and higher N than P demand in cell metabolism indicates that N limitation can severely affect plant growth and consequently biomass production. However, to what extent stoichiometric N:P availability in soil affects root systems and overall plant growth, and how the observed effect depends on the presence of intraspecific competition has been rarely tested. It is not clear whether plants (especially roots) respond similarly to varying N and P availability and if such responses are affected by intraspecific competition. Hence, it becomes important to understand the root foraging responses to stoichiometric availability of both N and P during early plant establishment in a factorial manner with altered N:P mass ratio of low/high N and P. Differences in mobility between N and P affect their availability to plants, and root responses are likely to be specific to nutrient distribution in soil. For example, P (as orthophosphate) is highly immobile in the soil and accumulates in the topsoil strata via plant residue and fertilizer inputs. Therefore, wide dispersion of lateral roots, enhanced adventitious rooting, and shallower root growth angles are among the key root responses that are associated with enhanced topsoil foraging for P (Lynch and Brown, 2001; Lynch, 2011). In contrast, N (as nitrate) is relatively mobile in the soil compared to P and moves down the soil strata with irrigation and precipitation events. Fewer crown roots in maize, for example, can potentially improve N acquisition by exploring deep soil strata, a key root system response (Saengwilai et al. 2014; Guo and York, 2019). Therefore, the coordinated uptake and utilization of both N and P are essential in relation to optimal plant growth. However, very little is known about how plants adjust their

75 biomass allocation and root growth responses to soil N:P stoichiometry. It is further not clear how co-limitation of

76 both N and P will direct the plant's response for their uptake (Venterink and Güsewell, 2010; Hu and Chu, 2019).

77 Root responses not only depend on soil nutrient availability but also on the presence of neighbors (whether of the

78 same or different species) through root-root competition for available nutrients (Cahill et al. 2010; Faget et al., 2013;

79 McNickle and Brown, 2014, Weidlich et al. 2018). Nutrient requirements are important determinant of plant-plant

80 competition. This is particularly true in mono-cropping systems where there is strong intraspecific competition for

81 soil nutrients, mainly because neighbors share the same life-history strategies and have similar resource demands.

82 Intense competition results in a direct negative effect on plant growth and ultimately on yield (Craine and Dybzinski,

83 2013; Bennett et al. 2016). Bennett et al. (2016) have shown interactive effects of nutrients with or without inter- and

84 intraspecific competition on plant biomass allocation and root system responses for grasses, legumes, and forbs.

85 Further, Hecht et al. (2016) showed for barley that roots respond to greater intraspecific competition (via manipulating

86 sowing density) by increasing root length density and specific root length through increased fine root production.

87 Later, Hecht et al. (2019) showed that the greater root length density under intraspecific competition was attributed to

88 greater main root numbers. Moreover, root responses to the intraspecific competition may also include root segregation

89 and aggregation to maximize the acquisition of nutrients (Cahill et al. 2010; Weidlich et al. 2018; Zhang et al. 2019).

90 Regardless of understanding how the availability of either N or P interacts with belowground competition to affect

91 plant growth (Thuynsma et al. 2016; Sun et al. 2016), it is still unclear how plants integrate the responses to differential

92 nutrient availability and the presence or absence of intraspecific competition during early growth stages. Therefore,

93 the aim of this study was twofold: (1) investigating how N:P stoichiometry in the soil solution affects plant

94 performance and root system responses of barley (*Hordeum vulgare* L.); and (2) determining if intraspecific

95 competition interacts with N:P stoichiometry in shaping plant performance.

96 We hypothesized that:

97   (1) From the nutrient stoichiometry perspective, N is more limiting than P for plant growth and low availability of N

98 has stronger effects than that of P on plant performance (both below- and aboveground).

99 (2) The intraspecific competition will lead to strong nutrient depletion, resulting in overall biomass reduction per plant.

100 (3) Root distribution and foraging strategy will be affected by N:P stoichiometry, with plants rooting deeper when N

101 is limiting and shallower when P is limiting, and the strength of the response will be modulated by intraspecific

102 competition.

103

104 **Materials and methods**

105 *Experimental setup*

106 The experiment was conducted in the greenhouse of the Leuphana University Lüneburg (Lüneburg, Germany,

107 53°14'23.8"N 10°24'45.5"E) from August 18th 2017 to September 11th 2017 for a total of 24 days. The average

108 day/night temperature and relative humidity were 22.3/15.3°C and 60/73%, respectively. A homogenous soil mixture

109 was prepared using sand, loess soil (nutrient-poor, collected from a lignite mine near Jackerath, Germany), and peat

110 potting soil (Nullerde, Einheitserde Werkverband e.V., Germany) in 8:2:1 ratio, respectively. Rhizoboxes (Height: 58

111 cm × Width: 26.6 cm × Thickness: 2 cm; volume: 3 L) were filled with ~ 5 kg of soil mixture. Pre-germinated (pre-

germination time: 24 h on a wet tissue paper) barley (*Hordeum vulgare* L. cv. Barke, Saatzucht Breun, Germany) seedlings were transplanted in rhizoboxes as shown in Fig.1. Each rhizobox received 1 seedling for absence and 3 seedlings (7.5 cm apart from each other) for the presence of intraspecific competition (hereafter competition). Rhizoboxes were placed in containers at a 45° angle and each container contained five rhizoboxes. In each container, the front rhizobox was covered with a black plastic plate and the last rhizobox was covered with a white polystyrene plate to maintain similar light and temperature conditions, respectively. Rhizobox position was randomly changed every fourth day.

The experiment was designed using a full factorial design to test how N:P stoichiometry (four levels: low N+low P (LN-LP), low N+high P (LN-HP), high N+low P (HN-LP), and high N+high P (HN-HP)) (based on pre-test showing that shoot growth was limited by N only above a 'threshold' of low P availability) and intraspecific competition (two levels: absence or presence of competitors) affect biomass production and allocation, soil exploration by roots, and N:P uptake of barley. In total, 8 treatment combinations were tested (4 levels of N:P stoichiometry × 2 levels of intraspecific competition) and each treatment was replicated five times resulting in a total of 40 experimental units (rhizoboxes). The mass ratio of 4 levels of N:P stoichiometry were 5.81 (for LN-LP), 1.45 (LN-HP), 22.47 (HN-LP), and 5.81 (HN-HP). Rhizoboxes were provided with 800 mL of half Hoagland concentration per rhizobox before transplanting. The composition of the Hoagland solution was adjusted for each N:P stoichiometry level (low/high N, low/high P) (Supplementary table 1). To maintain the osmotic potential, we used K_2SO_4 and $CaCl_2.2H_2O$ as a replacement for KH_2PO_4, $Ca(NO_3)_2.4H_2O$ and KNO_3 as mentioned in table 1. Rhizoboxes were left to drain for 24h and subsequently weighed. For each rhizobox, water loss was estimated as the difference between the mass of a rhizobox at a given time and its mass recorded at the beginning of the experiment.

Harvest and measurements

At harvest, shoots were cut at the base and oven-dried at 80 °C (for 48 h) until a constant mass was reached. Afterward, we carefully removed the front window of each rhizobox and divided the soil into six 10-cm layers (0-10, 10-20, 20-30, 30-40, 40-50, 50-58 cm). For each soil layer, roots were washed with tap water and stored at -20 °C until further measurements. We followed the protocol of Delory et al. (2017) for root trait measurements. Briefly, material adhering to roots was removed with brush and tweezers. In order to improve fine root detection during image analysis, clean roots were stained with a 1.7 mM neutral red solution for ~24 h. Excess stain was removed by continuously rinsing roots with distilled water, and big root segments were cut into small pieces to avoid root overlaps during scanning. Stained roots were spread in a thin layer of distilled water in a transparent tray and scanned at 600 dpi using a commercial scanner (Epson Perfection V800 Photo, Epson, Japan). Scanned images were then analyzed with an image analysis software (WinRhizo, Regent Instruments, Quebec, Canada) using a global threshold method. Interactive modifications to grey level pixel classification were made to improve root detection and root length estimation (Delory et al. 2017). Afterward, roots were dried at 60 °C (for 48 h) until a constant mass was reached. Root mass fraction (RMF) was calculated as the ratio of root biomass to the total plant biomass, and specific root length (SRL) was calculated as root length per unit of root biomass.

148 All shoot material was ground with a ball mill (MM 400, Retsch, Germany), and measured for total C and N with an

149 elemental analyzer (Vario EL, Elementar, Germany). For shoot P concentration, 70 mg ground samples were spiked

150 with 2 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) before microwave extraction, using a MARS 5 microwave system

151 (CEM GmbH, Germany) at 800W (80%) power, a linear temperature gradient from RT to 160°C in 20 min, holding

152 the end temperature for 15 min. Afterward, each sample was filled up to 14 mL with ultrapure water. For P

153 concentration determination, two aliquots of the obtained solution were diluted 1:20 with ultrapure water and analyzed.

154 The relative standard deviation between the two repetitions was ± 10%. Total P was measured with inductively

155 coupled plasma optical emission spectrometry (iCAP™ 7600 ICP-OES Analysator, Thermo Scientific, Germany).

156 *Vertical root distribution*

157 The vertical root distribution in each rhizobox was modeled using the following asymptotic equation (Gale and Grigal,

158 1987; Jackson et al. 1996; Oram et al. 2018):

$$Y = 1 - \beta^d$$

160 Where *Y* is the cumulative proportion [0,1] of the total root biomass located above depth *d* (in this case 0 – 58 cm),

161 and *β* is a fitted model parameter used as a simple numerical index of vertical root distribution (Schnepf et al. 2019).

162 Lower *β* values correspond to higher root mass allocation to surface layers, whereas higher values correspond to higher

163 root mass allocation to deeper soil strata (Fig. 2).

164 *Statistical analyses*

165 All statistical analyses were performed in R 3.5.0 (R Core Team, 2018) and graphs were prepared with 'ggplot2'

166 library (Wickham, 2016) and R-base. We followed the protocol for data exploration in Zuur et al (2010). Potential

167 outliers were detected using a combination of boxplots and Cleveland plots. Presented in graphs are mean values of 5

168 replicates (4 replicates for specific root length except for LN-HP where n = 5) ± standard error (SE). Two-way

169 ANOVA models were used to test if N:P stoichiometry, intraspecific competition, and their interaction affected shoot

170 and root biomass, vertical root distribution (β), specific root length, and shoot N and P concentrations. Residual plots

171 were visually checked for any mean-variance relationship ("V shape"). As we did not observe any pattern suggesting

172 heteroscedasticity in our data, we did not transform the data prior to analysis. Pairwise comparisons were performed

173 on estimated marginal means computed by *lsmeans* using Tukey contrasts (lsmeans; Lenth, 2016). In case there was

174 no interaction between N:P stoichiometry and competition, we show only N:P stoichiometry effects (for shoot biomass

175 and shoot P concentration). The linear relationship between shoot N concentration and specific root length was

176 analyzed using standard major axis (SMA) regression using the *smatr* package (Warton et al. 2012). SMA regression

177 examines the relationship between two variables that are both measured with errors (Warton et al. 2012).

178

179 **Results**

180 *Shoot biomass*

181 Both N:P stoichiometry ($F_{3,32} = 53.08$, $P < 0.001$) and competition ($F_{1,32} = 52.07$, $P < 0.001$) had a significant effect

182 on shoot biomass (per plant) production. The effect of N:P stoichiometry did not depend on the level of intraspecific

183 competition ($F_{3,32} = 0.48$, $P = 0.69$). Looking at the effect of N:P stoichiometry, shoot biomass (per plant) increased

184 in the following order: LN-LP < LN-HP < HN-LP < HN-HP. Compared to LN-LP, shoot biomass was on average

185   12%, 32%, and 58% greater under LN-HP, HN-LP, and HN-HP, respectively (Fig. 3A). For plants grown in the

186   presence of competitors, shoot biomass was on average 22% lower than plants grown in the absence of competition.

187   *Root system responses*

188   Even though the greater amount of either N, P, or both increased shoot biomass, neither N:P stoichiometry ($F_{3,32}$ =

189   0.79, $P = 0.51$) nor competition ($F_{1,32} = 1.49$, $P = 0.24$) had an effect on total root biomass production (per plant) (Fig.

190   3B). However, biomass allocation as measured by the RMF was affected by N:P stoichiometry ($F_{3,32} = 32.62$, $P <$

191   0.001), competition ($F_{1,32} = 26.01$, $P < 0.001$), and their interaction ($F_{3,32} = 5.77$, $P = 0.002$). Irrespective of the

192   presence or absence of competition, RMF was greater when both N and P were provided in low amounts (LN-LP)

193   (Fig. 4). A high amount of either N, P, or both decreased RMF when plants were grown without competition. In

194   contrast, when plants were grown in competition, providing high P (LN-HP) had no effect on RMF as compared to

195   LN-LP (Fig. 4). In addition, providing high N with low or high P (HN-LP and HN-HP) reduced RMF both in the

196   presence and absence of competitors.

197   Vertical root distribution (*β*) was affected differently across N:P stoichiometry levels for plants growing alone or in

198   competition (N:P stoichiometry: $F_{3,32} = 22.19$, $P < 0.001$; competition: $F_{1,32} = 59.46$, $P < 0.001$; N:P stoichiometry ×

199   competition: $F_{3,32} = 4.85$, $P = 0.006$). Vertical root distribution was governed by the identity of the nutrient being the

200   most limiting (either N, P, or both) only for individually grown barley plants (in absence of competition). Without

201   competition, a greater proportion of root biomass was found in deeper soil layers (greatest *β* value) when both N and

202   P were provided in low amounts (LN-LP). On average, plants grown without competition in the LN-HP treatment also

203   had a greater proportion of root biomass deeper in the soil as compared to HN-LP and HN-HP treatments (Fig. 5A).

204   Interestingly, the presence of competitors had a strong effect on the vertical root distribution. In this situation, the

205   identity of the nutrient being the most limiting did not have any impact on root distribution. Overall, plants tended to

206   increase root biomass allocation to deeper soil layers (greater *β* values) when growing in competition (Fig. 5A, see

207   supplementary Fig.2 for depth-wise root biomass).

208   Even though the belowground biomass production remained similar between experimental treatments, root

209   morphology was clearly impacted. Specific root length (SRL) was affected by N:P stoichiometry ($F_{3,32} = 7.06$, $P =$

210   0.001) and interacted with competition ($F_{3,32} = 5.70$, $P = 0.003$), but competition alone had no effect on SRL (Fig.

211   5B). Without competition, SRL was greater when either N, P, or both were provided in low amounts and did not

212   depend on the identity of the nutrient being the most limiting. In contrast, in the presence of competition, SRL was

213   greater only when P was the only limiting nutrient (HN-LP) (Fig. 5B).

214   *Shoot N:P concentrations*

215   N:P stoichiometry and competition (presence/absence) had distinct effects on shoot N and P concentrations. Providing

216   more N (HN-LP and HN-HP) or more P (LN-HP and HN-HP) resulted in greater shoot N and P concentrations,

217   respectively. Shoot N concentration was significantly altered by N:P stoichiometry ($F_{3,32} = 222.9$, $P < 0.001$),

218   competition ($F_{1,32} = 259.3$, $P < 0.001$), and their interaction ($F_{3,32} = 10.9$, $P < 0.001$) (Fig. 6A). Without competition,

219   shoot N remained similar for both HN-LP and HN-HP, whereas, in the presence of competition, plant shoots had a

220   greater N concentration under HN-LP than HN-HP (Fig. 6A). On the other hand, shoot P concentration was altered

221   only by N:P stoichiometry ($F_{3,32} = 9.19$, $P < 0.001$). Providing more P increased its concentration in shoots on average

222 by 53% (LN-HP) and 42% (HN-HP) (Fig. 6B). Our results also showed the existence of a positive correlation between

223 SRL and shoot N concentration ($R^2 = 0.51$; $P = 0.001$), but only under intraspecific competition (Fig. 7). Further,

224 shoot N:P mass ratio was affected by N:P stoichiometry ($F_{3,32} = 21.72$, $P < 0.001$) and competition ($F_{1,32} = 5.50$, $P =$

225 0.025). Compared to LN-LP, lower shoot N:P values were observed when N was the only limiting nutrient, while

226 greater shoot N:P values were observed when P was the only limiting nutrient in the soil solution (Supplementary Fig.

227 1). Shoot N:P ratios decreased from 24.6±2.3 to 20.1±2.1 in the presence of intraspecific competition.

228

229 **Discussion**

230 *Shoot but not root biomass production is more limited by N than by P*

231 There is a general consensus that plants respond to nutrient shortage by changing their allocation patterns both below-

232 and aboveground (Hermans et al. 2006). When the availability of both macronutrients was low (LN-LP), aboveground

233 productivity was the lowest, indicative of nutrient limitation. On the other hand, providing extra P or not, did not

234 increase the shoot biomass production if N was the limiting nutrient (both in LN-LP and LN-HP), highlighting higher

235 N demand for biomass production and supports synergistic response to N and P availability (Harpole et al. 2011). Leaf

236 N content is generally related to C assimilation during photosynthesis (Gastal and Lemaire, 2002). If reduced leaf N

237 content leads to a reduction in the plant's photosynthetic activity, a lower shoot biomass production can be expected

238 when N is limiting in the soil (Fig.1). Andrew et al. (1999) showed for *Pisum sativum*, *Triticum aestivum*, and

239 *Phaseolus vulgaris* that N shortage effects on plant growth are through its effects on protein synthesis. This further

240 demonstrates that N limitation is more severe than P limitation for plant growth (see Capek et al. 2018) as the

241 availability of extra P (LN-HP) in our study did not lead to higher shoot biomass production, probably due to N-

242 mediated decrease in photosynthetic activity. Increased availability of both N and P (HN-HP), on the other hand,

243 resulted in the greatest shoot biomass production because of greater N and P uptake that might ultimately lead to

244 higher photosynthetic activity (Kumar et al. 2019).

245 Interestingly, root biomass production remained similar across N:P stoichiometry levels, but the RMF was greater

246 when both N and P availability was low (LN-LP) in the absence of competition. This follows the general plant response

247 to increasing C investment belowground when nutrient availability in the environment is low (Poorter et al. 2012).

248 Nutrient availability can strongly direct resource allocation patterns in plants (Gastal and Lemaire, 2002). More C

249 allocation to roots under low nutrient availability is a well-known plant response as a potential mechanism to optimize

250 growth by exploring a greater proportion of the soil volume for nutrients (de Groot et al. 2003; Hammond et al. 2006;

251 Lambers et al. 2006). This is in line with optimal resource allocation theory, which predicts higher resource

252 partitioning in organs that maximize the plant growth (Bloom et al. 1985). Increased RMF due to nutrient shortage

253 allows plants to forage more effectively, yet it trades off with resource allocation in shoot biomass production (Garnett

254 et al. 2009). We are aware that RMF only provides information about resource allocation to root growth component

255 and does not necessarily include other carbon investments such as root respiration and exudation, yet it provides a hint

256 about plant investments belowground for nutrient foraging. Greater availability of both N and P (HN-HP) has

257 potentially led to lower investment belowground as shown in various studies for different vegetation (Aerts et al. 1991;

258 Klimes and Klimesova, 1994; Wright et al. 2014). This further supports the notion of preferential uptake of available

259 nutrients by roots, thereby minimizing their resource investments belowground for nutrient acquisition. These findings

260 partly support our first hypothesis as the response to N limitation was only seen for the shoot but not root biomass.

261 *Intraspecific competition reduces shoot but not root biomass production*

262 It has been shown that plant-plant competition decreases the total biomass production both for interspecific

263 (Heuermann et al. 2019) and intraspecific competition (Zhou et al. 2018). We also showed that shoot biomass

264 decreased in the presence of competition. A common underlying reason for this decline in biomass production when

265 plants are competing is due to quick uptake of available nutrients leading to soil nutrient shortage (Tilman, 1990;

266 Craine and Dybzinski, 2013). Surprisingly, we did not observe any change in root biomass production with or without

267 competition. When plants are competing, and if plant growth is mostly affected by nutrient availability in soil, we

268 would expect a greater resource investment in belowground organs to enhance nutrient uptake. In the presence of

269 competition, a strong decrease in shoot biomass without altering root biomass per plant is confirmatory of increasing

270 competitive ability for belowground resources, but at the expense of shoot biomass production. This also hints towards

271 the plant's phenotypic plasticity in biomass partitioning between shoots and roots. Since the duration of our experiment

272 was short and the plants were of the same age and size when grown in competition (3 plants rhizobox^{-1}), we believe

273 that aboveground competition (which is usually size-asymmetric) was low in this study (Weiner and Thomas, 1986).

274 According to the competition model for limiting resources (Van Wijk et al. 2003), a lower investment belowground

275 cannot sustain plant growth due to lower nutrient availability when plants are competing with each other. To maintain

276 growth, therefore, higher investment in roots should be favored. In a recent study focusing on interspecific competition

277 (growing oat with clover), increased root to shoot ratio without affecting shoot biomass production highlights that

278 competition favored root biomass production for nutrient access (Heuermann et al. 2019). Further, the observed

279 increase in RMF without affecting total root biomass under low N availability (LN-LP and LN-HP) supports our first

280 hypothesis that N is more limiting plant growth than P limitation. Secondly, our second hypothesis is partly supported

281 as only shoot biomass but not root biomass decreased with the intraspecific competition.

282 *Root biomass allocation to deeper soil layers increased under N limitation, but only when growing without*

283 *competitors*

284 Root biomass may not always be indicative of the absorptive capacity of roots, and significant modifications in root

285 morphology, anatomy, and architecture are possible with or without altering the total root biomass (Hodge, 2004). In

286 our study, although the total root biomass remained similar between experimental treatments, we showed that the

287 effect of N:P stoichiometry affected root system responses differently depending on the presence or absence of

288 competitors. Such root system responses can be driven by relative mobility and, therefore, availability of N and P in

289 soil strata. Vertical root distribution depended strongly on the identity of the limiting nutrient (either N, P, or both) in

290 the absence of competition. For example, plants invested more root biomass in top soil layers (lower β value) when P

291 availability was low (HN-LP) whereas this allocation shifted to deeper soil layers (higher β value) when N was the

292 most limiting nutrient (LN-HP). Interestingly, when both nutrients were limiting (LN-LP), β was greatest thus

293 suggesting that vertical root distribution was more likely driven by N limitation than P limitation and higher N than P

294 demand. Given that P is less mobile than N in the soil matrix (Harrison, 1987), we expect more P to be present in the

295 topsoil and more N to be present in deeper soil layers, and their relative limitations may have guided root responses.

296 Plants respond to P shortage by reducing the primary root elongation but an increased number of lateral roots (Vance
297 et al. 2003; Sanchez-Calderon et al. 2005). Further, Jia et al. (2018) showed that increasing the lateral root branching
298 enhanced maize P acquisition. Gruber et al. (2013) also showed a shallower yet highly branched root system for
299 *Arabidopsis* under P deficiency. On the other hand, when N is limiting plant growth, the plant's investment in deep
300 root systems is favored (Koevoets et al. 2016). In the presence of competition, β values did not change across N:P
301 stoichiometry levels. Competition most likely resulted in faster depletion of nutrients in soil through plant uptake.
302 Therefore, roots foraged throughout the rhizobox to their maximum extent to get excess to both N and P. In support
303 of our third hypothesis, we show that plants root deeper when N is the most limiting nutrient, whereas shallower when
304 P is the most limiting nutrient, but only in the absence of competition. Further, in the presence of intraspecific
305 competition, root foraging is modulated by deeper soil exploration.

306 We also showed that, in the absence of competition, the SRL was greater when either N, P, or both were available in
307 low amounts relative to HN-HP (Fig. 5B). Changes in SRL are general root morphological responses to lower
308 availability of nutrients in the soil (Kong et al. 2014). By increasing SRL without altering the overall root biomass,
309 plants are able to increase their foraging capacity. However, this may also be an apparent strategy of plants for nutrient
310 acquisition as thinner roots have a lower life span and faster turnover (McCormack et al. 2012). On the contrary, when
311 both N and P are not limiting plant growth (under HN-HP), it is more favorable for plants to invest less in increasing
312 SRL due to associated aboveground allocation trades off. We expected the same effect of N:P stoichiometry on SRL
313 in the presence of competition. However, we found contrasting effects, and SRL was lower when only N (LN-HP) or
314 both N and P (LN-LP) were available in low amounts, whereas it increased only under HN-LP (high N but low P
315 availability). As P is less mobile than N in the soil, increasing P foraging by greater SRL is likely one efficient strategy
316 to increase its uptake. In contrast, greater N mobility would rather result in a deeper rooting system than increasing
317 SRL locally to increase its uptake efficiently. Greater SRL with low P but high N availability (HN-LP) resulted in
318 higher shoot N concentration and associated higher P requirement. However, increased SRL did not result in higher
319 shoot P concentration due to its low availability. This further explains the positive relationship between SRL and shoot
320 N concentration (probably as an indirect consequence of P limitation) (Fig. 7). These findings contrast strongly with
321 results from a study in grasslands by Mommer et al. (2010), where interspecific competition with neighbors caused
322 both higher investment of plants in root biomass as well as an accumulation of roots in the topsoil. This contrasting
323 result could be driven by differences in root responses depending on whether neighbors are of the same or different
324 species. Clearly, the presence of neighbors, whether of the same species or not, can drive this partly unexpected
325 responses of roots. Whether experimental conditions are controlled (in the greenhouse) or not (in the field) will also
326 probably affect the outcome.
327
328 *Effect of N:P stoichiometry and competition on shoot N and P concentrations*
329 Shoot N and P concentrations were in line with what was expected. Providing high N (HN-LP and HN-HP) or high P
330 (LN-HP and HN-HP) resulted in greater N and P concentrations in shoots, respectively. Intriguingly, in the presence
331 of competition, we found that when both N and P availability was high (HN-HP), shoot N concentration was slightly
332 lower than in plants grown under high N and low P (HN-LP) availability. This can most likely be explained by the

333 fact that when both N and P were high, plants grew better (higher shoot biomass under HN-HP than HN-LP) and, as

334 a consequence, exacerbated greater N demand. On the other hand, shoot P concentration was driven only by its

335 availability in the soil and was similar for both with or without competition. This further supports our first hypothesis

336 that soil N availability has a stronger effect in regulating plant performance more than P.

337

338 **Conclusions**

339 Plant responses to soil nutrient availability and plant-plant competition are decisive for plant performance. Lower

340 shoot biomass under low N availability irrespective of P availability (both for LN-LP and LN-HP) indicates N

341 limitation for shoot biomass production most likely due to higher N demand for photosynthesis. Higher investments

342 belowground as a response to nutrient limitation pose a tradeoff with shoot biomass production. Roots foraged

343 differently for N or P uptake. A greater proportion of the total root biomass was found deeper in the soil when N was

344 limiting, while a greater proportion of the root biomass was found closer to the soil surface when P was limiting plant

345 growth. However, when plants were competing for N and P in soil solution, no decrease in root biomass but lower

346 shoot biomass per plant indicated differential resource allocation pattern by plants for maximizing nutrient uptake.

347 When competing, plants rooted deeper indicating higher N demand and associated root acquisition strategy under

348 these conditions. Such shift in plant resource allocation and root growth are key determinants for early plant nutrient

349 acquisition and establishment, and illustrate the importance of biotic as well as abiotic drivers of plant responses to

350 their environment. Field studies that manipulate N:P stoichiometry and focus on root foraging responses would move

351 the field further forward now.

364 **Data availability**
365 Raw data and R scripts used for data analyses can be freely accessed at https://doi.org/10.5281/zenodo.3613623

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506

507

508

Without competition
(1 plant rhizobox^{-1})

With competition
(3 plants rhizobox^{-1})

7.5 cm

0-10 cm

10-20 cm
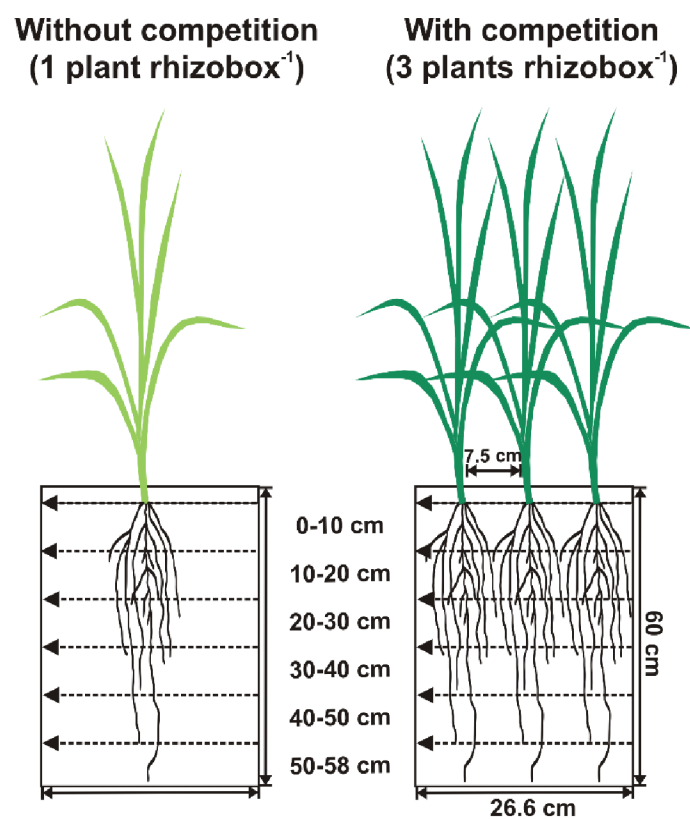
20-30 cm

30-40 cm

40-50 cm

50-58 cm

60 cm

26.6 cm

Fig. 1: Schematic diagram representing barley grown with or without competition in rhizoboxes and showing the rooting depths sampled to assess differential root foraging responses to four N:P stoichiometry levels: low N+low P, low N+high P, high N+low P, and high N+high P.
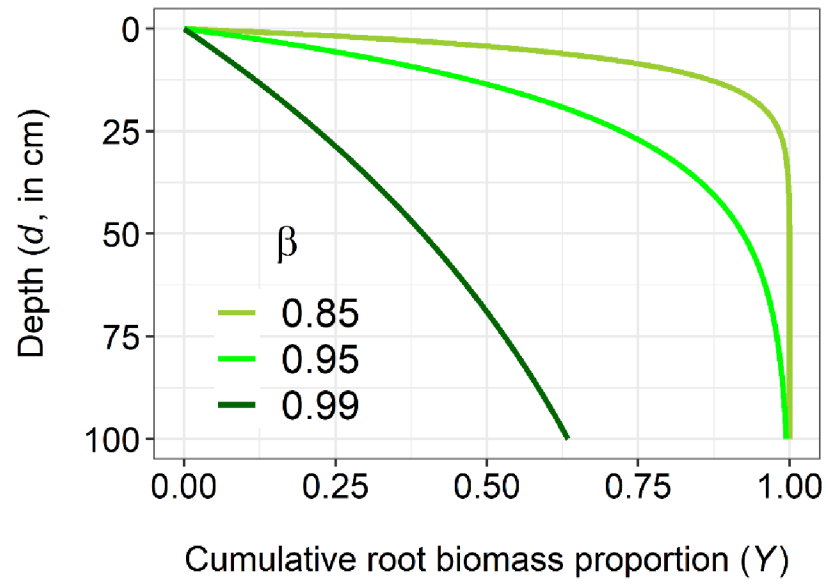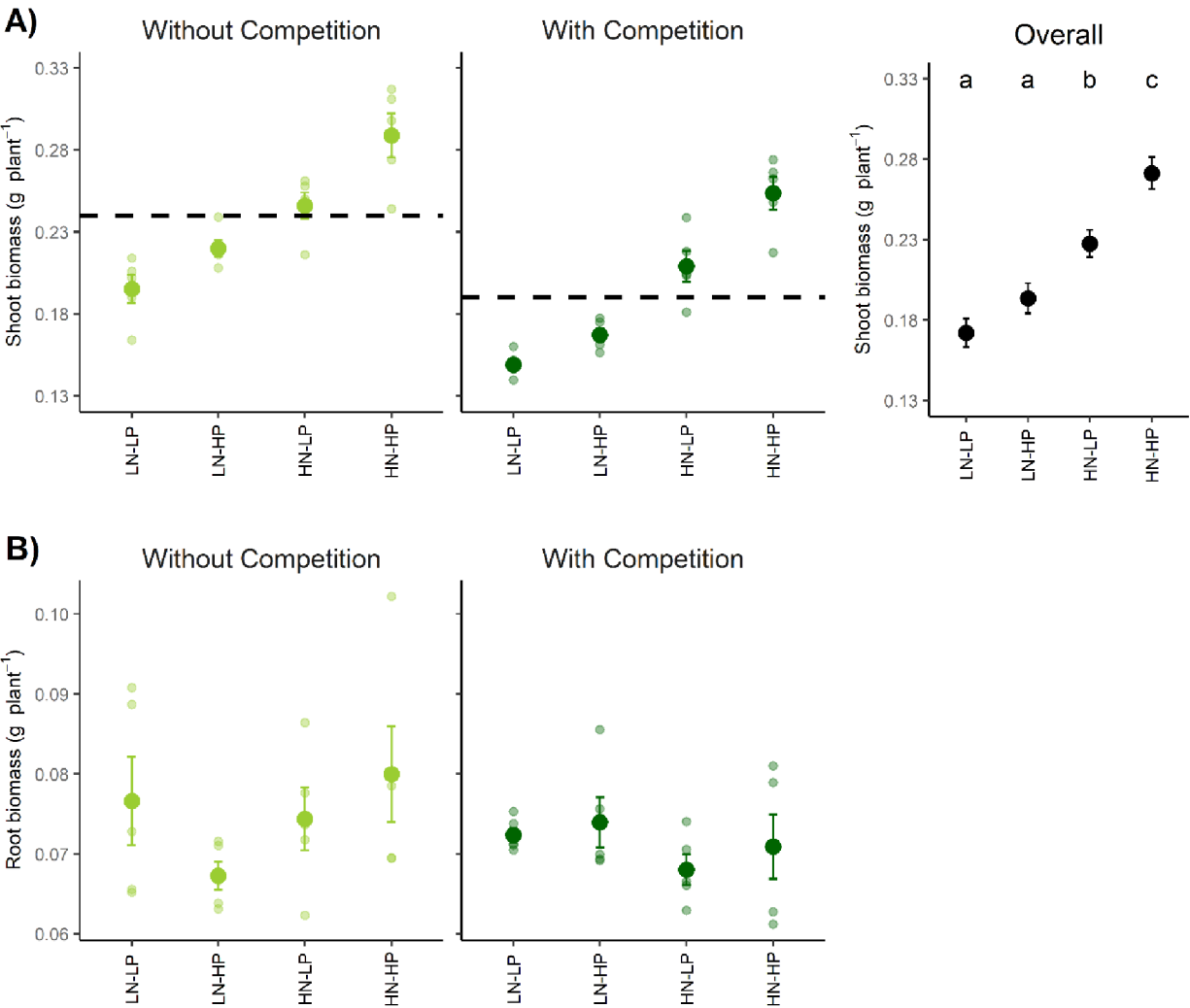
514
515 Fig. 2: Cumulative root biomass distribution as a function of soil depth as per Gale and Grigal (1987). Higher β values
516 imply that a greater proportion of root biomass is located in deeper soil layers, whereas lower β values imply that a
517 greater proportion of root biomass is located in shallower soil layers.

A)

Without Competition / With Competition / Overall — Shoot biomass (g plant⁻¹)

B)

Without Competition / With Competition — Root biomass (g plant⁻¹)

Fig. 3: A) shoot and B) root biomass (g plant^{-1}±SE) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P. For shoot biomass, there was no interaction between N:P stoichiometry and competition. Therefore, a graph showing the results for each N:P stoichiometry level (across competition levels) is also displayed. For shoot biomass, dashed lines show mean shoot biomass values without and with competition. Different letters indicate significant differences (Tukey's post-hoc, $P < 0.05$) between N:P stoichiometry levels only.
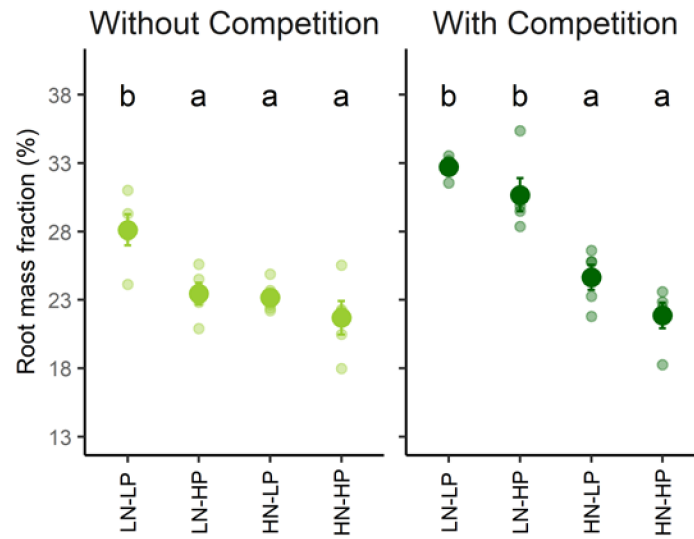
526

Fig. 4: Root mass fraction (%) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P. For each competition level, different letters indicate significant differences (Tukey's post-hoc test, $P < 0.05$) between N:P stoichiometry levels.
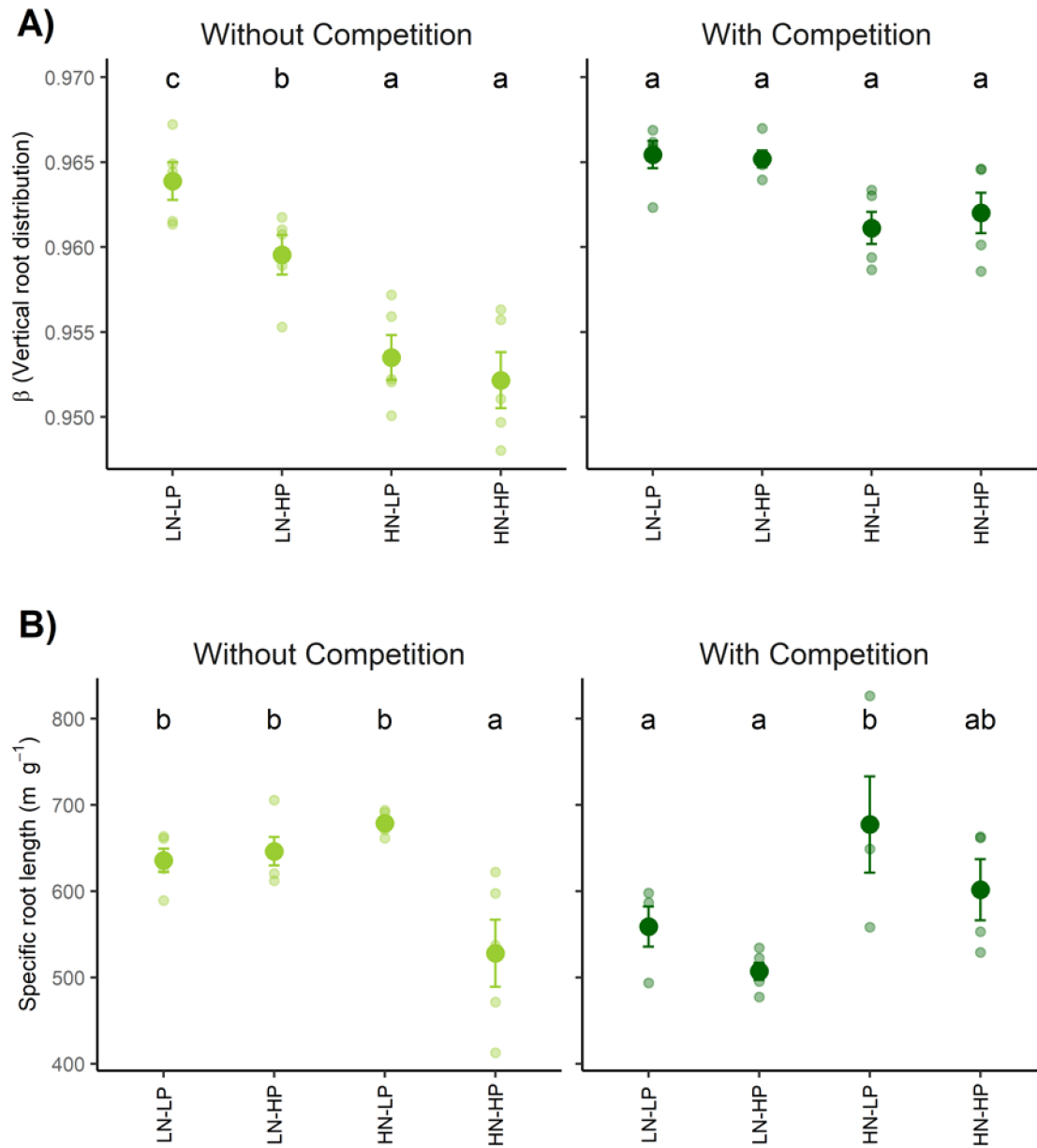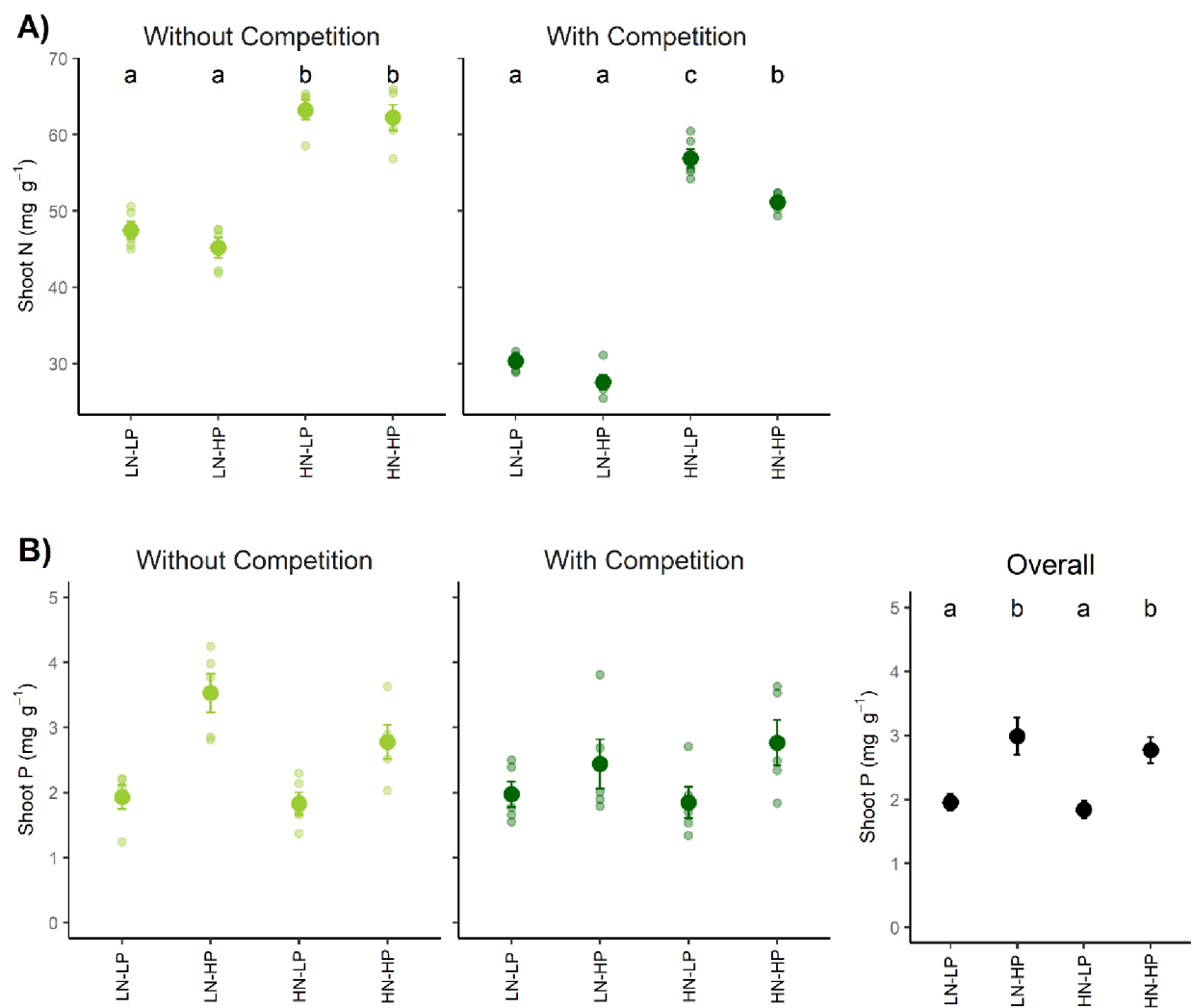
Fig. 5: A) Vertical root distribution (β±SE, see methods) and B) specific root length (m g⁻¹±SE) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P. For each competition level, different letters indicate significant differences (Tukey's post-hoc test, $P < 0.05$) between N:P stoichiometry levels.
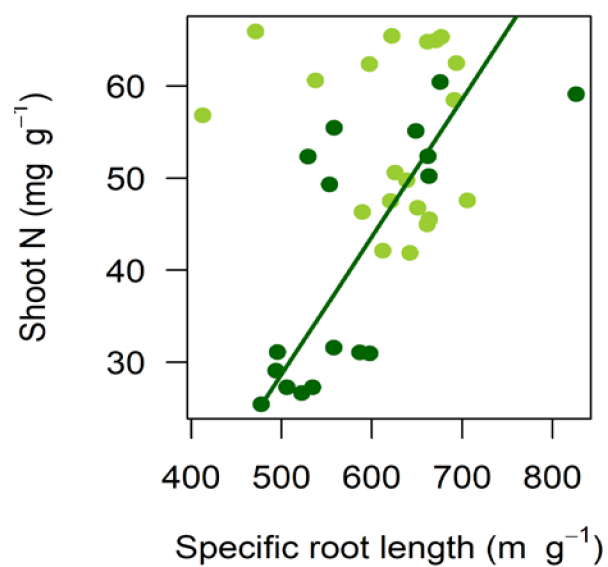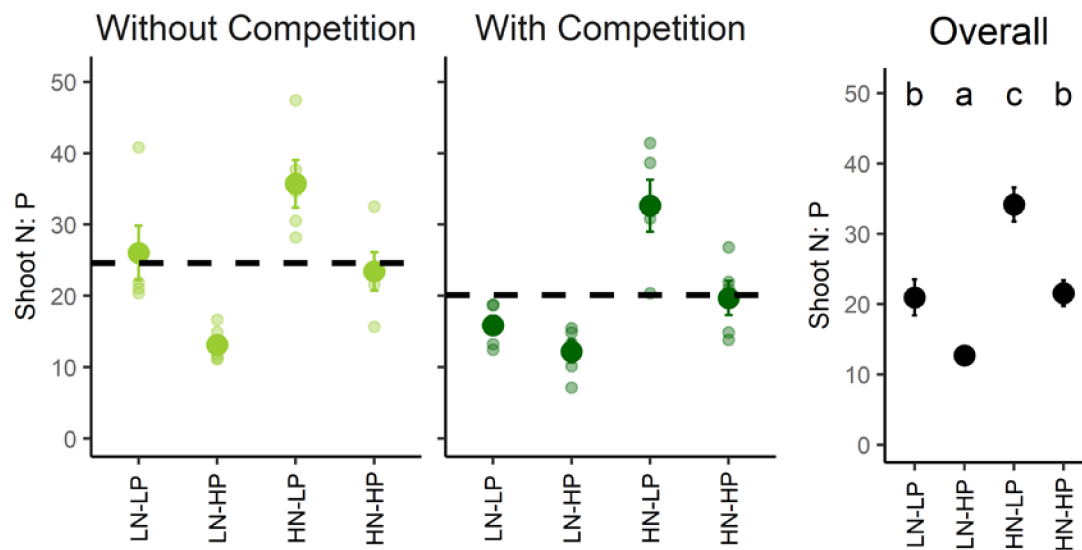
535 .

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Fig. 6: A) shoot N and B) shoot P (mg g^{-1}±SE) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P. For shoot P, there was no interaction between N:P stoichiometry and competition. Therefore, a graph showing the results for each N:P stoichiometry level (across competition levels) is also displayed. For shoot N, different letters indicate significant differences (Tukey's post-hoc test, $P < 0.05$) between N:P stoichiometry levels for each competition scenario. For shoot P, different letters indicate significant differences (Tukey's post-hoc test, $P < 0.05$) between N:P stoichiometry levels across competition scenarios.

544   .

545

546   Fig. 7: Linear relationship between specific root length (m g^{-1}) and shoot N concentration (mg g^{-1}). Light green circles
547   represent absence whereas dark green circles represent presence of competition, respectively. Regression line between
548   shoot N and specific root length is shown only when competition was present (for dark green).

Supplementary Fig. 1: Shoot N:P mass ratio (±SE) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P. For shoot P, there was no interaction between N:P stoichiometry and competition. Therefore, a graph showing the results for each N:P stoichiometry level (across competition levels) is also displayed. Dashed lines show mean shoot N:P values without and with competition. Different letters indicate significant differences (Tukey's post-hoc, $P < 0.05$).
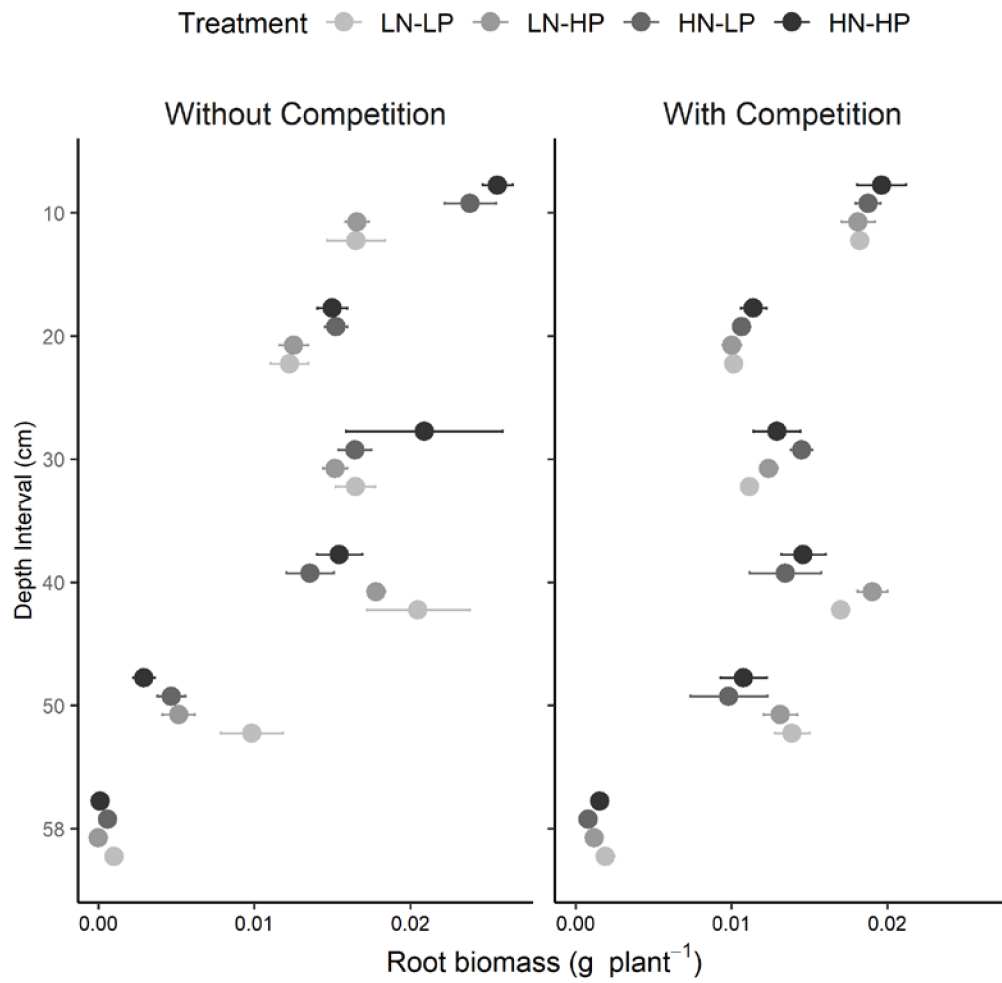
Supplementary Fig. 2: Vertical root biomass distribution (g plant[-1]) across N:P stoichiometry and with and without competition. LN-LP: low N and low P, LN-HP: low N and high P, HN-LP: high N and low P, and HN-HP: high N and high P.

12   Supplementary table 1: Chemical concentration of nutrient solutions provided to plants. The N:P
13   mass ratio of each stoichiometry of N and P is also provided in the last row of the table.

Macronutrients	Stock (M)	Low N-Low P (LN-LP)	Low N-High P (LN-HP)	High N-Low P (HN-LP)	High N-High P (HN-HP)
KNO_3	1	0.625	0.625	2.875	2.5
$Ca(NO_3)_2 \cdot 4H_2O$	1	0.625	0.625	2.125	2.5
KH_2PO_4	1	0.125	0.5	0.125	0.5
$MgSO_4 \cdot 7H_2O$	1	1.0	1.0	1.000	1.0
Micronutrients	Stock (mM)				
$H_3BO_3 \cdot H_2O$	46.3	0.5	0.5	0.5	0.5
$MnCl_2 \cdot 4H_2O$	9.2				
$ZnSO_4 \cdot 7H_2O$	0.77				
$CuSO_4 \cdot 5H_2O$	0.36				
MoO_3 (85% molybdic acid)	0.01				
Fe-Na-EDTA	50.12	0.5	0.5	0.5	0.5
Replacements	Stock (M)				
K_2SO_4	0.5	2.25	1.875		
$CaCl_2 * 2H_2O$	1	1.875	1.875		
N:P mass ratio		**5.81**	**1.45**	**22.47**	**5.81**

14