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Root production in a subtropical pasture is mediated by

cultivar and defoliation severity

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Abstract

Background. Grasslands occupy significant land area and account for a large proportion of the global soil carbon stock, yet the direct effects of grazing and genotypic composition on relationships between shoot and root production are poorly resolved. This lack of understanding hinders the development of models for predicting root production in managed grasslands, a critical variable for determining soil carbon stocks.

Methods. We quantified the effects of season-long defoliation treatments on both shoot and root production across four cultivars of a widely-planted pasture grass species (*Paspalum notatum Fluegge*) in a common garden setting in South Florida, USA.

Results. We found that infrequently applied (4 week) severe defoliation (to 5 cm) substantially enhanced shoot production for all cultivars, while severe defoliation reduced root production across cultivars, regardless of frequency. Overall, cultivars varied substantially in root production across the range of defoliation treatments in our study. However, there was no significant relationship between shoot and root production.

Conclusions. Our results find that aboveground and belowground productivity are only weakly coupled, suggesting caution against use of simple aboveground proxies to predict variations in root production in grasslands. More broadly, our results demonstrate that improved modeling and management of grasslands for belowground ecosystem services, including soil carbon sequestration/stocks, will need to account for intraspecific genetic variations and responses to defoliation management.

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Introduction

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59   Grassland ecosystems occupy more than a fifth of earth's land area and account for a

60   large proportion of the global SOC stock (1,2). However, there is considerable uncertainty in

61   predictions of net ecosystem exchange, and hence carbon sequestration services from grasslands

62   (3,4). One significant source of uncertainty is that while large herbivore grazing is known to

63   mediate patterns of plant species composition, diversity, and aboveground primary productivity

64   (5–7), the effects of grazing on belowground processes and soil carbon is less clear (8–11). In

65   particular, there are limited field studies where the impact of grazing on root production in

66   grassland systems has been directly measured (e.g., via root ingrowth cores or minirhizotron

67   technology, but see Ziter and MacDougall (12) Balogianni et al. (11) and Cooley et al. (13)).

68   Since belowground production may be the largest component of total NPP for many grasslands

69   (14,15), determining how grazing affects root production will help to predict if and when

70   grassland ecosystems will behave as carbon sinks, and whether grazing is likely to promote or

71   inhibit carbon sequestration services.

72   Root carbon inputs may constitute a disproportionate amount of the total SOC stock

73   compared with shoot carbon (16–18), and are especially critical in grassland ecosystems where

74   aboveground tissue is susceptible to frequent removal by fire and grazing (19). Current

75   understanding of how grazing affects root production is ambiguous. For example, one temperate

76   mesocosm study showed that intense defoliation inhibited root production and accelerated the

77   loss of SOC (20), whereas some field studies have documented greater belowground allocation

78   and root production under grazing (Hafner et al. (21) in the Tibetan plateau; Wilson et al. (22) in

79   subtropical pasture).. Augustine et al. (23) found that defoliation reduced belowground carbon

80   allocation in one grazing-adapted North American grass species (*Pascopyrum smithii,* western

81   wheatgrass) but not in another (*Bouteloa gracilis,* blue grama), highlighting interspecific

82   variations in response to a given defoliation regime. In general, laboratory and mesocosm studies

83   have found that frequent grazing/defoliation leads to declines in standing root biomass over the

84   long term (24), whereas a global synthesis of data comparing grazed and ungrazed grasslands

85   found a mix of positive and negative effects on standing root biomass (25). Overall, this

86   discordance suggests that variations in plant composition, underlying environmental factors,

87   grazing intensity, or some combination of these factors significantly mediate the effect of grazing

88   on root production.

89   Grazing effects on belowground production may not only vary based on plant species, but

90   also on the genotypic composition of a grazed stand, given the increasing evidence of the

91   importance of intraspecific variation in driving ecosystem structure and function (26,27). In

92   general, some literature suggests that reduced root allocation (and increased shoot allocation)

93   following grazing may represent an evolutionarily adaptive trait for grazing tolerance (28). For

94   instance, Carman (1985) (29) noted that short-leaved genotypes of *Schizachyrium scoparium,*

95   selected from a long-term grazed site, exhibited lower rates of root elongation post-grazing than

96   longer-leaved genotypes from a long-term grazing excluded site. Planted pasture grasses also

97   have been shown to exhibit genotypic variability in shoot and root production in response to

98   grazing (e.g. Dawson et al. (30)). For example, Interrante et al. (31) observed significantly less

99   plant cover in recently selected, upright-growing *Paspalum notatum* (bahiagrass) cultivars in

100   response to severe, frequent defoliation, but did not observe less cover with the same defoliation

101   treatments applied to widely naturalized cultivars, suggesting significant intraspecific variability

102   in grazing tolerance and belowground allocation.

103   Although root production is a critical component of predicting the carbon cycle in

104   grassland ecosystems, it is difficult to monitor or predict over large spatial scales. Thus, regional-

105   scale grassland models have been developed that predict total NPP and/or greenhouse gas

106   exchange on the basis of aboveground canopy characteristics estimated from remote sensing

107   (32–34). Similarly, some previous work has sought to predict BNPP on the basis of readily

108   obtained aboveground measurements in both grasslands (14) and forests (35). Recently,

109   concerted efforts have been made to link fine root traits with other plant traits, across species and

110   environments, by compiling and analyzing global-scale big datasets (36). The goal is to have

111   reliable aboveground proxies for predicting critical belowground root processes (37). However,

112   given the evidence for potentially significant genotypic and defoliation effects on belowground

113   carbon allocation, it is unclear whether aboveground proxies can ever reliably approximate root

114   production. Given the central importance of root system carbon inputs to maintaining SOC,

115   especially in grasslands, we need more data from experimental systems where genotypic

116   composition and grazing management have been manipulated, and the relationship between

117   above and belowground allocation have been quantified.

118   In this study, we tested the independent and combined effects of defoliation intensity and

119   frequency, and cultivar on root production of a widely utilized pasture grass species of the

120   southeastern United States, *Paspalum notatum* Flüegge (bahiagrass). For Bahiagrass, we can

121   broadly delineate cultivars on the basis of growth habit where historically older, widely

122   naturalized cultivars tend to have a more prostrate growth pattern, whereas recently selected

123   cultivars tend to have a more upright growth pattern, reflecting selection for improved forage

124   growth characteristics (38). Previous work, and considerable producer experience, suggests that

125   bahiagrass has a remarkable resilience to intense grazing, wherein forage growth and quality is

126   maximized with severe defoliation (close to ground level) so long as regrowth intervals are

127   adequate (39,40). However, the impact of defoliation severity on root production across

128   cultivars, and their associated growth habits, has not been directly studied, reflecting a general

129   lack of information on belowground growth responses in warm season subtropical pasture (13).

130   To redress this gap in knowledge we conducted an experiment in a common garden setting under

131   realistic conditions of limited soil fertility to isolate the effects of defoliation intensity, frequency

132   and cultivar on belowground production, and to evaluate the relationship between aboveground

133   and belowground growth.

134   Consistent with the literature on compensatory growth responses from natural and

135   planted pastures (40–42), and the literature on genotypic variability (e.g. Dawson et al. (30)) we

136   hypothesized that:

137   1)   Severe defoliation, applied infrequently, would stimulate increases in

138   aboveground primary productivity (via compensatory response mechanisms),

139   but would have neutral effects on root productivity across all cultivars;

140   2)   Severe defoliation, applied frequently, would significantly suppress root

141   production across all cultivars as a consequence of plant requirements to

142   prioritize photosynthate allocation to regrowing shoots;

143   3)   Widely naturalized, decumbent cultivars would show proportionally greater

144   reductions in root production under severe defoliation compared to the more

145   upright cultivars, reflecting a beneficial adaptation for increased shoot

146   allocation following severe defoliation events; and

147   4)   Despite alterations to belowground allocation on the basis of cultivar and

148   defoliation treatment, shoot production and root production would positively

149   correlate at the plot level reflecting variations in underlying soil factors

150   determining total productivity.

151

152 **Materials & Methods**

153

154   To evaluate the independent and potential interactive effects of defoliation intensity and

155 plant cultivar on root production, we established 32 3 m x 7 m experimental plots at the

156 University of Florida Range Cattle Research and Education Center, Ona, FL (27°26' N,

157 82°55'W) in 2009. The soils were uniform and classified as Pomona fine sand (sandy, siliceous,

158 hyperthermic Ultic Alaquod). First, we seeded plots with one of four bahiagrass cultivars

159 (Argentine, Pensacola, Tifton-9, and UF-Riata). Bahiagrassis a perennial C4 pasture grass with

160 improved germplasm that was introduced to Florida in the 1920s from South America and

161 constitutes the primary forage for the Florida cow-calf industry (43). 'Argentine' and 'Pensacola'

162 are widely-distributed, naturalized cultivars in the state of Florida with a decumbent growth

163 habit, whereas 'Tifton-9' and 'UF-Riata' are recently-released cultivars selected for improved

164 agronomic characteristics including more upright growth habits and less photoperiod sensitivity

165 (31,38). Plots were fully established by the onset of the 2010 summer growing season with

166 complete, uniform plant cover. More details, including soil fertility characteristics can be found

167 in Vendramini et al. (38). Site weather data for this period were accessed from the Florida

168 Automated Weather Network (FAWN, http://fawn.ifas.ufl.edu/data/), including temperature,

169 precipitation, and evapotranspiration, and all fell within normal ranges (Table A1).

170   We initiated defoliation treatments on June 13th 2013 and concluded field sampling 16

171 weeks later on October 5th 2013. Although we did not measure soil moisture, the soils were all

172 visibly waterlogged from July until the end of the experiment, as is typical in Florida Spodosol

173   soils (43). We therefore assumed that plant growth was not limited by water availability during

174   the sampling period, or at the very least that water availability was essentially constant across

175   plots. Each plot (n = 32) was randomly assigned to either a frequent (2 week) or infrequent (4

176   week) defoliation treatment to simulate grazing stress. Each plot was divided in half and received

177   two defoliation intensities (a severe defoliation to 5 cm residual height, and a mild defoliation to

178   15 cm residual height) resulting in n = 64 experimental units (Figure A1). Thus, our design was

179   effectively split-plot with two main-plot treatments (cultivar and defoliation frequency), while

180   our subplot factor was defoliation intensity. Overall, each cultivar X defoliation severity X

181   defoliation frequency treatment was replicated 4 times.

182   We harvested a 0.92-m^2 quadrat from each subplot during each defoliation treatment with

183   a rotary mower (Sensation Mow-Blo Model 11F4-0) at the target cutting heights: 5 cm for the

184   severe defoliation, 15 cm for the mild defoliation, values chosen based on personal observation

185   (C.H. Wilson, L.E Sollenberger, J.M. Vendramini) to represent the extremes of pasture

186   defoliation under grazing by beef cattle in Florida. To quantify aboveground production,

187   harvested material was oven-dried at 60°C to constant mass and weighed on an analytical scale.

188   During the final harvest, all subplots were harvested at 5 cm. Total aboveground production was

189   determined by summing values for each subplot across all dates including the final harvest.

190   Aboveground production values are presented in gm^{-2} (dry biomass).

191   To quantify root primary production in response to the defoliation treatments, we

192   installed 2-mm mesh root in-growth cores (44) on June 7th, 2013, prior to imposing the

193   defoliation treatments. Cores were 7.5 cm diameter x 25 cm deep and constructed of fiberglass

194   mesh. They were installed by first excavating a cylinder of soil with a soil auger to target

195   dimensions, placing the mesh bags into the cylinder so that the upper edge of the bags was just

196   below the soil surface, and then re-filling the cores with sieved, root-free soil from the same plot.

197   We retrieved the cores at the end of the growing season on October 5th 2013, 16 weeks after

198   installation. The final volume of soil contained in each core was quantified prior to washing the

199   roots free of soil on a 250-uM sieve. Root samples were then oven-dried at 60°C to constant

200   mass and weighed on an analytical scale. To correct for variation in core volume, root biomass

201   was multiplied by a correction factor determined as the inverse of the ratio of each core volume

202   to a reference core (a cylinder of 7.5 cm diameter and 25 cm depth). Finally, we visually

203   determined that almost all root biomass was contained within the depth we evaluated (i.e. 25 cm

204   depth) by digging several test pits around our study area. We note from personal observation that

205   wet pastures tend to result in shallower root distribution, consistent with early literature such as

206   (45). Therefore, we multiplied root biomass by a constant (10000/(pi*3.75^2)) to convert our

207   measures to g/m^2, putting them on an easily interpretable scale.

208

209   Statistical Analysis

210   Response variables for analyses were shoot and root production, and a measure of root

211   allocation defined as:

212
$$\frac{Root\ production}{Root\ production + Shoot\ production}$$

213   To analyze among-cultivar variability in response to our treatments, we parameterized a

214   varying-intercept/varying-slope Bayesian hierarchical model that we applied to both of our

215   response variables. In this model, we estimate intercept and slope (i.e., treatment effects)

216   coefficients for each cultivar, where each batch of coefficients is modeled as a draw from a

217   normal distribution with an estimated variance component (46). We included binary predictor

218   variables using a -0.5/0.5 "effect coding" for our experimentally imposed treatments: lenient (15

219   cm) and infrequent (4 wk) defoliation were assigned -0.5 values, while frequent (2 wk) and

220   severe (5 cm) defoliation were assigned 0.5 values. Under this coding, the model intercept

221   represents the grand mean, and the coefficients for defoliation severity and frequency represent

222   the main effects of severe and/or frequent defoliation across both levels of the other treatment

223   (see e.g. Schabenberger et al. (47)). We also included a term for the interaction of severe and

224   frequent defoliation treatments and a random effect of plot to allow for correlation in

225   observations from the same plot. Our varying-intercept/varying-slope model therefore included

226   four separate estimates of grand means (one for each cultivar), each of which represents an

227   estimate of performance for that cultivar across all defoliation treatment conditions, and four

228   treatment effect estimates (one for each cultivar) for frequent defoliation, severe defoliation, and

229   their interaction. Since these coefficients were drawn from distributions with estimated variance

230   components, the separate estimates were partially pooled towards their common mean, which

231   also was estimated from the data, a property that built in an automatic correction for multiple

232   comparisons among cultivars and obviated the need for arbitrary post-hoc adjustments such as

233   the Bonferonni correction (48). Finally, because growth data are naturally constrained to be

234   positive only and because we observed a pattern of variance increasing with the mean, we used a

235   gamma distribution to model our data, which naturally accounts for this nearly universal pattern

236   in biomass data. We used the standard log-link in our parameterization of the gamma regression

237   model, and thus our model coefficients represent multiplicative effects, and are reported on the

238   log link scale (46). Values greater than zero indicate positive effects on the response variable,

239   whereas values less than zero indicate negative effects. As in all cases where the log-link is used,

240   exponentiation of these regression coefficients returns the multiplicative effect which can be

241   naturally interpreted as a % effect.

242   We display treatment effects graphically by first plotting estimated fixed effect

243   coefficients (i.e. frequency, severity and frequency X severity) centered on the median, and

244   include both a 50% (thick) and a 95% (thin line) uncertainty (credible) interval. These

245   coefficients represent the overall average effects of treatment or the interaction effect across all

246   cultivars. In addition, we graphically present the varying intercepts portion of our model, which

247   represents the overall average deviation of each cultivar from the grand mean across all cultivars,

248   and is thus naturally centered at zero. Here again, we include both 50% (thick) and a 95% (thin

249   line) credible intervals. The proportion of the credible interval above or below zero can be

250   interpreted as the Bayesian probability of that cultivar differing in response from the average

251   across all cultivars. In the case of root allocation, we further analyze all the pairwise contrasts

252   among cultivars (n=6 contrasts), by taking the difference between each coefficient at each

253   iteration of the MCMC sampler. These pairwise contrasts thus represent the differences between

254   each pair of cultivars in their overall root allocation, averaged across all treatment conditions.

255   We estimated these models in a Bayesian framework via Hamiltonian Monte Carlo in the

256   packaged "rstanarm" (v2.18.2) called from R (v3.5.3) via Rstudio (v1.1.463). Prior to analysis,

257   shoot and root production responses were standardized by dividing by their mean, resulting in

258   this case with response variables with scale $\sim O(1)$ to facilitate faster sampling, and to help

259   specify weakly-regularizing Normal(0,1) priors for all treatment effects. For all models we

260   sampled the target (posterior) distribution with four chains of 2000 iterations each. Model

261   convergence was assessed via use of the R-hat < 1.01 criterion (46) as well as by visual

262   inspection for chain blending and stability, and monitoring of the powerful diagnostics built into

263   rstanarm (i.e. divergent transitions and E-BFMI, citation).

264   To understand the relative importance of defoliation treatment and cultivar compared

265   with shoot production for predicting root production, we first fit a simple univariate regression

266   model using only aboveground biomass from each subplot (n=64) as a continuous covariate. We

267   then refit our varying-intercepts/varying-slopes model while including shoot production as a

268   continuous covariate alongside treatment and cultivar effects. We compare a Bayesian R^2 metric

269   between the models (49). Because the visual and R^2 comparisons were so clear, we had no need

270   to evaluate additional metrics of model predictive performance.

271

272   **Results**

273   *Shoot production model*

274   Average shoot production across all cultivars and treatment combinations in our study

275   was 290 gm^{-2}, with the highest values observed in the infrequent severe defoliation treatment,

276   which averaged 384 gm^{-2} (Fig 1). The fixed main effect estimate (on log-link scale, and reported

277   as posterior median +/- posterior standard error) for severe defoliation was positive [0.28 +/-

278   0.07, Fig 2a], while the estimate for frequent defoliation was negative [-0.18 +/- 0.08, Fig 2a];

279   however, the interaction was negative as well [-0.25 +/- 0.15, Fig 2a], consistent with readily

280   observable pattern (Fig 1) that it is the combination of severe + infrequent (4 wk) defoliation that

281   leads to over-yielding. Overall, we did not estimate substantial variability in shoot production

282   *among* cultivars across all treatments, although the upright cultivars (UF-Riata and Tifton-9) had

283   slightly higher production than the decumbent cultivars Argentine and Pensacola (Fig. 3a).

284   **Fig. 1: Raw data (gm^{-2}) plotted as circles (shoots) and triangles (roots).** Error bars show
285   mean biomass (gm^{-2}) +/- 1 SE for shoots (purple error bars) and roots (brown error bars). The
286   panels are faceted by treatment combinations: intensity of defoliation on top (lenient 15 cm or
287   severe 5 cm on top), and frequency of defoliation labeled on the right hand side (2 wk or 4 wk).
288   The x-axis groups responses by cultivar: A = Argentine, P = Pensacola, T9 = Tifton-9, and UF-R
289   = UF-Riata.
290

Fig. 2: Fixed effects from varying-intercepts/varying-slopes Gamma regression model.
Coefficients are plotted on the log-link scale and include a median (point), 50% (thick line) and 95% (thin line) credible intervals for a) shoot production, b) root production and c) root allocation. Where the entire 95% credible interval falls above or below zero, we can interpret that as a 97.5+% Bayesian probability of that coefficient having a positive or negative effect on the response, respectively.

Fig. 3: Varying-intercepts from the Gamma regression model for root production.
Coefficients represent deviations of each cultivar (A = Argentine, P = Pensacola, T9 = Tifton-9, and UF-R = UF-Riata) from the overall mean (fixed effect coefficient), and are thus naturally centered at 0, where negative values represent lower than average performance, and positive values higher than average performance. Plots include a median (point), and 50% (thick line) and 95% (thin line) credible intervals. Where the entire 95% credible interval falls above or below zero, we can interpret that as a 97.5+% Bayesian probability of the cultivar having a higher or lower overall root production compared to the mean among all cultivars.

Root production model

We observed an average root production of 224 gm^{-2}, where mild defoliation treatments were the highest with 262 gm^{-2} averaged across 2 wk and 4 wk defoliation frequencies, compared with severe defoliation with an average of 186 gm^{-2} (Fig 1). The fixed main effect estimate for severe defoliation was negative (-0.33 +/- 0.12, Fig 2b), with >97.5% of posterior probability below 0, while the main effects of frequent defoliation and the interaction of frequent X severe defoliation were highly uncertain, with 95% credible intervals spanning a similar range above and below zero. Average root production across all treatment groups varied by cultivar more substantially than shoot production (Fig 3b), with the decumbent cultivars Argentine and Pensacola having greater root production than the upright cultivars UF-Riata and Tifton-9 (Fig 3b, Fig. 4). The greatest contrast was between Argentine and UF-Riata, which had a median posterior difference of -0.36 on the log-link scale (Fig. 4), which represents a 30% lower root production.

320 **Fig. 4: Pairwise contrasts among each cultivar for the varying intercepts of the root**
321 **allocation model.** Key: A = Argentine, P = Pensacola, T9 = Tifton-9, and UF-R = UF-Riata.
322 Plots include a median (point), and 50% (thick line) and 95% (thin line) credible intervals.
323 Where the entire 95% credible interval falls above or below zero, we can interpret that as a
324 97.5+% Bayesian probability of the first cultivar having a higher root allocation than the second
325 cultivar.
326
327 *Root allocation*

328   The fixed main effect estimate for severe defoliation on root allocation proportion was -

329 0.34 +/- 0.09 (Fig 2c), a very similar median estimate to that for root production, although with a

330 smaller uncertainty (SE = 0.09 versus 0.12). This result represents a median estimate of 29%

331 reduced allocation proportion to roots overall among cultivars and across both frequencies of

332 defoliation with severe defoliation. Variation among cultivars was also similar to that observed

333 for root production (Fig 3c versus 3b), and thus we did not repeat the pairwise analysis since it

334 would convey redundant information.

335

336 *Root production predictions*

337   The univariate regression between shoot and root production revealed a very weak (R^2 =

338 0.09) relationship (Fig 5a). The full model that included treatment indicators and cultivar identity

339 (as in the analyses above), yielded a median R^2 of 0.45 (Fig 5b). After removing the varying

340 intercepts/slopes by cultivar, this R^2 value declined to 0.21 (see supplement), indicating that

341 accounting for cultivar identity doubles model fit. Close examination of Fig 5b reveals that the

342 full model accounted for observed variations in root production quite well in the range of 100-

343 300 gm^{-2} but severely underpredicted root production > 300 gm^{-2}.

344 **Fig. 5: Shoot production does not predict root production.** a) Predicted versus observed
345 scatterplot for root production as predicted by shoot production as an aboveground proxy, and b)
346 predicted versus observed scatterplot for root production as predicted by defoliation treatment,

347 cultivar identity, and shoot production. For reference, the 1:1 line of "perfect fit" is plotted along

348 with an in-sample median Bayesian R^2 for both predictive models.

349

Discussion

351

352   Severe defoliation resulted in substantially greater shoot production when applied

353 infrequently, but reduced root production among the bahiagrass cultivars. Averaged across all

354 defoliation treatments, root production was also more strongly variable among cultivars than was

355 shoot production. Thus, our results suggest that severe defoliation can trigger a tradeoff between

356 aboveground and belowground allocation in managed subtropical pastures, and that the extent of

357 this tradeoff depends in part on cultivar identity. Contrary to Georgiadis et al. (50) and Briske

358 and Richards (28) who suggested that overcompensation is only likely to occur under water-

359 limitation, or given concomitant fertilization, we found significantly greater shoot production in

360 response to severe defoliation under limited fertility and abundant soil water. Compared with

361 mild defoliation, all cultivars exhibited this compensatory aboveground growth response to

362 severe defoliation, but only when defoliation was applied infrequently (similar to Gates et al.

363 (51)). However, the severe, but infrequent defoliation treatment that led to aboveground

364 compensatory growth also suppressed root production. Thus, under low-input conditions,

365 manipulating defoliation intensity and frequency to enhance forage production could evoke a

366 tradeoff between shoot and root production.. Given the substantial literature demonstrating the

367 importance of root carbon for maintenance of soil carbon pools (17,18,22), these altered

368 allocation patterns may have significant consequences for carbon cycling, and hence soil carbon

369 sequestration services, in managed subtropical pastures. Moreover, use of simple aboveground

370 proxies, such as leaf area/biomass, are unlikely to help constrain predictions of root production

371 over large spatial scales.

372   Our results differ from the short-term responses measured by Ziter and Macdougall (12)

373   and Hamilton III et al. (52) where a single defoliation event stimulated root production and root

374   exudation, respectively. Moreover, the results reported here appear to conflict with

375   measurements of standing root biomass, root exudation rates, and their connections to microbial

376   biomass and soil carbon, across a system of long-term grazing exclosures on a similar pasture

377   site, as reported in Wilson et al. (22). These discrepancies suggest that root responses to short-

378   term grazing/defoliation events can strongly differ from season-long responses to grazing

379   regimens where both intensity and frequency of defoliation are expected to mediate plant

380   regrowth strategies (28). Moreover, long-term impacts of grazing exclusion in bahiagrass-

381   dominated subtropical pasture appear to involve pronounced phenotypic shifts in root:shoot

382   ratios, whereby absence of grazing favors lower root:shoot ratios, even when holding species

383   composition constant (22)On the other hand, Thornton and Millard (53) found that greater

384   severity of defoliation resulted in lower root mass (but greater N uptake per unit of root mass),

385   which is consistent with our findings. Meanwhile, Dawson et al. (30) report that weekly

386   defoliation over a growing season reduced root biomass compared with no defoliation, but

387   infrequent defoliation (every 8 weeks) had no effect. Our ambivalent findings on the role of

388   frequency of defoliation were thus somewhat surprising. Although we observed marked

389   suppression of variability of production under our severe + frequent treatment (see e.g., Fig 1),

390   root production was not markedly lower than in our severe + infrequent treatment. Overall, it

391   appears that in our system, severity, not frequency, of grazing is the more important determinant

392   of grass root production.

393   We observed substantial overall variability in root production among the grass cultivars.

394   However, it does not appear possible to predict cultivar-level belowground responses to specific

395   grazing regimens based on observations of aboveground compensatory growth responses. As we

396   hypothesized, the cultivars selected for enhanced upright growth habit (Tifton 9, UF-Riata, (31))

397   exhibited less overall root production, especially Tifton-9, compared with the widely naturalized

398   decumbent types (Argentine, Pensacola), especially Argentine. On the other hand, all cultivars

399   responded equally negatively to severe defoliation *per se*, and we observed similar total root

400   production among all cultivars in the severe + frequent defoliation treatment, a scenario

401   reasonably representative of overstocked pastures. These results contradict the theory that more

402   grazing-tolerant genotypes, in our case Argentine and Pensacola, will have lower root production

403   as a consequence of greater post-grazing allocation to shoot regrowth (28,30). Instead, it appears

404   that cultivars simply vary in root growth potential, but that severe defoliation, especially when

405   applied frequently, overwhelms this variability.

406   Contrary to hypothesis, our study revealed that shoot and root production are decoupled

407   at fine spatial scales, at least in our experimental plots, with shoot production explaining only 8%

408   of the in-sample variation in root production. By contrast, defoliation treatment and especially

409   cultivar identity appear to be very important for predicting root production in this system,

410   together accounting for roughly half the observed variance in root production. Gill et al. (14)

411   reported some success in predicting belowground NPP using an algorithm based only on

412   aboveground biomass and climate. However, their model consistently under-predicted root

413   production in more productive sites. Interestingly, we observed a similar severe underprediction

414   of root production in our more productive plots. Thus, we caution against using aboveground

415   proxies to predict belowground production, even within uniform and homogeneous ecosystems,

416   such as the planted pasture system where we worked. Our results suggest that knowledge of

417   grazing management and cultivar identity (in addition to species-level variations in composition,

418   (54,55)) are critical for generating accurate predictions of BNPP. Moreover, half of the variance

419   in belowground production was unexplained, even in our best model, suggesting significant

420   spatial heterogeneity in root system productivity that should be further investigated. Given recent

421   calls highlighting the importance of plant roots to future progress in biogeochemical modeling

422   and the quest to find reliable, scalable aboveground proxies to indirectly infer root processes

423   (36,37), our results are a sobering reminder of the challenges inherent to linking production

424   above and belowground. Accordingly, we suggest that a high priority for future research is to

425   study belowground root-rhizosphere processes using spatially-explicit sampling protocols

426   designed to maximize insight into heterogeneity at various spatial and temporal scales.

427   At the large scale, McNaughton (1998) (8) found that grazing intensity is uncorrelated

428   with standing root biomass or productivity in the Serengeti. However, in speciose natural

429   grasslands plant diversity may confer a stabilizing influence on root production (55,56). By

430   contrast, monoculture pasture systems may respond more like mesocosm systems where high

431   defoliation intensity is associated with reduced root biomass (24). Moreover, since a large

432   proportion of managed grasslands are dominated by single species, variation in root production

433   among cultivars may represent an especially important component of diversity. Grazing

434   management may need to be matched to cultivar-level characteristics to optimize both forage and

435   root production, and establishment of planted pastures with multiple cultivars or genotypes may

436   be a viable, yet underappreciated, strategy for enhancing functional diversity. For instance,

437   combining upright and decumbent cultivars may introduce beneficial genotypic diversity that

438   could maximize utilization of both above and belowground resources via niche complementarity

439   (57,58). Additionally, cultivar-level variability suggests the potential for ecologists to collaborate

440   with plant breeders to improve the sustainability of grassland agroecosystems by development of

441   improved forage cultivars selected for superior belowground traits.

442   Overall, our results suggest that intermittent severe defoliation can elicit much greater

443   shoot growth, but have neutral or negative effects on root production. It is possible that a more

444   moderate defoliation intensity than we tested would have led to similar stimulation of

445   aboveground compensation without the negative consequence for root production, a possibility

446   our study was not designed to test. Neither did our study consider impacts of defoliation on

447   rhizome biomass, but we note that our intent was to focus on root production since it appears to

448   be of greater relevance for soil carbon sequestration than other compartments of plant biomass

449   (17). Likewise, it is also possible that the lower fine root production we measured may have been

450   compensated for by greater rhizodeposition/root exudation. However, this possibility seems

451   unlikely given that rates of root exudation generally correlate to fine root surface area (22,59).

452

Conclusions

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455   Root production is critical for maintaining and increasing soil carbon pools in grassland

456   ecosystems, yet findings on the immediate and long-term effects of grazing on root production

457   remain variable. We hypothesized that severe defoliation, if applied infrequently, might lead to

458   overyielding of shoots, but would have only small impacts on root production. Moreover, we

459   hypothesized that cultivars selected for an upright growth habit would show less root production

460   overall, and would be more sensitive to defoliation stress. Overall, we found that severe

461   defoliation *per se*, regardless of frequency, suppressed root production, even as infrequently

462   applied severe defoliation increased shoot production. Thus, it appears that manipulating timing

463   and intensity of grazing to optimize forage production might evoke a negative tradeoff with root

464   production. We did find support for the hypothesis that recently developed upright cultivars have

465   lower root production, and a lower root:shoot ratio, than widely naturalized decumbent cultivars.

466   The main limitation of our work is that realistic animal grazing management can differ from

467   experimentally imposed defoliation in two major ways: 1) grazing impacts will fall along a

468   spectrum of timing and intensity with more intermediate values than can be tested in a

469   randomized factorial experiment, and 2) grazers will return a certain fraction of consumed

470   carbon and nutrients in the form of manure and urine, creating heterogeneous patches of varying

471   nutrient availability. Moreover, we also caution that year-year variability in growing conditions

472   can induce variability in experimental effects. Ideally, we recommend long-term (3+ year) field

473   studies of controlled grazing (or defoliation) to begin to properly estimate the random effects of

474   such year-year environmental fluctuations.

475   In addition to recommending greater future consideration of intraspecific variations in

476   belowground responses to grazing, our work supports the need to perform season-long measures

477   of belowground productivity to obtain reliable estimates of belowground production that can be

478   used to parameterize soil carbon models. Our data also suggest that reliance on aboveground

479   proxies is, unfortunately, not justified at least for subtropical pastures. In addition, given the

480   limitations of observational and comparative work, we suggest that longer-term field

481   manipulations are necessary to evaluate a suite of grazing management scenarios across plant

482   composition treatments. Such experiments will significantly improve our ability to inform the

483   design and management of grassland agroecosystems for meeting aboveground (forage)

484   production goals while also optimizing belowground production, and thus soil carbon

485   sequestration and other soil carbon mediated ecosystem services such as nutrient retention and

486   water cycling (2).

487

488

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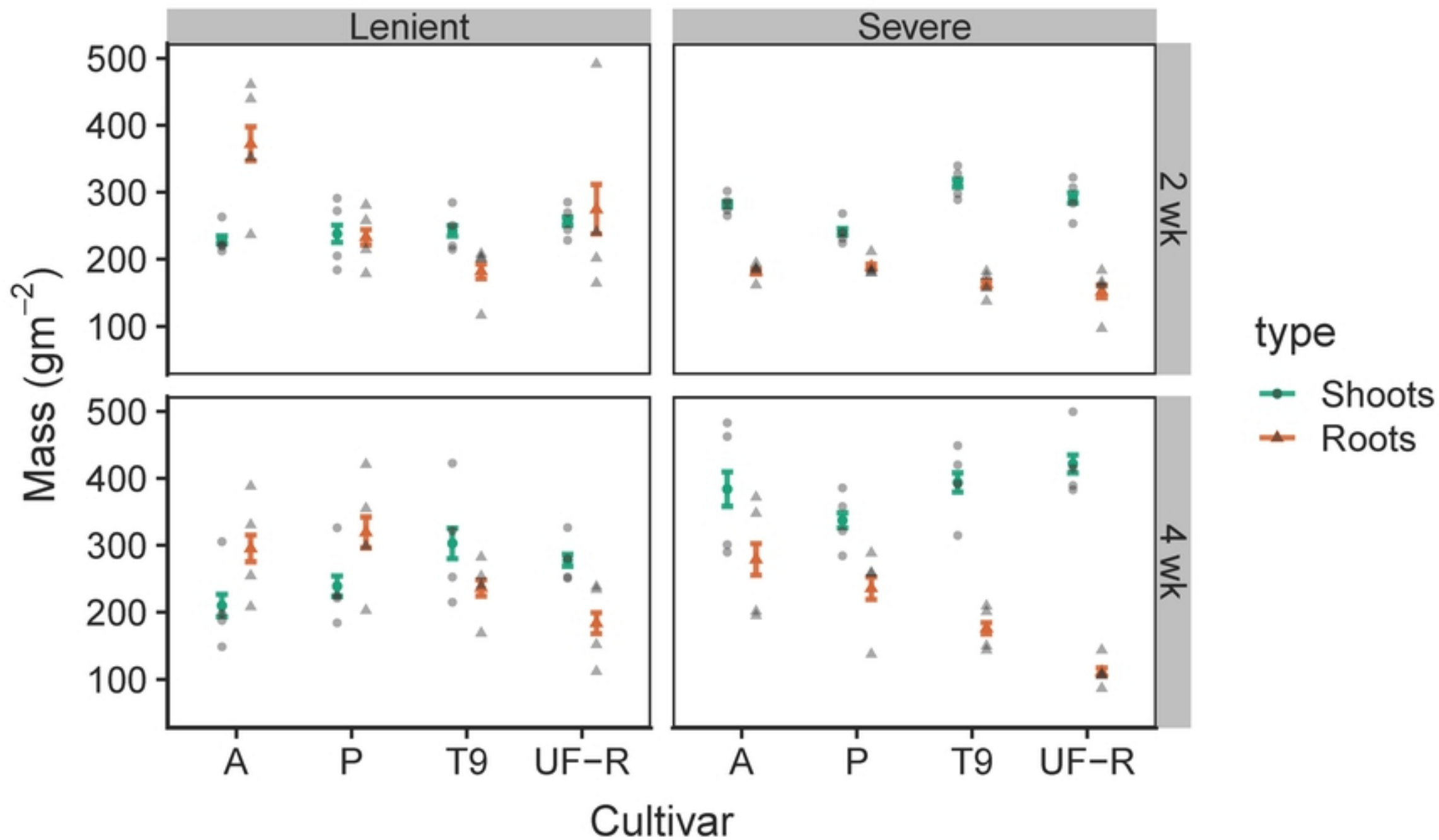620   1;54(3):210–4.

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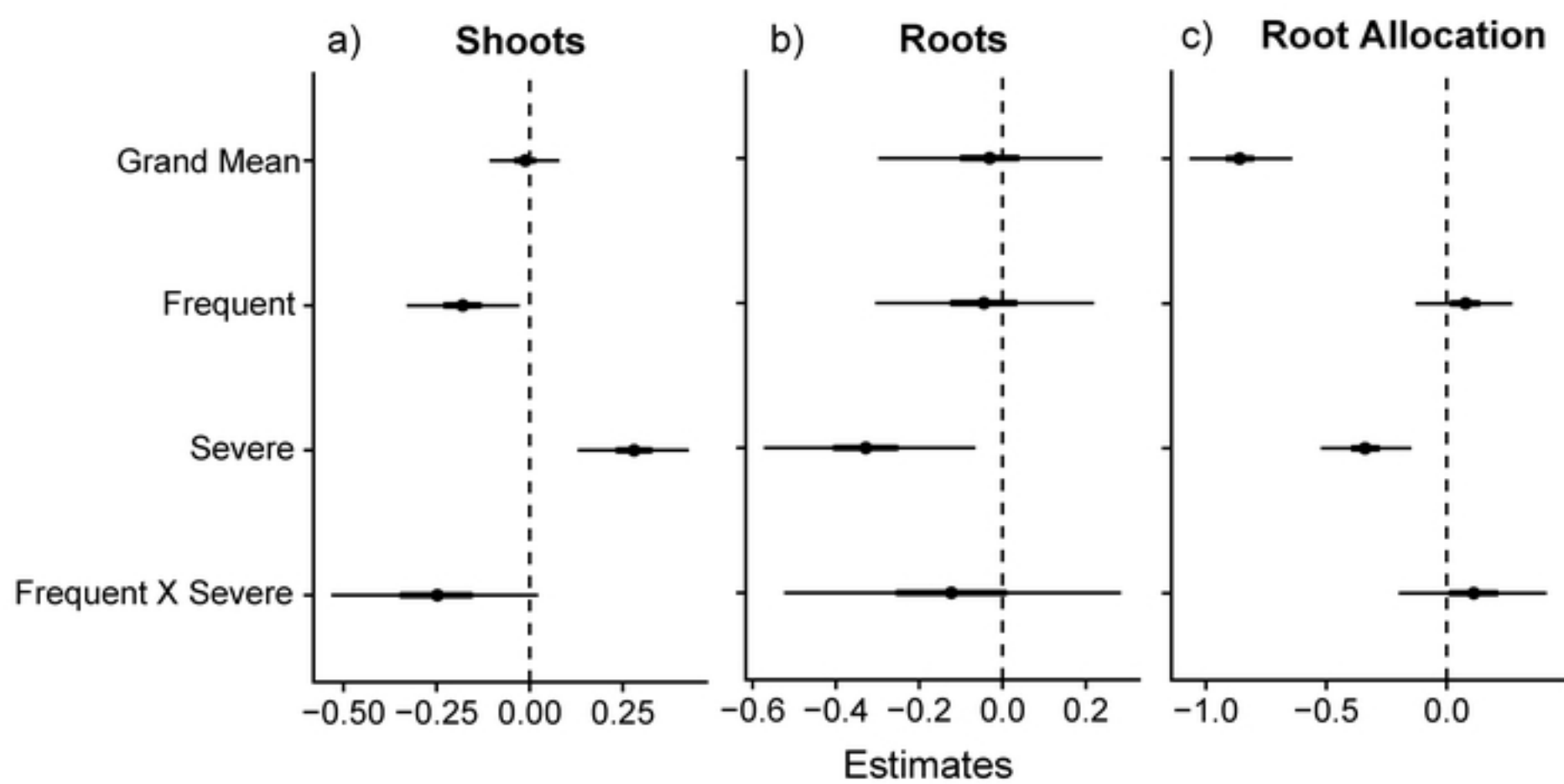
SUPPORTING INFORMATION

S1 Fig. Diagram showing layout of plots. North is top of the page. Legend: Defoliation severity- Red = Severe Defoliation (5 cm), Blue = Lenient Defoliation (15 cm). Defoliation frequency - 2wk = Defoliated every 2 weeks, 4wk = Defoliated every 4 weeks. Bahia cultivar identity - A = Argentine, P = Pensacola, T9 = Tifton 9, R = UF-Riata.

S1 Table. Meterogical data from our study site during study season. Ona Range Cattle Research and Education Center. Accessed from the Florida Automated Weather Network (FAWN), http://fawn.ifas.ufl.edu/.
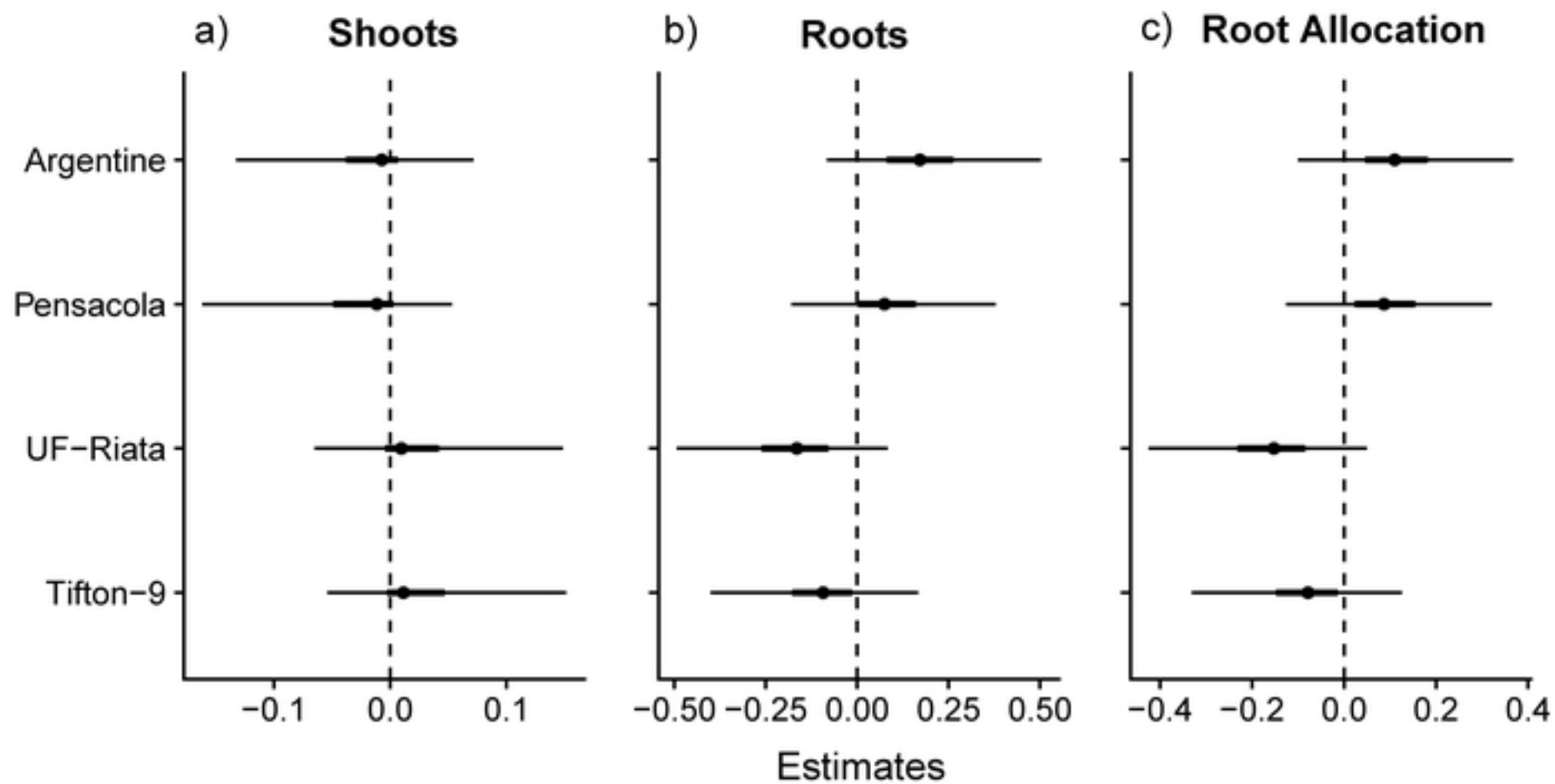
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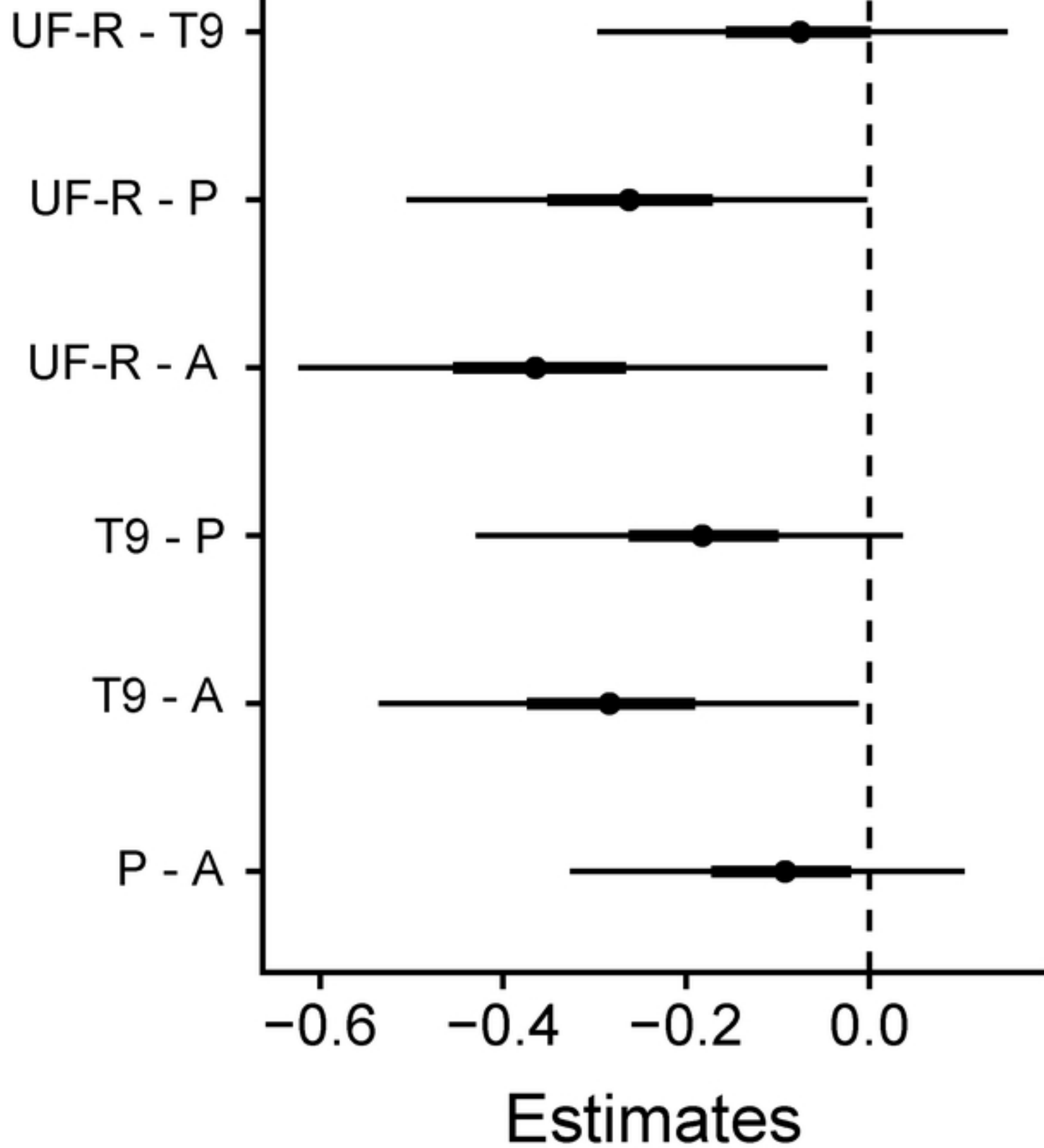
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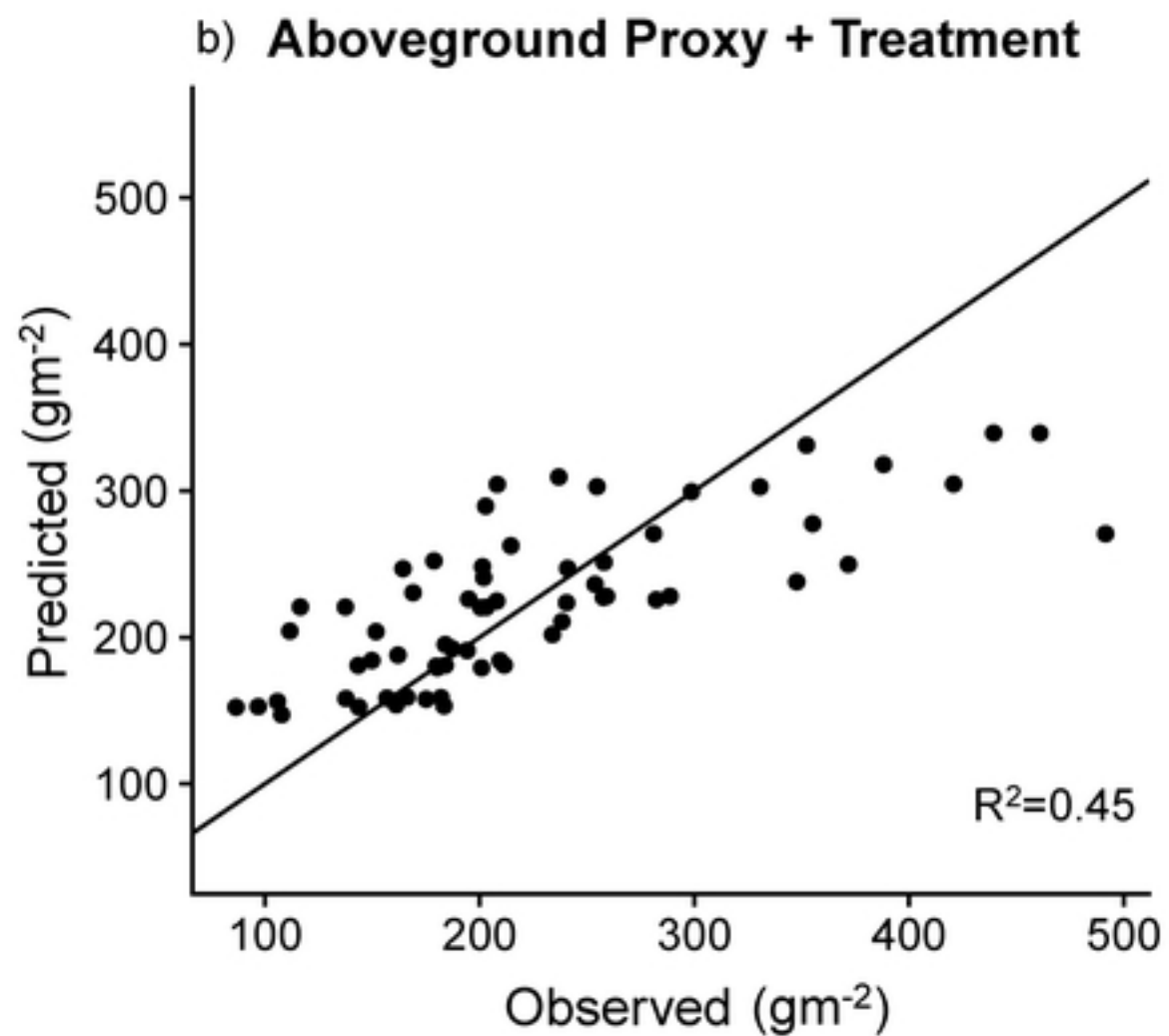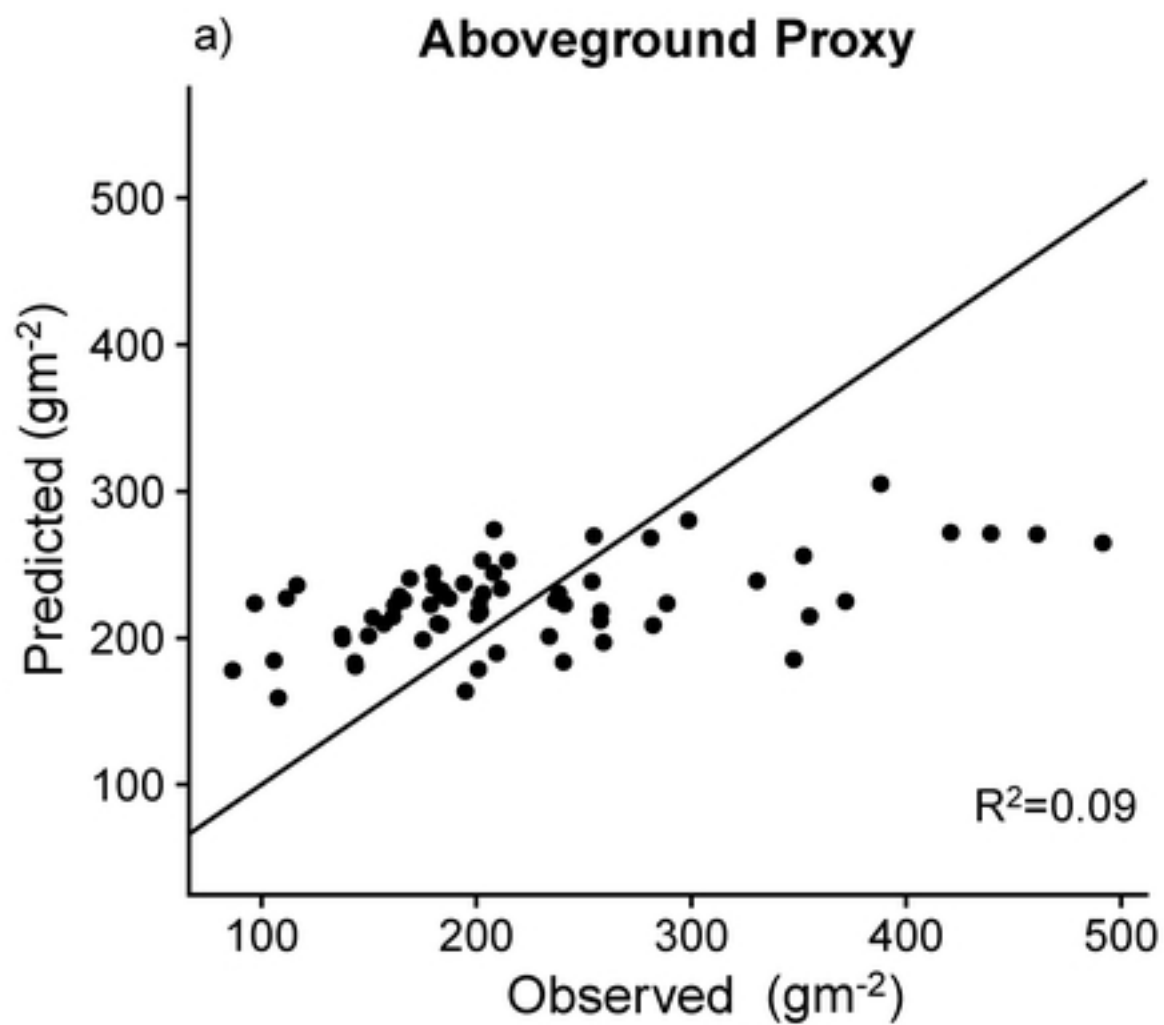683

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Figure

Figure

a) **Shoots**   b) **Roots**   c) **Root Allocation**

Estimates

Figure

Pairwise Differences

Figure

a) **Aboveground Proxy**
b) **Aboveground Proxy + Treatment**

$R^2=0.09$

$R^2=0.45$

Figure