Inoculation with the mycorrhizal fungus *Rhizophagus irregularis* modulates the relationship between root growth and nutrient content in maize (*Zea mays* ssp. *mays* L.)

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ABSTRACT

Plant root systems play an essential role in nutrient and water acquisition. In resource-limited soils, modification of root system architecture is an important strategy to optimize plant performance. Most terrestrial plants also form symbiotic associations with arbuscular mycorrhizal fungi to maximize nutrient uptake. In addition to direct delivery of nutrients, arbuscular mycorrhizal fungi benefit the plant host by promoting root growth. Here, we aimed to quantify the impact of arbuscular mycorrhizal symbiosis on root growth and nutrient uptake in maize. Inoculated plants showed an increase in both biomass and the total content of twenty quantified elements. In addition, image analysis showed mycorrhizal plants to have denser, more branched root systems. For most of the quantified elements, the increase in content in mycorrhizal plants was proportional to root and overall plant growth. However, the increase in boron, calcium, magnesium, phosphorus, sulfur and strontium was greater than predicted by root system size alone, indicating fungal delivery to be supplementing root uptake.

KEYWORDS: mycorrhiza, root development, ionome, maize

35 **INTRODUCTION**

36 Plant productivity is typically limited by the availability of nutrients, in both natural and
37 agricultural ecosystems. Under nutrient-poor conditions, plants have the capacity to modulate the
38 architecture and functionality of their root system, potentially increasing nutrient uptake (Lynch,
39 1995). Beyond a general reallocation of resources from leaf to root, there are nutrient-specific
40 changes in the development of primary (PR) and lateral (LR) roots, building a root system
41 architecture (RSA) that may better optimize resource acquisition (López-Bucio et al., 2003;
42 Osmont et al., 2007; Gruber et al., 2013). Nutrients are heterogeneously distributed in the soil,
43 and plants respond to local concentration, allocating greater root production to regions of higher
44 availability (Campbell et al., 1991; Farley and Fitter, 1999; Grossman and Rice, 2012). In
45 addition, nutrient distribution varies across soil horizons: poorly-mobile nutrients such as
46 phosphorus (P), potassium (K), magnesium (Mg) or calcium (Ca) are typically enriched in
47 topsoil, while more mobile nutrients, such as nitrogen (N), are typically more abundant deeper in
48 the soil (Rubio et al., 2003; Ho et al., 2004; Lynch and Brown, 2008; Postma et al., 2014;
49 Rangarajan et al., 2018). As a consequence, researchers have distinguished RSAs optimized for
50 topsoil versus deeper foraging (Lynch, 2019).

51 Root development is regulated by the combined action of internal developmental
52 pathways and external environmental stimuli (Malamy and Ryan, 2001), conditioning both
53 plasticity and intra- and inter-specific variation. These pathways are based on the action of plant
54 hormones, signal receptors, transcription factors and secondary messengers, including Ca^{2+},
55 nitric oxide and reactive oxygen species (Fukaki and Tasaka, 2009; Garay-Arroyo et al., 2012;
56 Jung and McCouch, 2013; Schlicht et al., 2013; Zhang et al., 2015; Shahzad and Amtmann,
57 2017). Auxin works with cytokinin to regulate LR initiation (Aloni et al., 2006), and with

58 gibberellin to modulate cell proliferation and elongation (Fu and Harberd, 2003). Strigolactones
59 impact LR formation and root hair development in a dose-dependent manner (Koltai, 2011), and,
60 in maize, promote nodal root development (Guan et al., 2012). RSA is largely a product of the
61 balance between root elongation and branching (Postma et al., 2014). Primary root growth will
62 continue if the root meristem is active and populations of stem cells within the quiescent center
63 are maintained. In *Arabidopsis*, the exhaustion of the primary root meristem under low P is
64 considered the classic example of a plastic response to optimize topsoil foraging (Williamson et
65 al., 2001; López-Bucio et al., 2002; Mora-Macías et al., 2017). Root branching, through the
66 production of first- or higher- order LRs, is regulated at the level of the formation of LR
67 primordia and their subsequent expansion. Low availability of N, P, sulfur (S) or zinc (Zn)
68 promotes an increase in the density and/or length of LRs, although, as mentioned above, when
69 the nutrient distribution is patchy, LRs may proliferate in regions of high local nutrient
70 abundance (Zhang et al., 1999; Kutz et al., 2002; López-Bucio et al., 2002; Bouranis et al., 2008;
71 Gruber et al., 2013). In cereal crops, the bulk of the adult root system is comprised of shoot-
72 borne crown (CR) roots and associated LRs (Hochholdinger and Tuberosa, 2009). While greater
73 branching will increase the surface area for nutrient uptake, it becomes inefficient if placement
74 of roots in close proximity results in competition for the same nutrients, an effect that will be
75 greater for more mobile nutrients (Postma et al., 2014).
76 Significant intra-specific variation has been observed in RSA and plasticity, and has been
77 linked to superior agronomic performance in crops under specific edaphic conditions. In
78 common bean, varieties producing shallow basal roots and a large number of adventitious roots
79 explore the topsoil more efficiently, performing better in low P soils (Rubio et al., 2003; Lynch
80 and Brown, 2008). In rice, the PHOSPHATE STARVATION TOLERANCE 1 (PSTOl1) kinase,

81 identified from the traditional variety Kasalath, is associated with enhanced early root growth,
82 and increases yield under low P conditions (Wissuwa et al., 2005; Gamuyao et al., 2012).
83 In addition to relying on their roots to acquire nutrients, most terrestrial plants also form
84 mutualistic symbioses with arbuscular mycorrhizal (AM) fungi of the Phylum Glomeromycota
85 (Parniske, 2008; Smith and Read, 2010). The association with AM fungi is one of the oldest
86 plant symbioses, and AM symbioses were likely important during plant colonization of the land,
87 performing essential functions before the evolution of the vascular root system (Schüßler et al.,
88 2001). Establishment of AM symbiosis requires a complex interchange of signals between plant
89 and fungus that results in the entry of the fungus into the root and the development of highly
90 branched arbuscules within the root cortical cells, providing the primary site of nutrient exchange
91 (Parniske, 2008). AM fungi are obligate symbionts, obtaining carbon from their plant host in
92 return for providing nutrients and water that are acquired by an extensive network of root-
93 external hyphae (Bago et al., 2003). The clearest benefit to the plant is enhanced P uptake (Chiu
94 and Paszkowski, 2019), although AM fungi have been reported to promote uptake of other
95 elements, including N, Fe, S and Zn (Liu et al., 2000; González-Guerrero et al., 2005;
96 Govindarajulu et al., 2005; López-Pedrosa and González-Guerrero, 2006; Allen and Shachar-
97 Hill, 2009). In practice, complex interactions between nutrients influence the outcome of the
98 symbiosis with respect to any given element (Liu et al., 2000; Gerlach et al., 2015; Ramírez-
99 Flores et al., 2017). Although AM symbioses are widespread in both natural ecosystems and
100 cultivated fields, the plant host maintains a degree of control over establishment and the extent of
101 colonization, rejecting the fungus under high nutrient conditions (Nouri et al., 2014).
102 AM fungi not only directly deliver nutrients, but also promote growth of the host plant
103 roots, with secondary effects on nutrient uptake. General improvements in plant health under

104 AM symbiosis may be correlated with increased root system size (Berta et al., 1995; Tisserant et
105 al., 1996; Gutjahr et al., 2009; Sawers et al., 2017). AM symbiosis is also associated with
106 developmental changes, such as an increase in LR production and greater root branching (Berta
107 et al., 1990; Paszkowski and Boller, 2002; Oláh et al., 2005; Gutjahr et al., 2009). The failure to
108 form LR primordia in the maize *lateral rootless1* (*lrt1*) mutant can be partially overcome by AM
109 symbiosis, indicating an influence on plant developmental pathways (Hochholdinger and Feix,
110 1998; Paszkowski and Boller, 2002). In *Medicago truncatula,* developing fungal spores are
111 sufficient to trigger LR formation (Oláh et al., 2005; Gutjahr et al., 2009). In rice, AM fungi can
112 induce LR formation in *pollux*, *ccamk* and *cyclops* mutants, even though symbiosis is not
113 established, confirming that RSA modification need not be dependent on enhanced host plant
114 nutrition (Gutjahr et al., 2009).

115 The impact of AM symbiosis on nutrient uptake can be quantified by comparing the
116 concentration or content of a given element in the aerial portion of mycorrhizal (M) and non-
117 colonized (NC) plants (Gerlach et al., 2015; Ramírez-Flores et al., 2017). Such a comparison,
118 however, does not distinguish direct hyphal nutrient delivery from the secondary consequences
119 of fungus-induced modification of root architecture or the effects of greater root growth resulting
120 from a general increase in plant vigor. In the case of P, elegant labelling experiments and
121 extensive functional studies have clearly demonstrated the direct role of root-external hyphae in
122 nutrient delivery (Pearson and Jakobsen, 1993; Smith et al., 2003; Chiu and Paszkowski, 2019).
123 The picture, however, remains less clear with respect to other mineral nutrients.

124 In this study, we characterized RSA and leaf ionome in young maize plants, grown with
125 or without inoculation with AM fungi. For each nutrient, we asked whether any increase in
126 uptake was explainable by changes in the root system alone, or whether there was evidence of

127 additional hyphal foraging. For an increase resulting from greater root growth, we expected the
128 relationship between root surface area and uptake to be maintained, albeit that mycorrhizal plants
129 themselves were larger. If, however, there was a significant effect of hyphal foraging, we
130 predicted an increase in uptake per unit surface area of root.
131

132 **MATERIALS AND METHODS**
133
134 *Plant material and growth conditions*

135 Two maize inbred lines (*Zea mays* ssp. *mays* var. B73 and W22) were grown with (M) or
136 without (NC) inoculation with fungus, in PVC tubes (1 m in height, 15 cm in diameter). A total
137 of 64 plants (2 genotypes x 2 treatments x 16 replicates) were planted in complete blocks across
138 four planting dates. Each planting consisted of 16 plants (2 genotypes x 2 treatments x 4
139 replicates), with a one week interval between planting dates. B73 seed was produced in the
140 winter of 2015 in Valle de Banderas, Nayarit, México. W22 seed was produced in the summer of
141 2013 in Aurora, New York, USA. Each tube was filled with 17 L of a sterilized substrate mix
142 consisting of sand:perlite:silt (4.5:1.5:1, v/v). The substrate Olsen P concentration was 4.8 ppm.
143 For M plants, we inoculated each tube with ~700 *Rhizophagus irregularis* spores collected from
144 a commercial liquid inoculant (AGTIV®) and delivered to the middle of the tube at 15 cm depth
145 before planting. The experiment was conducted under greenhouse conditions, at an average
146 temperature of 24 ºC and humidity of 48%. Maize seeds were surface sterilized with 1% sodium
147 hypochloride solution for 10 min and rinsed five times with sterile distilled water. Seeds were
148 then placed in 30 ml of sterile distilled water and shaken for 48 hours before planting. From 5
149 days after emergence (DAE), plants were watered every other day with 200mL of ⅓ Hoagland

150 solution, modified to a final P concentration of 25 µM (Hoagland and Broyer, 1936). Plants were
151 harvested at 56 DAE.
152
153 *Evaluation of plant growth*
154 Leaf length and width were measured manually, and leaf area estimated as 0.75 x length x width
155 (Mollier and Pellerin, 1999). Shoot and root fresh weights were measured after cutting the aerial
156 part and washing the root. Dry weight was determined by oven drying tissue at 70º for 48 hrs.
157 Root volume was measured by submergence in a test tube and recording of the volume of water
158 displaced.
159
160 *Estimation of fungal colonization in seedling roots*
161 Lateral root segments were collected at random from the upper 15 cm of the root system, placed
162 in 10% KOH solution, autoclaved for 10 min, and stained with 0.05% trypan blue in
163 acetoglycerol. The percentage of root length colonized was quantified in 15 root pieces per plant
164 using a modified grid-line intersect method (McGonigle et al., 1990).
165
166 *Characterization of root system architecture by image analysis*
167 The cleaned root system was placed in a water-filled tub and photographed using a digital Nikon
168 camera D3500. Raw images were individually processed using Adobe® Photoshop® CC (Version
169 14.0) to remove background and increase contrast. Processed images were scaled and analyzed
170 using GiA Roots software (Galkovskyi et al. 2012). After scale calibration, the images were
171 segmented using the adaptive thresholding method and basic parameter settings were manually
172 optimized. Finally, all binary images were analyzed to quantify the root system traits. Stacked

173 (overlay) root system images were generated using the stacks tool in ImageJ/Fiji (version 2.0.0).
174 Root traits are described in Supplementary Table S1.
175
176 *Analysis of the leaf ionome*
177 The third youngest leaf (variously, leaf 5 or 6 for different individuals) was collected for ionomic
178 analysis using inductively coupled plasma mass spectrometry (ICP-MS) as described previously
179 (Ziegler et al. 2013, Ramírez-Flores et al., 2017). Briefly, weighed tissue samples were digested
180 in 2.5 ml of concentrated nitric acid (AR Select Grade, VWR) with an added internal standard
181 (20 p.p.b. In, BDH Aristar Plus). The concentration of the elements B, Na, Mg, Al, P, S, K, Ca,
182 Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo and Cd was measured using an Elan 6000 DRC-e
183 mass spectrometer (Perkin-Elmer SCIEX) connected to a PFA microflow nebulizer (Elemental
184 Scientific) and Apex HF desolvator (Elemental Scientific). A control solution was run every 10th
185 sample to correct for machine drift both during a single run and between runs. Given that
186 samples were digested in concentrated nitric acid prior to analysis and run in a 70% N
187 atmosphere, it was not possible to accurately estimate N concentration.
188
189 *Statistical analyses and data visualization*
190 All statistical analyses were performed in R (R Core Team, 2019). From the initial planting, a
191 small number of plants did not germinate. In addition, we removed clear outliers (assessed by
192 visual inspection of plant growth and health) and any plants assigned to the non-inoculated group
193 that showed evidence of root-internal hyphal structures. The final dataset consisted of 53
194 individuals (Table S2). Leaf surface area estimates were square root transformed for analysis. A
195 linear fixed-effect model was applied on a trait-by-trait basis to control for differences between

the four planting dates. Principal component (PC) analysis was performed on Gia Roots traits along with root fresh (RFW) and dry weight (RDW), using R/ade4::dudi.pca (Dray and Dufour, 2007) with centered and scaled values. Linear Discriminant (LD) analysis on PC scores was performed with R/MASS::lda (Venables and Ripley, 2002). Ionomic analysis generated element concentration data; in addition, total element content was estimated as the product of concentration and shoot dry weight (SDW).

Data were analyzed using R/stats::lm to fit inoculation status and genotype in a complete model. After adjustment for multiple testing (R/stats::p.adjust; method = “BH”), no significant ($p < 0.05$) interaction between inoculation x genotype was detected for any trait, and we present here the additive model for simplicity. We also performed a one-way ANOVA with a single four-level treatment factor (B73.NC, B73.M, W22.NC and W22.M) that was used to assign means groups with R/agricolae::HSD.test (de Mendiburu, 2019) for presentation in Fig. S1. Box plots were generated with base R using default settings. Root images were pre-processed using imagemagick (www.imagemagick.org), converting them to png format, setting the background to transparent and reducing the alpha level. Images were imported into R using R/png and plotted onto the PCA/LD space using the rasterImage function. Pearson’s correlation coefficients were calculated using R/Hmisc::rcorr (Harrell, 2019) and visualized using R/gplots::heatmap.2 (Warnes et al., 2019). The impact of root system size and inoculation on element content, was assessed using the model *Content* = *NSA* + *Inoculation*, evaluating the *Inoculation* term using sequential (Type I) sum-of-squares (implemented by default in R). For comparison, partial (Type III) sum-of-squares were calculated using R/car::Anova (Fox & Weisberg, 2011), setting the “contrasts” option to c("contr.sum", "contr.poly").

RESULTS

Growth of maize seedlings increased when inoculated with *Rhizophagus irregularis*

To evaluate the relationship between root system architecture (RSA) and nutrient acquisition in mycorrhiza plants, we grew the two maize inbred lines B73 and W22 under low phosphorus (P) conditions, with (M) or without (NC) inoculation with the AM fungus *Rhizophagus irregularis* (Fig. 1A). These two widely used inbred lines were selected to provide a reference for future work, and to allow greater generalization than would be possible based on a single genotype. B73 is the reference for the most complete maize genome assembly. W22 is the background for the primary publicly available maize reverse genetics collections. Inoculation with AM fungus significantly enhanced plant growth in both genotypes (Fig. 1B, S1, S2. Table 1). Fungal inoculation and plant genotype were both significant ($p < 0.001$) predictors of plant biomass but there was no evidence of an interaction between the two (Fig. S2, Table 1; S2). By harvest (55 DAE), the marginal effect of inoculation on shoot dry weight (SDW) was an increase of 142% (Table 1). In both M and NC treatments, B73 tended to be smaller than W22, the range of SDW overlapping between B73 M and W22 NC treatments (Fig. 1, S1, S2). The inclusion of plants similar in size, yet differing in inoculation status, was important for our subsequent interpretation of element content.

To quantify mycorrhizal colonization, the percentage of root length containing different fungal structures was estimated by microscopic inspection. NC plants were confirmed to be free from colonization (fungal structures were observed in three plants in the NC group; these individuals were not included in the analysis), while inoculated plants showed fungal structures typical of the symbiosis (hyphae, arbuscules and vesicles). M plants were well colonized (Fig.

241 1C-E), and there was no difference in colonization rate between the two plant genotypes (Fig S1.
242 Table S2).
243
244 **The root system was modified by inoculation with *Rhizophagus irregularis***
245 Root fresh and dry weight (RFW and RDW) and root volume (RV) increased significantly in
246 inoculated plants (Fig. 2A. Table 1). To characterize RSA, we photographed the plants and
247 analyzed the images with GiA Roots (General Image Analysis of Roots; Galkovskyi et al.,
248 2012). Ten GiA Roots traits showed a significant response to inoculation (Table 1. Fig. S1). In a
249 principal component (PC) analysis using all 19 GiA Roots traits, the first four PCs explained
250 90% of the total variance (Fig. 2B; 2C). PC1 (explaining 59% of the total variance) was
251 dominated by variables associated with overall root system and plant size (Fig. 2; 4A). PC2
252 (explaining 12% of the total variance) was associated with overall root system shape and aspect
253 ratio (Fig. 2; 4A). PC3 (7%) and PC4 (7%) captured aspects of root branching and root system
254 solidity (Fig. 3; 4A). NC and M plants were well differentiated by PC1 and, to a lesser extent, by
255 PC3, indicating a shift towards larger, more branched/solid root systems in M plants. GiA Roots
256 evaluated solidity as the total network area divided by the network convex area. Although,
257 greater solidity was clear in M W22, the root system in the smallest B73 NC plants was also
258 considered relatively solid because the convex area itself was so low. We performed a linear
259 discriminant (LD) analysis using the root PC values, reinforcing the separation of M and NC
260 plants by size and solidity of the root system and better distinguishing the W22 M and B73 NC
261 treatments (Fig. 3).
262
263 **Nutrient content was correlated with root system size and mycorrhizal colonization**

264 AM fungi can increase plant nutrient uptake by delivery of nutrients via the hyphal network and
265 as a secondary consequence of promoting root growth. To better understand the relative
266 importance of these two factors, we considered the relationship between root system size and
267 mineral nutrient content. We determined the concentration of 20 different elements, including P,
268 in leaf tissue using inductively coupled plasma mass spectrometry (ICP-MS). Although leaf
269 element concentration provides an endpoint readout of various aspects of plant nutrient relations
270 (Salt et al., 2008), it was used here as a proxy for nutrient uptake. We estimated nutrient content
271 as the product of leaf concentration and total shoot dry weight and investigated the relationship
272 with the GiaRoots trait NSA (Network Surface Area), used as our measure of root system size.
273 As expected, an increase in NSA (associated with larger plants) was correlated with greater
274 nutrient content (Fig. 4B). Of greater interest, however, was the degree to which this relationship
275 was modified by AM colonization; *i.e.* for any given nutrient, was NSA sufficient to explain
276 element content in M plants? We ran a linear model using NSA and inoculation status as
277 predictors, assessing inoculation based on sequential (Type I) sum-of-squares, evaluating any
278 fungal effect beyond that explained by NSA alone (Fig. 5). A significant positive effect of
279 inoculation was associated with boron (B), Ca, Mg, P, S and strontium (Sr), indicating a unit of
280 root surface area to be associated with a greater leaf content of these elements in inoculated
281 plants.
282
283 **DISCUSSION**
284 Inoculation with *Rhizophagus irregularis* resulted in increased growth of maize grown under low
285 P availability. In comparison with a previous small pot evaluation (Sawers et al., 2017), plants
286 were larger, and the proportional increase in M plants was greater, presumably reflecting less

287 growth inhibition in the larger tubes that we used here. A difference in leaf surface area was
288 observed by day 40 - 45 after emergence, broadly consistent with the timing reported in rice for
289 the first observation of arbuscules and the accumulation of transcripts encoding mycorrhiza-
290 associated P transporters (Gutjahr et al., 2008). AM colonization was correlated not only with an
291 increase in root system size, but also in the degree of branching and solidity of the root system
292 (as captured by the GiA Roots traits MaxNR, MNR, NS). Inoculation with AM fungi has
293 previously been shown to promote root growth and branching in diverse plant hosts (Berta et al.,
294 1990; Paszkowski and Boller, 2002; Oláh et al., 2005; Gutjahr et al., 2009).

295 Increased growth was accompanied by greater concentration of Ca, Mn, P and Sr in the
296 leaves of M plants. The leaf concentration of K and Se was reduced in M plants, but for all
297 elements, taking biomass into account, total content increased. The content of B, Ca, Mg, P, S
298 and Sr following inoculation exceeded expectation based on root surface area alone. We interpret
299 this to reflect the impact of hyphal foraging, although we do not discount additional
300 contributions of enhanced root function (*e.g.* greater density or length of root hairs; stimulation
301 of the plant uptake pathways, production of exudates by plant or fungus) or changes in nutrient
302 partitioning. For other elements, the content in M plants could be explained by the larger root
303 system alone, although there is the possibility of a "hidden" mycorrhizal contribution (*i.e.* fungal
304 nutrient delivery balanced by an equivalent reduction in direct root uptake. Smith et al., 2003).

305 The growth increase in inoculated plants was likely driven by increased P uptake, given
306 that the experiment was conducted under low P availability. The route of P from soil to fungus,
307 and subsequent delivery to the plant host is well characterized (Chiu and Paszkowski, 2019). We
308 saw clear evidence that a unit of mycorrhizal root translates to a greater quantity of P obtained
309 than an equivalent unit of non-colonized root, as would be anticipated as a consequence of

310 hyphal foraging (Fig. 5). In comparison to P, the impact of AM colonization on the uptake of
311 Ca, Mg and S (the other macronutrients for which our observations were consistent with hyphal
312 foraging) is less well characterized (Gerlach et al., 2015; Ramírez-Flores et al., 2017). In the case
313 of S, it has been shown that AM fungi have the capacity to transfer S from soil to their plant
314 hosts (Gray and Gerdemann, 1973; Allen and Shachar-Hill, 2009), and that accumulation of
315 plant sulphate transporter transcripts increases in colonized roots (Casieri et al., 2012;
316 Giovannetti et al., 2014). Furthermore, the promoter of the *Lotus japonicus* sulphate transporter
317 gene *LjSULTR1;2* is active in arbusculated cells (Giovannetti et al., 2014), suggesting a function
318 in uptake from the peri-arbuscular space analogous to that of the PT4 high-affinity P transporter.
319 With regard to Mg, transcripts encoding Mg transporters are known to accumulate to higher
320 levels in wheat following mycorrhizal inoculation (Li et al., 2018). In common with P, the
321 elements S, Ca and Mg are often poorly available due to low-mobility and formation of
322 conjugates with other soil compounds (Kelly and Barber, 1991; Scherer, 2001; Lynch, 2019). As
323 such, hyphal foraging and delivery of these nutrients by mycorrhizal fungi would be predicted to
324 be of potential benefit to plants under field conditions. In the future, it will be informative to
325 combine evaluation of RSA in the field with assessment of AM fungal communities and
326 quantification of elements in reproductive stage plants and grain.
327

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519

520 **Table 1. Traits responsive to inoculation with *Rhizophagus irregularis***

Trait[1]	Description[2]	NC		M		MR[3] (%)
		mean	SE	mean	SE	
QLA45	Sqrt leaf surface area, day 45 (cm)	11.6	0.449	14.8	0.497	28
QLA50	Sqrt leaf surface area, day 50 (cm)	12.2	0.426	17.4	0.433	43
QLA55	Sqrt leaf surface area, day 55 (cm)	13.6	0.471	19.6	0.676	44
SFW	Shoot fresh weight (g)	14	1.3	35.8	2.16	156
RFW	Root fresh weight (g)	17.6	1.64	44.5	2.84	153
SDW	Shoot dry weight (g)	1.78	0.184	4.29	0.284	142
RDW	Root dry weight (g)	1.39	0.122	3.28	0.185	136
RV	Root volume (ml)	18.6	1.75	48.4	3.17	160
MaxNR	Maximum number of roots	36.3	1.78	53.9	2.03	49
MNR	Median number of roots	23.4	1.35	39.8	1.57	70
NB	Network bushiness	1.59	0.054	1.37	0.028	-14
NetA	Network area (cm^2)	95.1	6.89	181	9.72	90
NL	Network length (cm)	1870	150	3610	206	93
NLD	Network Length Distribution	0.937	0.058	0.661	0.033	-29
NP	Network perimeter (cm)	3640	281	6870	390	89

NS	Network solidity	0.129	0.004	0.185	0.006	43
NSA	Network surface area (cm^2)	361	27.1	698	38.4	93
NV	Network volume (cm^3)	6.84	0.536	13.3	0.808	95
Ca	Leaf Ca concentration (ppm)	9470	435	12900	589	36
K	Leaf K concentration (ppm)	24000	612	18300	1130	-24
Mn	Leaf Mn concentration (ppm)	115	5.25	149	6.26	29
P	Leaf P concentration (ppm)	612	19.3	1160	28.9	89
Se	Leaf Se concentration (ppm)	0.663	0.017	0.498	0.017	-25
Sr	Leaf Sr concentration (ppm)	39	1.86	51.3	2.85	32

[1] Traits listed showed a significant (adjP < 0.05) main effect of fungal inoculation.

[2] GiA Roots traits as described in full by Galkovskyi et al. 2012

[3] Mycorrhiza response calculated as M-NC/NC x 100

526 **FIGURE LEGENDS**

527 **Figure 1. Experimental set-up and plant growth response.** The maize inbred lines B73 and
528 W22 were grown with (M) or without (NC) inoculation with the AM fungus *Rhizophagus*
529 *irregularis*. A) Overall view of the growth system, the block in the foreground are inoculated
530 plants. B) Square root of leaf surface area (QLA) for M and NC individuals, quantified every 5
531 days from 5 days after emergence until harvest at day 55. Boxes show 1st quartile, median and
532 3rd quartile. Whiskers extend to the most extreme points within 1.5x box length; outlying values
533 beyond this range are shown as filled circles. Days at which M and NC groups were significantly
534 different for a given inbred (Tukey HSD; $p < 0.05$) are indicated with an asterisk. C) Root crown
535 on an M plant, illustrating the profusion of lateral roots and characteristic yellow pigmentation.
536 D) Trypan-blue stained root section of an M plant, showing root-internal hyphae (hyp), vesicles
537 (ves), and arbuscules (arb). Scale bar, 15 µm. E) Colonization (% root length colonized) for B73
538 and W22, determined with respect to fungal hyphae (H), arbuscules (A) and vesicles (V). Points
539 indicate individual plants. Boxes and whiskers as in B).
540
541 **Figure 2. Principal component analysis describes variation in root system architecture.** A)
542 Stacked (overlay) images of the root systems of all plants in the NC/M B73/W22 treatment
543 groups. Scale bar, 30 cm. B) The contribution of root system traits to principal components (PCs)
544 1 to 4. The variance explained by each PC is given in parentheses. See Table S1 for trait
545 abbreviations. C) Root system images arranged by loading on each of PCs 1 to 4. Coloured
546 points at the base of the image indicate genotype and fungal treatment as described in the key.
547 Traits with a major positive or negative contribution to each PC are listed on the right or left of
548 the plot, respectively. A single word description of the extremes of each PC is given at the side
549 of each plot.
550
551 **Figure 3. Inoculation with AM fungi is correlated with an increase in size and branching of**
552 **the root system.** Root system images projected by Linear Discriminants (LDs) 1 and 2. Colored
553 points indicate genotype and fungal treatment as described in the key.
554
555 **Figure 4. Root system architecture is correlated with total element content in the leaf.** A)
556 Heatmap representation of pairwise correlations of root system principal components (PC) with
557 their contributing GiARoot traits (Abbreviated as described in Materials and Methods. SFW,
558 shoot fresh weight and SDW, shoot dry weight also shown). B) Heatmap representation of
559 pairwise correlations of root system principal components (PC) and colonization measures (%
560 root length of hyphae (Hyp), vesicles (Ves) and arbuscules (Arb)) with total element content. In
561 both A) and B) significant correlations are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***,
562 $p < 0.001$).
563
564 **Figure 5. AM colonization modifies the relationship between root system size and element**
565 **content.** Scatter plot representation of the relationship between element content (shown in mg or

566 μg) and root system size (estimated as the GiaRoots trait NSA) for twenty named elements.
567 Colored points indicate genotype and fungal treatment of individual plants as described in the
568 key. Black and red lines indicate the fit for the additive model *Total ~ NSA + Fungus* for levels
569 NC and M of *Fungus*, respectively. The p-value for an effect of *Fungus* (calculated using Type I
570 SS; adjusted for multiple tests) is given in parentheses following the element name in the plot
571 title.
572

573 **Figure S1. Impact of AM colonization of growth, root architecture and nutrient levels.** The
574 maize inbred lines B73 and W22 were grown with (M) or without (NC) inoculation with the AM
575 fungus *Rhizophagus irregularis*. Traits are described in main text and Table S1, S2, S3. Boxes
576 show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within
577 1.5x box length; outlying values beyond this range are not shown. Line segments show the
578 reaction norm for each genotype, connecting median values for NC and M.
579

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587 *performance*.

588

589 **AUTHOR CONTRIBUTIONS**

590 RRF, RJHS and VOP designed the study. MRRF conducted the experiments. MRRF, EBB and
591 RRA performed image analysis. All authors contributed to data analysis and writing of the
592 manuscript.

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594 Sawers[2,4*] and Víctor Olalde-Portugal[1*]

595

596 **PREPRINT**

597 This article is available as a preprint at https://www.biorxiv.org/content/10.1101/695411v1

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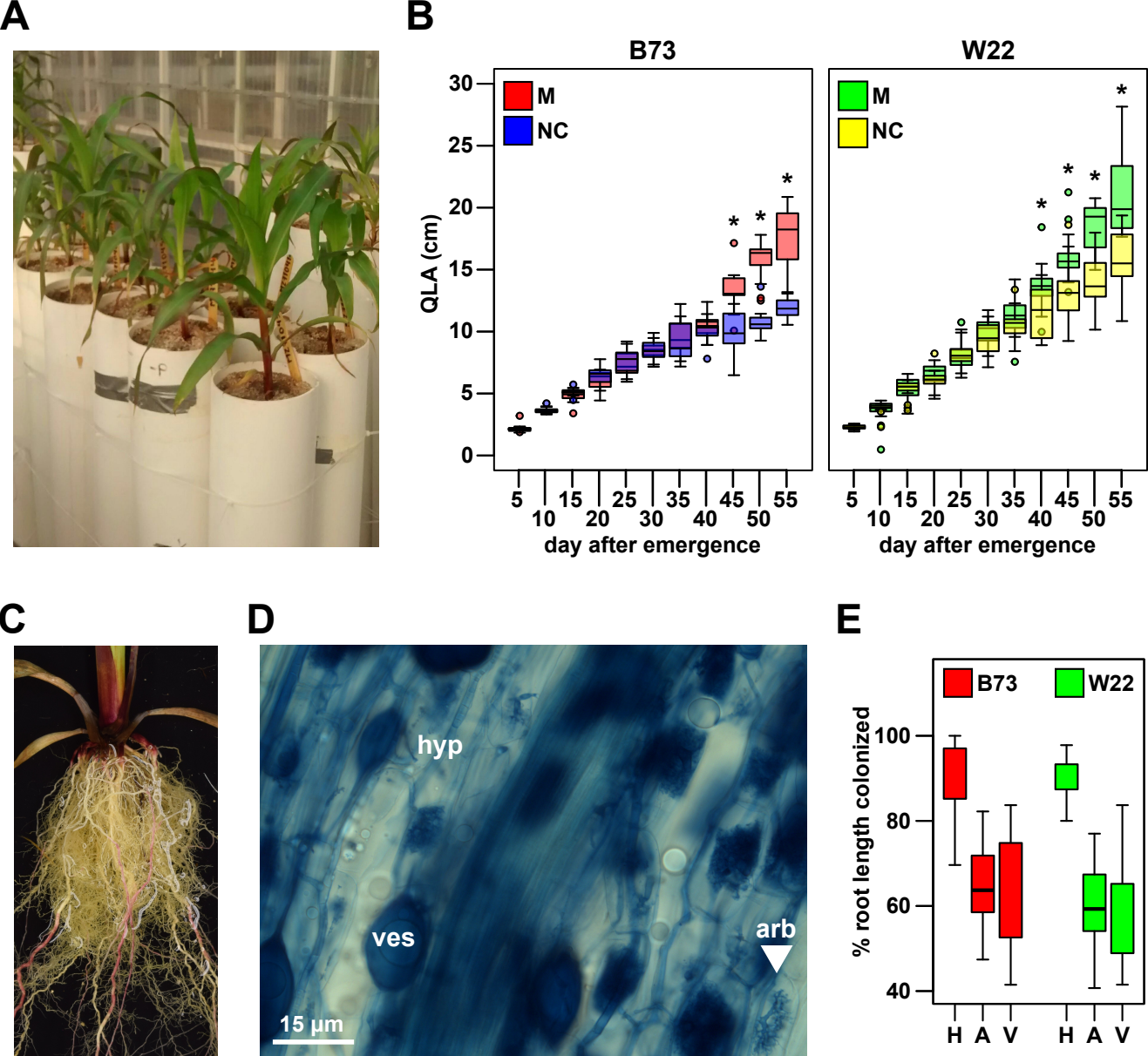
600 **CONFLICT OF INTEREST STATEMENT**

601 Rubén Rellán-Álvarez is a review editor for Plant Direct.


A
B
B73
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M
NC
QLA (cm)
day after emergence
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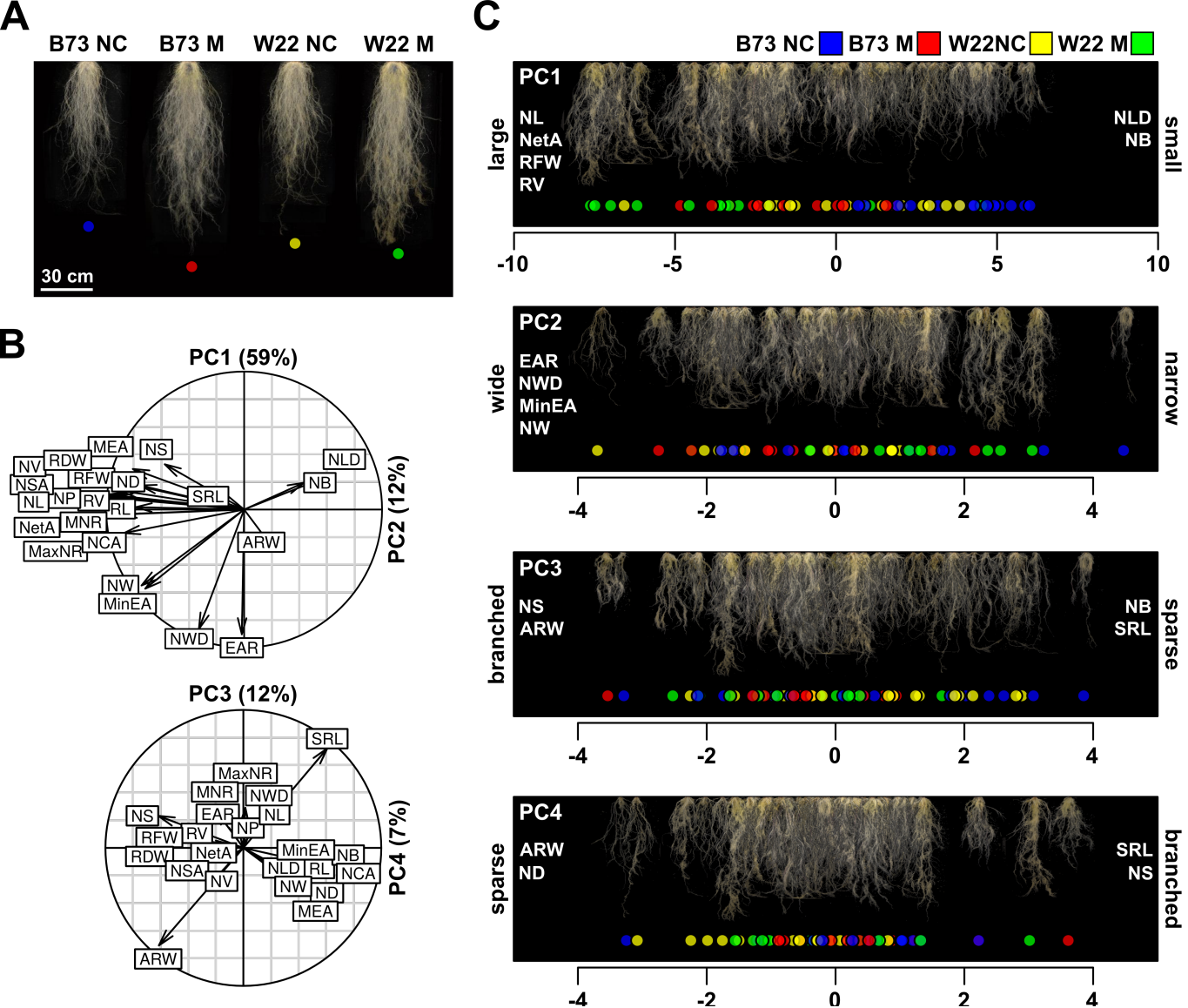
15 µm
E
B73
W22
% root length colonized
H A V
H A V


A
B73 NC
B73 M
W22 NC
W22 M
30 cm
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PC1 (59%)
PC2 (12%)
MEA
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NV
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PC3 (12%)
PC4 (7%)
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B73 M
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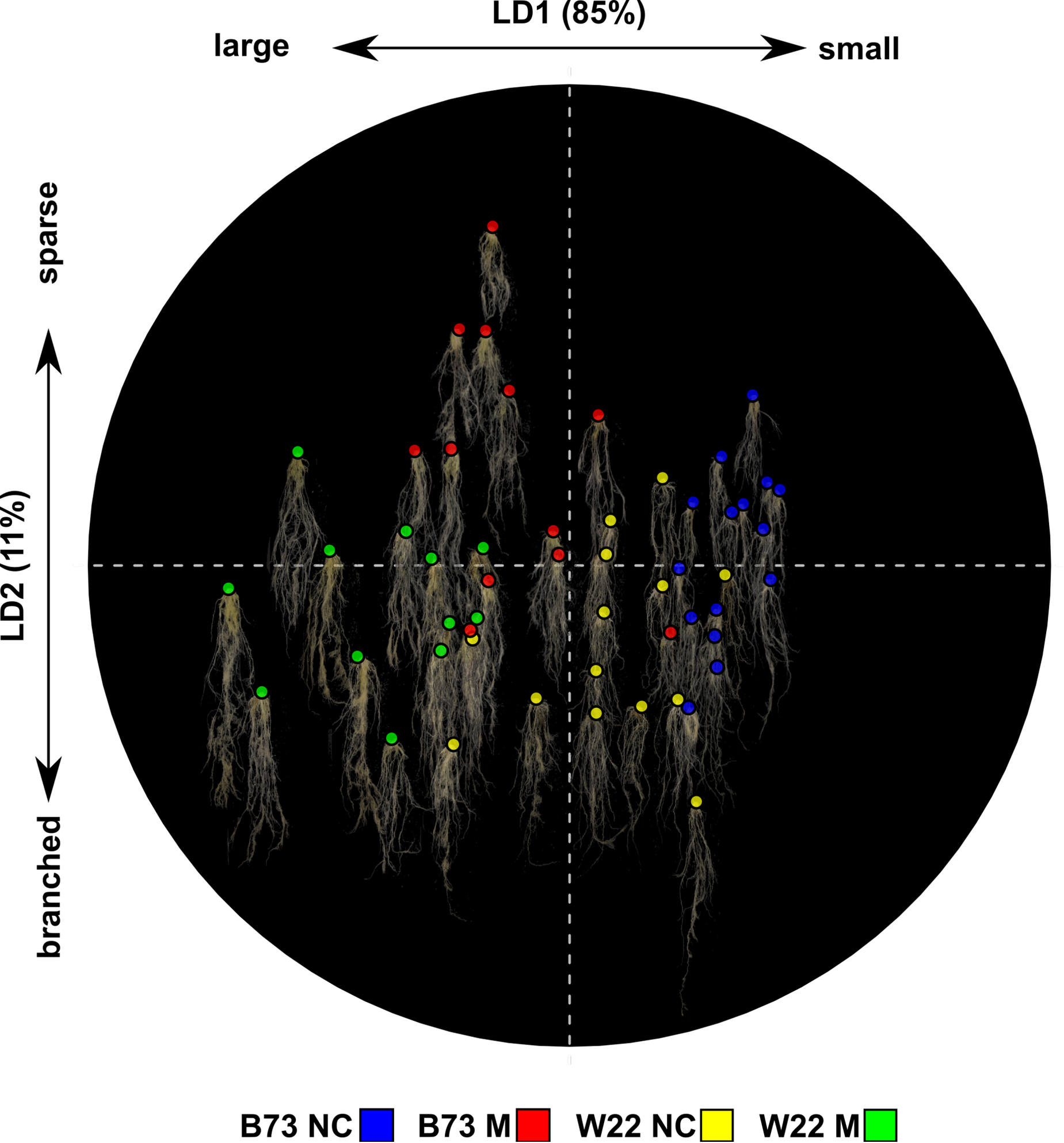
LD1 (85%)
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LD2 (11%)
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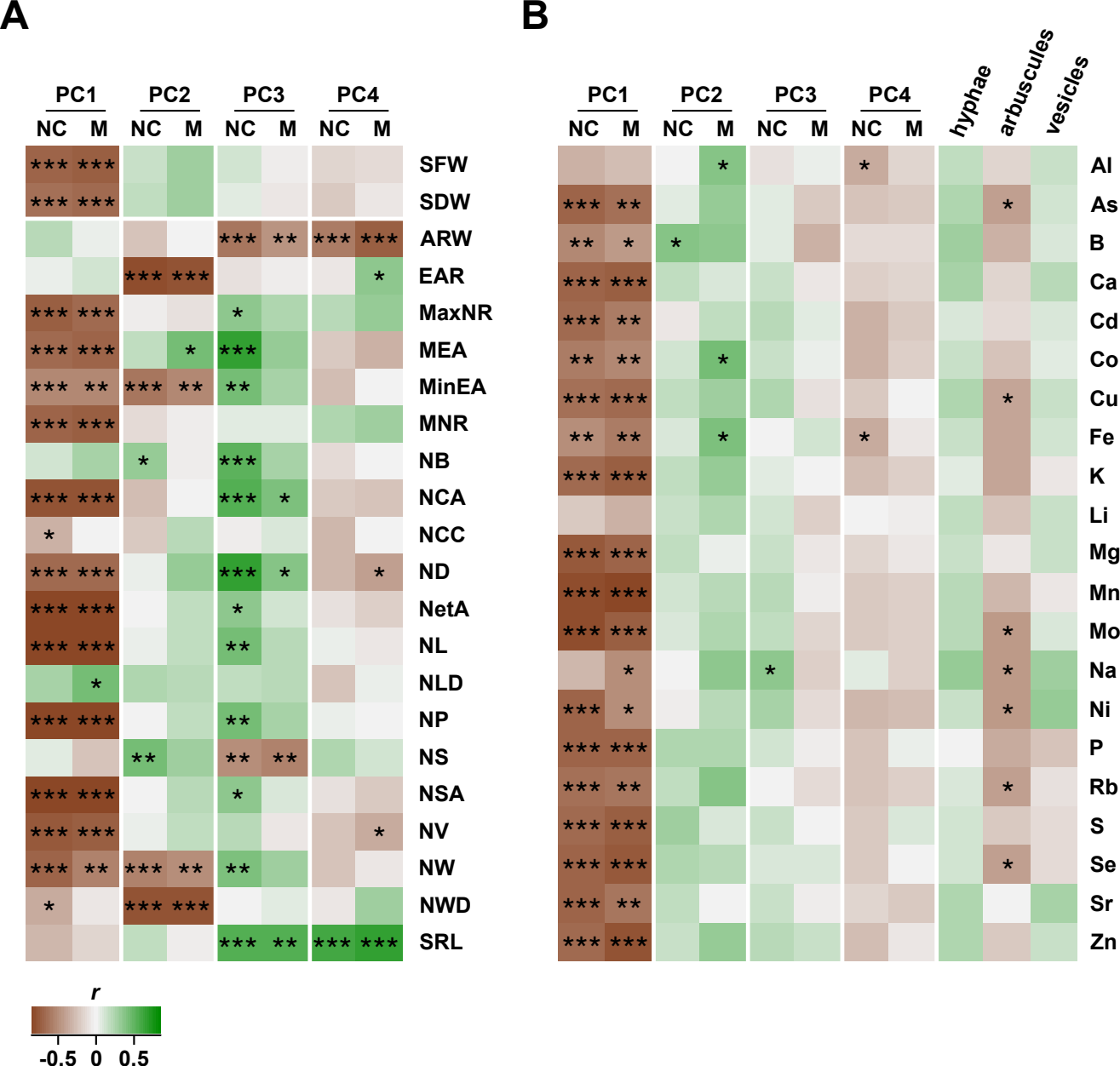
B73 NC
B73 M
W22 NC
W22 M


A
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SFW
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ARW
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MEA
MinEA
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NCC
ND
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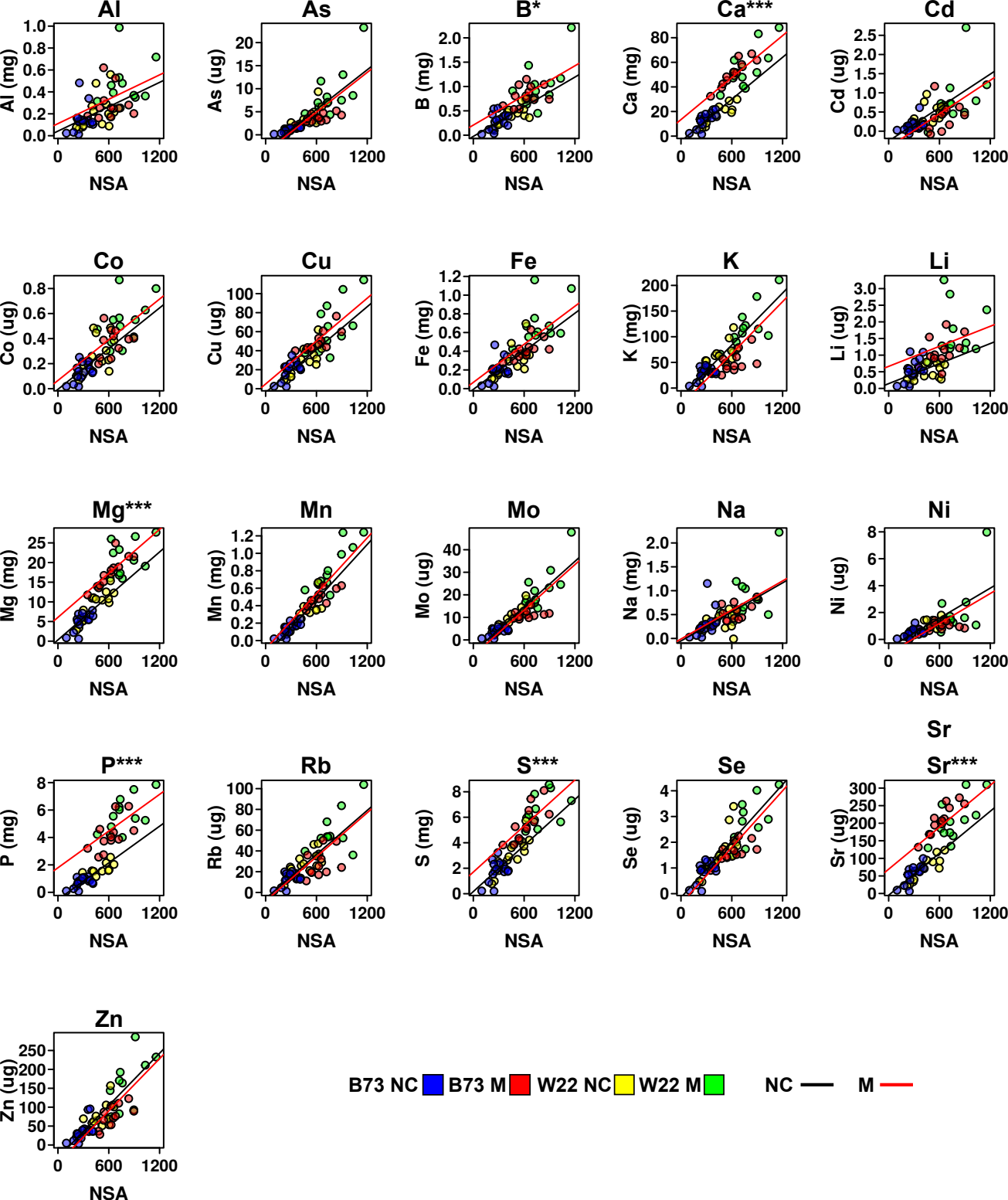
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B (mg)
Ca***
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Fe
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K
K (mg)
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Mg***
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Mn (mg)
Mo
Mo (ug)
Na
Na (mg)
Ni
Ni (ug)
P***
P (mg)
Rb
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Zn (ug)
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B73 M
W22 NC
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