Inoculation with the mycorrhizal fungus *Rhizophagus irregularis* increases nutrient uptake in maize (*Zea mays*) through hyphal foraging and promotion of root growth

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ABSTRACT

Plant root systems play an important role in nutrient and water acquisition. In resource-limited soils, modifications of the root system architecture is an important strategy to optimize plant performance. The majority of terrestrial plants also enter into symbiotic associations with arbuscular mycorrhizal fungi to maximize nutrient acquisition. In addition to direct delivery of nutrients, arbuscular mycorrhizal fungi provide secondary benefits by promoting root growth. Here, we investigated the interaction of nutrient limitation and arbuscular mycorrhizal symbiosis in their impact on root system development and nutrient uptake in maize. Inoculated plants showed an increase in biomass and total mineral content. In addition to greater biomass, mycorrhizal plants had denser, more branched root systems. We quantified twenty different mineral nutrients. For the majority of elements, the increased content in mycorrhizal plants was proportional to root system growth and development. Boron, calcium, magnesium, phosphorus, sulfur and strontium, however, accumulated to levels that indicated fungal delivery to be supplementing root uptake.

KEYWORDS: mycorrhizae, root development, maize, ionome

INTRODUCTION

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

Root development is regulated by the combined action of internal developmental pathways and external environmental stimuli (Malamy and Ryan, 2001), conditioning both plasticity and intra- and inter-specific variation. These pathways are based on the action of plant hormones, signal receptors, transcription factors and secondary messengers, including Ca^{2+}, nitric oxide and reactive oxygen species (Fukaki and Tasaka, 2009; Schlicht et al., 2013; Zhang

et al., 2015); (Garay-Arroyo et al., 2012; Jung and McCouch, 2013; Shahzad and Amtmann, 2017). Auxin works with cytokinin to regulate LR initiation (Aloni et al., 2006), and with gibberellin to modulate cell proliferation and elongation (Fu and Harberd, 2003). Strigolactones impact LR formation and root hair development in a dose-dependent manner (Koltai, 2011), and, in maize, promote nodal root development (Guan et al., 2012). RSA is largely a product of the balance between root growth and branching (Postma et al., 2014). Primary root growth will continue as long as the root meristem is active and populations of stem cells within the quiescent center are maintained. In *Arabidopsis*, the exhaustion of the primary root meristem under low P is considered the classic example of a plastic response to optimize topsoil foraging (Williamson et al., 2001; López-Bucio et al., 2002)(López-Bucio et al., 2002; Mora-Macías et al., 2017). Root branching, through the production of first- or higher- order LRs, is regulated at the level of the formation of LR primordia and their subsequent expansion. Low availability of N, P, sulfur (S) or zinc (Zn) promotes an increase in the density and/or length of LRs, although, as mentioned above, when the nutrient distribution is patchy, LRs may proliferate in regions of high local nutrient abundance (Zhang et al., 1999; Kutz et al., 2002; López-Bucio et al., 2002; Bouranis et al., 2008; Gruber et al., 2013). In cereal crops, the bulk of the adult root system is comprised of shoot-borne crown (CR) roots and associated LRs (Hochholdinger and Tuberosa, 2009). While greater root branching will increase the surface area for nutrient uptake, it becomes inefficient if placement of roots in close proximity results in competition for the same nutrients, an effect that will be greater for more mobile nutrients (Postma et al., 2014).

Significant intra-specific variation has been observed in RSA and plasticity. In crop plants, such variation has been linked to superior agronomic performance under specific edaphic

conditions. In common bean, varieties producing shallow basal roots and a large number of adventitious roots explore the topsoil more efficiently, resulting in superior performance in low P soils (Rubio et al., 2003; Lynch and Brown, 2008). In rice, the PHOSPHATE STARVATION TOLERANCE 1 (PSTOl1) kinase, identified from the traditional variety Kasalath, is linked to enhanced early root growth, and increases yield under low P conditions (Wissuwa et al., 2005); (Gamuyao et al., 2012).

In addition to relying on the action of their own roots for nutrient uptake, the majority of terrestrial plants have the capacity to form mutualistic symbioses with arbuscular mycorrhizal (AM) fungi of the Phylum Glomeromycota (Parniske, 2008; Smith and Read, 2010). The association with AM fungi is one of the oldest plant symbioses, and AM symbioses were likely instrumental in plant colonization of the land, performing essential functions before the development of the vascular root system (Schüßler et al., 2001). Establishment of AM symbiosis requires a complex interchange of signals between plant and fungus, that proceed the entry of the fungus into the root and the development of highly branched arbuscules within the root cortical cells that act as the primary site of nutrient exchange (Parniske, 2008). AM fungi are obligate symbionts, obtaining carbon from their plant host in return for providing nutrients and water that are acquired by an extensive network of root-external hyphae (Bago et al., 2003). The clearest benefit to the plant is enhanced P uptake (Chiu and Paszkowski, 2019). In addition, AM fungi have been reported to promote uptake of N, S, Zn and Fe (Liu et al., 2000; González-Guerrero et al., 2005; Govindarajulu et al., 2005; López-Pedrosa and González-Guerrero, 2006; Allen and Shachar-Hill, 2009). A relationship between P availability and the concentration of other ions has been observed. Although AM symbioses are widespread in both natural ecosystems and

cultivated fields, the plant host maintains a degree of control over establishment and the extent of colonization, rejecting the fungus under high nutrient conditions (Nouri et al., 2014).

AM fungi not only deliver nutrients obtained through hyphal foraging, but also promote root growth, with secondary effects on nutrient uptake. General improvements in plant health under AM symbiosis may be correlated with increased root system size (Berta et al., 1995; Tisserant et al., 1996; Gutjahr et al., 2009; Sawers et al., 2017). AM symbiosis is also associated with an increase in LR production and greater root branching (Berta et al., 1990; Paszkowski and Boller, 2002; Oláh et al., 2005; Gutjahr et al., 2009). The failure to form LR primordia in the maize *lateral rootless1* (*lrt1*) mutant can be partially overcome by AM symbiosis, indicating an influence on plant developmental pathways (Hochholdinger and Feix, 1998; Paszkowski and Boller, 2002). In *Medicago truncatula,* developing fungal spores are sufficient to trigger LR formation (Oláh et al., 2005; Gutjahr et al., 2009). In rice, AM fungi can induce LR formation in *pollux*, *ccamk* and *cyclops* mutants, even though symbiosis is not established, confirming that RSA modification is not dependent on enhanced host plant nutrition in this species (Gutjahr et al., 2009).

AM fungi improve plant nutrition by directly delivering nutrients and by promoting greater root growth. In turn, the impact on the roots is a consequence both of greater plant vigor and of fungus-induced developmental reprogramming. Although a comparison of nutrient concentration or quantity in the aerial portion of mycorrhizal (M) and non-colonized (NC) plants is indicative of mycorrhizal effect, it cannot distinguish direct hyphal nutrient delivery from the secondary consequences of root modification (Gerlach et al., 2015; Ramírez-Flores et al., 2017). Furthermore, nutrient levels may be equivalent in M and NC plants, even though uptake has

shifted from plant root to fungus (Smith et al., 2003). In the specific case of P, elegant labelling experiments and extensive functional studies have clearly demonstrated the direct role of root-external hyphae in nutrient delivery (Pearson and Jakobsen, 1993; Smith et al., 2003)(Chiu and Paszkowski, 2019)(Pearson and Jakobsen, 1993; Smith et al., 2003). The picture remains less clear with respect to other mineral nutrients.

In this study, we characterized RSA and the leaf ionome in vegetative stage maize plants, grown with or without inoculation with AM fungi. For each nutrient, we asked whether any increase in uptake was explained by changes in the root system alone, or whether there was evidence of additional hyphal foraging. Our expectation was that extensive hyphal foraging would result in an increased uptake per unit surface area of root. Conversely, for an increase resulting solely from root growth, we expected the relationship between surface area and uptake to be maintained, albeit that mycorrhizal plants themselves were larger.

MATERIALS AND METHODS

Plant material and growth conditions

Two maize inbred lines (*Zea mays* ssp. *mays* var. B73 and W22) were grown with (M) or without (NC) inoculation with fungus, in PVC tubes (1 m in height, 15 cm in diameter). A total of 64 plants (2 genotypes x 2 treatments x 16 replicates) were planted in complete blocks across four planting dates. B73 seed was produced in the winter of 2015 in Valle de banderas, Nayarit, México. W22 seed was produced in the summer of 2013 in Aurora, New York, USA. Each tube was filled with 19L of a sterilized substrate mix consisting of sand:perlite:silt (4.5:1.5:1, v/v). The substrate Olsen P concentration was 4.8 ppm. For M plants, we inoculated each tube with

~700 *Rhizophagus irregularis* spores obtained from a commercial liquid inoculant (AGTIV®). The experiment was conducted under greenhouse conditions, at 24 ºC (average temperature) and humidity of 48%. Maize seeds were surface sterilized with 1% sodium hypochloride solution for 10 min, and rinsed five times with sterile distilled water. Seeds were soaked in 30 ml of sterile distilled water and were shaken for 48 hours before planting. From 5 days after emergence (DAE), plants were watered, every other day, with 200mL of ⅓ Hoagland solution, modified to a final P concentration of 25 µM (Hoagland and Broyer, 1936). Plants were harvested at 56 DAE.

Evaluation of plant growth

Leaf length and width were measured manually, and leaf area estimated as 0.75 x length x width. Fresh weight of shoot and root were measured after cutting the aerial part and washing the root. Dry weight was determined by drying shoot and root tissue in the oven at 70º for 48 hrs.

Estimation of fungal colonization in seedling roots

To estimate fungal colonization, segments of fine root were collected at random from the upper 15 cm of the root system, cleared with 10% KOH solution and autoclaved for 10 min, and stained in 0.05% trypan blue in acetoglycerol. The percentage of root length colonized was quantified in 15 root pieces per plant using a modified grid-line intersect method (McGONIGLE et al., 1990).

Characterization of root system architecture

The cleaned root system was placed in a water-filled tub and photographed using a digital Nikon camera (Nikon Corp., Tokyo, Japan). Raw images were individually processed using Adobe® Photoshop® CC (Version 14.0) to remove the background and obtain a good contrast between foreground and background non- root pixels. Processed images were scaled and analyzed using GiA Roots software (Galkovskyi et al. 2012). Root traits are described in Supplementary Table S1.

Analysis of the leaf ionome

The third youngest leaf was collected for ionomic analysis using ICP-MS as described previously (Ramírez-Flores et al., 2017).

Statistical analyses and data visualization

All statistical analyses were performed in R statistics (R Core Team, 2019). From the initial planting, a small number of plants did not germinate. In addition, a number of plants of clear outliers were removed, as where any plants assigned to the non-inoculated group that, upon microscopic evaluation, showed any evidence of root-internal hyphal structures. The final dataset consisted of 56 individuals (Table S2). Leaf surface area estimates were square root transformed under the expectation of a linear increase over time. A 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 alone, and to fit inoculation status and genotype in a complete model. After adjustment for multiple testing (R/stats::p.adjust; method = "BH"), no inoculation x genotype interactions were considered significant at the 5% level. A one-way ANOVA was also performed with a single four-level treatment factor (B73.NC, B73.M, W22.NC and W22.M), and used to assign means groups with R/agricolae::HSD.test (de Mendiburu, 2019). 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 on the basis of sequential (Type I) sum-of-squares (implemented by default in R). For comparison, parital (Type III) sum-of-squares were calculated using R/car::Anova (Fox & Weisberg, 2011). The "contrasts" option was set to c("contr.sum", "contr.poly")).

RESULTS

Maize seedlings show increased growth under low phosphorus availability when inoculated with Rhizophagus irregularis

To evaluate the relationship between root system architecture (RSA) and nutrient acquisition in mycorrhiza response, 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). Inoculation with AM fungus significantly enhanced plant biomass in both genotypes, although W22 showed an earlier foliar response than B73 (40 and 45 DAE, respectively. Fig. 1B. Table 1). By harvest (55 DAE), shoot dry weight (SDW) showed an increase of 142% in M plants (Table 1). Fungal inoculation and plant genotype were both significant ($p < 0.001$) predictors of plant growth (Table 1; S2), but there was no evidence of an interaction between the two; *i. e.* although the two lines showed growth differences their response to inoculation was equivalent. B73 plants were smaller than W22, with B73 M plants being indistinguishable in size from W22 NC plants (Fig. 1, S2). The inclusion of plants similar in size, yet differing in inoculation status, was important for our subsequent interpretation of nutrient data.

To quantify mycorrhizal colonization, root fragments were collected randomly from the upper 15 cm of the root system, and the percentage of root length containing different fungal structures estimated by microscopic inspection. NC plants were confirmed to be free from colonization (fungal structures were observed in a small number of plants in the NC group; three 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. 1E), and there was no difference in colonization between the two plant genotypes (Fig S1. Table S2).

The root system is modified by inoculation with Rhizophagus irregularis

Root fresh and dry weight (RFW and RDW) and root volume (RV) increased significantly in inoculated plants (Fig. 2A. Table 1). To characterize RSA, we photographed the plants and analyzed the images with GiA Roots (General Image Analysis of Roots; (Galkovskyi et al., 2012)). Nine GiA Roots traits showed a significant response to inoculation (Table 1. Fig. S1). In a principal component (PC) analysis using all 19 GiA Roots raits, the first four PCs explaining 90% of the total variance (Fig. 2B; 2C). PC1 (explaining 59% of the total variance) was dominated by variables associated with overall root system size (Fig. 2; 4A). PC2 (explaining 12% of the total variance) was associated with the overall root system shape and aspect ratio (Fig. 2; 4A). PC3 (7%) and PC4 (7%) captured aspects of variation in root branching and the solidity of the root system (Fig. 3; 4A). The root systems of NC and M plants were well differentiated by PC1 ($p < 0.001$) and, to a lesser extent, by PC3 ($p = 0.054$ for the genotype x inoculation interaction). GiA Roots calculates Network Solidity as the total network area divided by the network convex area. The trend towards greater network solidity was clear in W22; in B73, the root systems of a number of the smallest NC plants were considered relatively solid, given that their total network area itself was low), indicating a shift towards larger, more branched root systems in M plants. We also performed a linear discriminant (LD) analysis using the root PC values, reinforcing the separation of M and NC plants on the basis of size and solidity of the root system (Fig. 3).

The extent of the root system and mycorrhizal colonization are correlated with total nutrient uptake

AM fungi can increase plant nutrient uptake by delivery of nutrients via the hyphal network and as a secondary consequence of promoting root growth. To better understand the relative importance of these two factors, we considered the relationship between the extent of the root system (quantified using the GiaRoots trait NSA, Network Surface Area) and total uptake of each element (estimated as the product of leaf concentration and total shoot dry weight). As expected, an increase in NSA (associated with larger plants) was correlated with greater total nutrient uptake (Fig. 4B). Of greater interest, however, was the degree to which this relationship was modified by AM colonization; *i.e.* for any given nutrient, was NSA alone sufficient to explain increased uptake in M plants? We ran a linear model using NSA and inoculation status as predictors, assessing inoculation on the basis of sequential (Type I) sum-of-squares, interrogating any fungal effect beyond that predicted by the extent of the root system alone (Fig. 5; Table SX). The fungal effect was significant with respect to the nutrients boron (B), Ca, Mg, P, S and strontium (Sr). In all cases for which the fungal effect was significant, the coefficient associated with M plants was positive; *i. e.* a unit of root surface area was associated with greater nutrient content in colonized plants.

DISCUSSION

Inoculation with *Rhizophagus irregularis* resulted in increased growth in the maize grown under low P availability. In comparison with previous characterization in small pots (Sawers et al., 2017), the plants were larger, and the proportional increase in M plants was greater, presumably reflecting less growth inhibition in the larger tubes. Nonetheless, the relative growth of the two varieties was similar to the previous report. A difference in leaf surface area was observed by

day 40 - 45 after emergence, broadly consistent with the timing reported in rice for the first observation of arbuscules and the accumulation of transcripts encoding mycorrhiza-associated P transporters (Gutjahr et al., 2008). AM colonization was correlated not only with an increase in root system size, but also in the degree of branching and solidity of the root system (as captured by the GiA Roots traits MaxNR, MNR, NS). Inoculation with AM fungi has previously been shown to promote root growth and branching in diverse plant hosts (Berta et al., 1990; Paszkowski and Boller, 2002; Oláh et al., 2005; Gutjahr et al., 2009).

Increased growth was accompanied by a greater concentration of Ca, Mn, P and Sr in the leaves of M plants. The leaf concentration of K and Se was reduced in M plants, but for all elements, taking greater biomass into account, total content increased. For B, Ca, Mg, P, S and Sr, the content in M plants was greater than could be explained on the basis of the root surface area alone. We interpret this to reflect the impact of hyphal foraging. In addition, enhanced root function (*e.g.* greater density or length of root hairs; stimulation of the plant uptake pathways) or changes in nutrient partitioning, might contribute to this effect. For other elements, the increase in M plants could be explained as a consequence of greater root growth alone, although we do not discount the possibility of "hidden" mycorrhizal uptake (*i.e.* a mycorrhizal contribution balanced by an equivalent reduction in direct root uptake. (Smith et al., 2003)).

Our experiment was conducted under P limitation, and enhanced P uptake was presumably the major factor underlying the growth increase in M plants. The route of P from soil to fungus, and subsequent delivery to the plant host is well characterized (Chiu and Paszkowski, 2019). As anticipated, we saw evidence that a unit of mycorrhizal root translates to a greater quantity of P obtained than an equivalent unit of non-colonized root (Fig. 5). In comparison to P,

the role of AM fungi in the uptake of Ca, Mg and S (the other macronutrients for which we saw evidence of hyphal foraging) is less well characterized, and previous studies report conflicting results with regard to levels of accumulation in mycorrhizal maize (Gerlach et al., 2015; Ramírez-Flores et al., 2017). It has been clearly shown that AM fungi have the capacity to transfer S from soil to their plant hosts (Gray and Gerdemann, 1973; Allen and Shachar-Hill, 2009), and that plant sulphate transporter transcripts increase accumulation in the roots of AM plants (Casieri et al., 2012; Giovannetti et al., 2014). Furthermore, the promoter of the *Lotus japonicus* sulphate transporter gene *LjSULTR1;2* is active in arbusculated cells (Giovannetti et al., 2014), suggesting a function in uptake from the peri-arbuscular space that is analogous to that of the PT4 high-affinity P transporters. With regard to Mg, it has been reported that transcripts encoding Mg transporter encoding transcripts accumulate to higher levels in mycorrhizal wheat (Li et al., 2018). In common with P, the elements S, Ca and Mg are often poorly available to plants due to low-mobility and formation of conjugates with other soil compounds (Kelly and Barber, 1991)(Scherer, 2001)(Lynch, 2019), suggesting that hyphal foraging would provide significant uptake benefits under field conditions.

Inoculation with arbuscular mycorrhizal fungi promoted increased element content in maize by stimulating root growth and by hyphal foraging.

LITERATURE CITED

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol **149**: 549–560

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance

and root gravitropism. Ann Bot **97**: 883–893

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol **131**: 1496–1507

Berta G, Fusconi A, Trotta A, Scannerini S (1990) Morphogenetic modifications induced by the mycorrhizal fungus Glomus strain E3 in the root system of Allium porrum L. New Phytologist **114**: 207–215

Berta G, Trotta A, Fusconi A, Hooker JE, Munro M, Atkinson D, Giovannetti M, Morini S, Fortuna P, Tisserant B, et al (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in Prunus cerasifera. Tree Physiol **15**: 281–293

Bouranis DL, Buchner P, Chorianopoulou SN, Hopkins L, Protonotarios VE, Siyiannis VF, Hawkesford MJ (2008) Responses to Sulfur Limitation in Maize. *In* NA Khan, S Singh, S Umar, eds, Sulfur Assimilation and Abiotic Stress in Plants. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1–19

Campbell BD, Grime JP, Mackey JML (1991) A trade-off between scale and precision in resource foraging. Oecologia **87**: 532–538

Casieri L, Gallardo K, Wipf D (2012) Transcriptional response of Medicago truncatula sulphate transporters to arbuscular mycorrhizal symbiosis with and without sulphur stress. Planta **235**: 1431–1447

Chiu CH, Paszkowski U (2019) Mechanisms and Impact of Symbiotic Phosphate Acquisition. Cold Spring Harb Perspect Biol. doi: 10.1101/cshperspect.a034603

Farley RA, Fitter AH (1999) The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. J Ecol **87**: 849–859

Fukaki H, Tasaka M (2009) Hormone interactions during lateral root formation. Plant Mol Biol **69**: 437–449

Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, et al (2012) GiA Roots: software for the high throughput analysis of plant root system architecture. BMC Plant Biol **12**: 116

Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature **488**: 535–539

Garay-Arroyo A, De La Paz Sánchez M, García-Ponce B, Azpeitia E, Alvarez-Buylla ER (2012) Hormone symphony during root growth and development. Dev Dyn **241**: 1867–1885

Gerlach N, Schmitz J, Polatajko A, Schlüter U, Fahnenstich H, Witt S, Fernie AR, Uroic K,

Scholz U, Sonnewald U, et al (2015) An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. Plant, Cell & Environment **38**: 1591–1612

Giovannetti M, Tolosano M, Volpe V, Kopriva S, Bonfante P (2014) Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in Lotus japonicus. New Phytol **204**: 609–619

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol N (2005) Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol **42**: 130–140

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature **435**: 819–823

Gray LE, Gerdemann JW (1973) Uptake of sulphur-35 by vesicular-arbuscular mycorrhizae. Plant and Soil **39**: 687–689

Grossman JD, Rice KJ (2012) Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evol Appl **5**: 850–857

Gruber BD, Giehl RFH, Friedel S, von Wirén N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. Plant Physiol **163**: 161–179

Guan JC, Koch KE, Suzuki M, Wu S, Latshaw S, Petruff T, Goulet C, Klee HJ, McCarty DR (2012) Diverse Roles of Strigolactone Signaling in Maize Architecture and the Uncoupling of a Branching-Specific Subnetwork. Plant Physiol **160**: 1303–1317

Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H, Paszkowski U (2008) Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. Plant Cell **20**: 2989–3005

Gutjahr C, Casieri L, Paszkowski U (2009) Glomus intraradices induces changes in root system architecture of rice independently of common symbiosis signaling. New Phytol **182**: 829–837

Hoagland DR, Broyer TC (1936) GENERAL NATURE OF THE PROCESS OF SALT ACCUMULATION BY ROOTS WITH DESCRIPTION OF EXPERIMENTAL METHODS. Plant Physiol **11**: 471–507

Hochholdinger F, Feix G (1998) Early post-embryonic root formation is specifically affected in the maize mutant lrt1. Plant J **16**: 247–255

Hochholdinger F, Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. Curr Opin Plant Biol **12**: 172–177

Ho MD, McCannon BC, Lynch JP (2004) Optimization modeling of plant root architecture for water and phosphorus acquisition. J Theor Biol **226**: 331–340

Jung JKH, McCouch S (2013) Getting to the roots of it: Genetic and hormonal control of root architecture. Front Plant Sci **4**: 186

Kelly JM, Barber SA (1991) Magnesium uptake kinetics in loblolly pine seedlings. Plant and Soil **134**: 227–232

Koltai H (2011) Strigolactones are regulators of root development. New Phytol **190**: 545–549

Kutz A, Muller A, Hennig P, Kaiser WM, Piotrowski M, Weiler EW (2002) A role for nitrilase 3 in the regulation of root morphology in sulphur-starving Arabidopsis thaliana. Plant J **30**: 95–106

Li M, Wang R, Tian H, Gao Y (2018) Transcriptome responses in wheat roots to colonization by the arbuscular mycorrhizal fungus Rhizophagus irregularis. Mycorrhiza **28**: 747–759

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (Zea mays L.) grown in soil at different P and micronutrient levels. Mycorrhiza **9**: 331–336

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Current Opinion in Plant Biology **6**: 280–287

López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol **129**: 244–256

López-Pedrosa A, González-Guerrero M (2006) GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of Glomus intraradices. Fungal Genet. Biol.

Lynch J (1995) Root Architecture and Plant Productivity. Plant Physiol **109**: 7–13

Lynch JP (2019) Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. New Phytol. doi: 10.1111/nph.15738

Lynch JP, Brown KM (2008) Root strategies for phosphorus acquisition. Plant Ecophysiology 83–116

Malamy JE, Ryan KS (2001) Environmental Regulation of Lateral Root Initiation in Arabidopsis. Plant Physiol **127**: 899–909

McGONIGLE TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular

mycorrhizal fungi. New Phytol **115**: 495–501

Mora-Macías J, Ojeda-Rivera JO, Gutiérrez-Alanís D, Yong-Villalobos L, Oropeza-Aburto A, Raya-González J, Jiménez-Domínguez G, Chávez-Calvillo G, Rellán-Álvarez R, Herrera-Estrella L (2017) Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. Proc Natl Acad Sci U S A **114**: E3563–E3572

Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in Petunia hybrida. PLoS One **9**: e90841

Oláh B, Brière C, Bécard G, Dénarié J, Gough C (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in Medicago truncatula via the DMI1/DMI2 signalling pathway. Plant J **44**: 195–207

Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root system architecture. Annu Rev Plant Biol **58**: 93–113

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol **6**: 763–775

Paszkowski U, Boller T (2002) The growth defect of lrt1 , a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. Planta **214**: 584–590

Pearson JN, Jakobsen I (1993) Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. New Phytologist **124**: 481–488

Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiol **166**: 590–602

Ramírez-Flores MR, Rellán-Álvarez R, Wozniak B, Gebreselassie M-N, Jakobsen I, Olalde-Portugal V, Baxter I, Paszkowski U, Sawers RJH (2017) Co-ordinated Changes in the Accumulation of Metal Ions in Maize (Zea mays ssp. mays L.) in Response to Inoculation with the Arbuscular Mycorrhizal Fungus Funneliformis mosseae. Plant Cell Physiol **58**: 1689–1699

Rangarajan H, Postma JA, Lynch JP (2018) Co-optimization of axial root phenotypes for nitrogen and phosphorus acquisition in common bean. Ann Bot **122**: 485–499

Rubio G, Liao H, Yan X, Lynch JP (2003) Topsoil Foraging and Its Role in Plant Competitiveness for Phosphorus in Common Bean. Crop Science **43**: 598

Sawers RJH, Svane SF, Quan C, Grønlund M, Wozniak B, Gebreselassie M-N, González-Muñoz E, Chávez Montes RA, Baxter I, Goudet J, et al (2017) Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate

transporters. New Phytol **214**: 632–643

Scherer HW (2001) Sulphur in crop production — invited paper. European Journal of Agronomy **14**: 81–111

Schlicht M, Ludwig-Müller J, Burbach C, Volkmann D, Baluska F (2013) Indole-3-butyric acid induces lateral root formation via peroxisome-derived indole-3-acetic acid and nitric oxide. New Phytol **200**: 473–482

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res **105**: 1413–1421

Shahzad Z, Amtmann A (2017) Food for thought: how nutrients regulate root system architecture. Curr Opin Plant Biol **39**: 80–87

Smith SE, Read DJ (2010) Mycorrhizal Symbiosis. Academic Press

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol **133**: 16–20

Tisserant B, Gianinazzi S, Gianinazzi-Pearson V (1996) Relationships between lateral root order, arbuscular mycorrhiza development, and the physiological state of the symbiotic fungus in Platanus acerifolia. Canadian Journal of Botany **74**: 1947–1955

Williamson LC, Ribrioux SP, Fitter AH, Leyser HM (2001) Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiol **126**: 875–882

Wissuwa M, Gatdula K, Ismail A (2005) Candidate gene characterization at the Pup1 locus: a major QTL increasing tolerance of phosphorus deficiency. Copyright International Rice

Zhang H, Jennings A, Barlow PW, Forde BG (1999) Dual pathways for regulation of root branching by nitrate. Proc Natl Acad Sci U S A **96**: 6529–6534

Zhang Y, von Behrens I, Zimmermann R, Ludwig Y, Hey S, Hochholdinger F (2015) LATERAL ROOT PRIMORDIA 1 of maize acts as a transcriptional activator in auxin signalling downstream of the Aux/IAA gene rootless with undetectable meristem 1. J Exp Bot **66**: 3855–3863

Add:

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Felipe de Mendiburu (2019). agricolae: Statistical Procedures for Agricultural

Research. R package version 1.3-0.

Frank E Harrell Jr, with contributions from Charles Dupont and many others. (2019). Hmisc: Harrell Miscellaneous. R package version 4.2-0.

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2019). gplots: Various R Programming Tools for Plotting Data. R package version 3.0.1.1.

John Fox and Sanford Weisberg (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL:

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

Trait[1]	Description[2]	NC		M		MR[3] (%)
		mean	SE	mean	SE	
SLA45	Sqrt leaf surface area, day 45 (cm)	11.6	0.449	14.8	0.497	28
SLA50	Sqrt leaf surface area, day 50 (cm)	12.2	0.426	17.4	0.433	43
SLA55	Sqrt leaf surface area, day 55 (cm)	13.6	0.471	19.6	0.676	44
SFW	Leaf 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	Leaf 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	95.1	6.89	181	9.72	90
NL	Network length	1870	150	3610	206	93
NLD	Network Length Density	0.937	0.058	0.661	0.033	-29
NP	Network perimeter	3640	281	6870	390	89
NS	Network solidity	0.129	0.004	0.185	0.006	43
NSA	Network surface area	361	27.1	698	38.4	93
NV	Network volume	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

FIGURE LEGENDS

Figure 1. Experimental set-up and plant growth response. The maize inbred lines B73 and W22 were grown with (M) or without (NC) inoculation with the AM fungus *Rhizophagus irregularis*. A) Overall view of the growth system, the block in the foreground are inoculated plants. B) Square root (sqrt) of leaf surface area for M and NC individuals, quantified every 5 days from 5 days after emergence until harvest at day 55. Boxes show 1st quartile, median and 3rd quartile. Whiskers extend to the most extreme points within 1.5x box length; outlying values beyond this range are shown as filled circles. Days at which M and NC groups were significantly different for a given inbred (Tukey HSD; $p < 0.05$) are indicated with an asterisk. C) Root crown on an M plant, illustrating the profusion of lateral roots and characteristic yellow pigmentation. D) Trypan-blue stained root section of an M plant, showing root-internal hyphae (hyp), vesicles (ves), and arbuscules (arb). Scale bar, 15 μM. E) Colonization (% root length colonized) for B73 and W22, determined with respect to fungal hyphae (H), arbuscules (A) and vesicles (V). Points indicate individual plants. Boxes and whiskers as in B).

Figure 2. Principal component analysis describes variation in root system architecture. A) Stacked images of the root systems of NC and M plants for B73 and W22 inbred lines. Scale bar, 30 cm. B) The contribution of root system traits to principal components (PC) 1 to 4. The variance explained by each PC is given in parentheses. See Materials and Methods for trait abbreviations. C) Root system images arranged by loading on each of PCs 1 to 4. Coloured points at the base of the image indicate genotype and fungal treatment as described in the key. Traits with a major positive or negative contribution to each PC are listed on the right or left of the plot, respectively. A single word description of the extremes of each PC is given at the side of the plot.

Figure 3. Inoculation with AM fungi is correlated with an increase in size and branching of the root system. Root system images projected by Linear Discriminants (LDs) 1 and 2. Colored points indicate genotype and fungal treatment as described in the key.

Figure 4. Root system architecture is correlated with total element content in the leaf. A) Heatmap representation of pairwise correlations of root system principal components (PC) with their contributing GiARoot traits (Abbreviated as described in Materials and Methods. SFW, shoot fresh weight and SDW, shoot dry weight also shown). B) Heatmap representation of pairwise correlations of root system principal components (PC) and colonization measures (% root length of hyphae (Hyp), vesicles (Ves) and arbuscules (Arb)) with the total quantity of each ion. In both A) and B) significant correlations are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 5. AM colonization modifies the relationship between root system architecture and nutrient uptake. Scatter plot representation of the relationship between total nutrient uptake (shown in mg or µg) and extent of the root system (estimated as the GiaRoots trait NSA) for twenty named elements. Colored points indicate genotype and fungal treatment of individual plants as described in the key. Black and Red lines indicate the fit for the simple additive model *Total* ~ *NSA* + *Fungus* for levels NC and M of *Fungus*, respectively. The p-value for an effect of *Fungus* (calculated using Type I SS; adjusted for multiple tests) is given in parentheses following the element name in the plot title.

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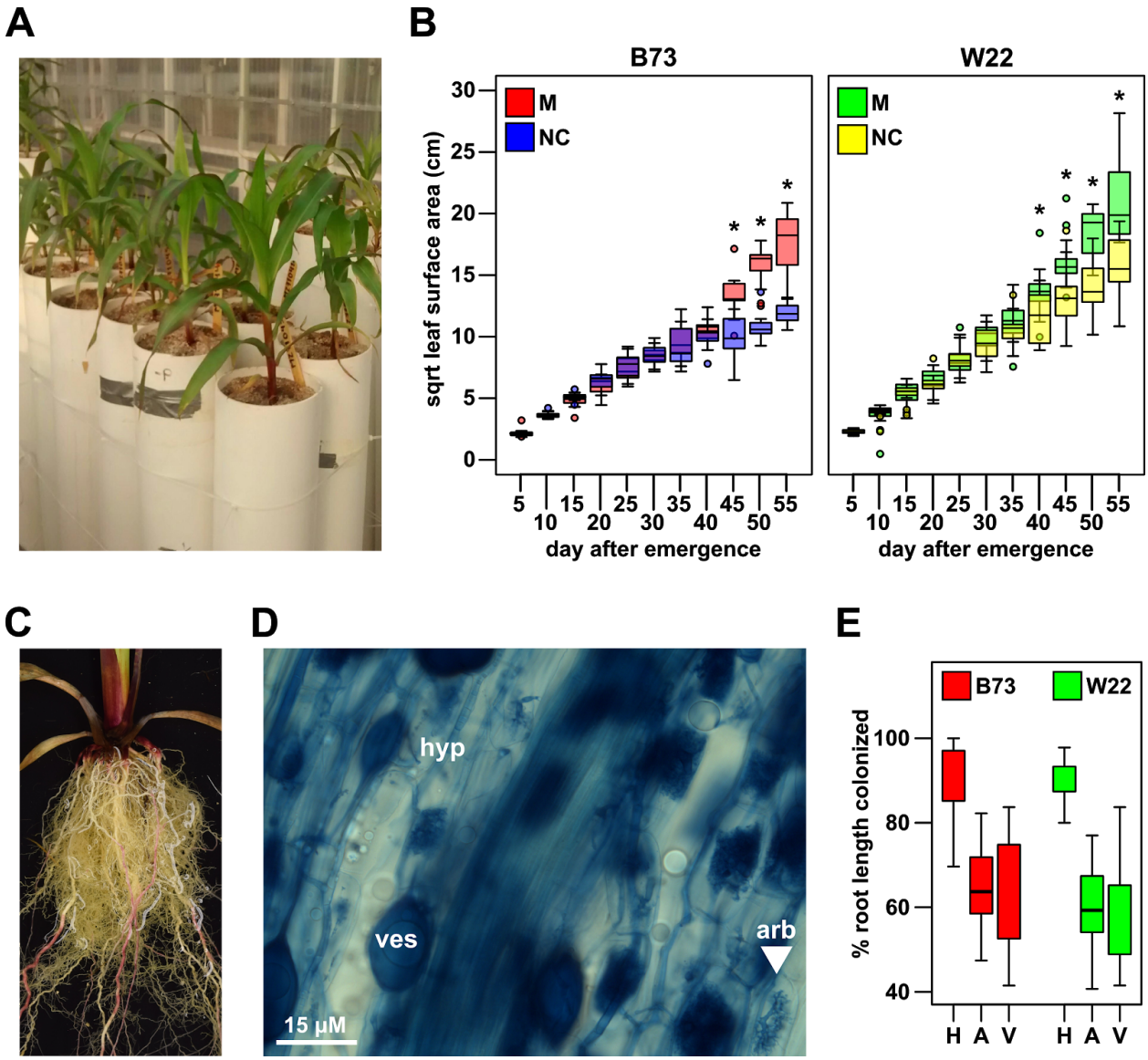
CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.



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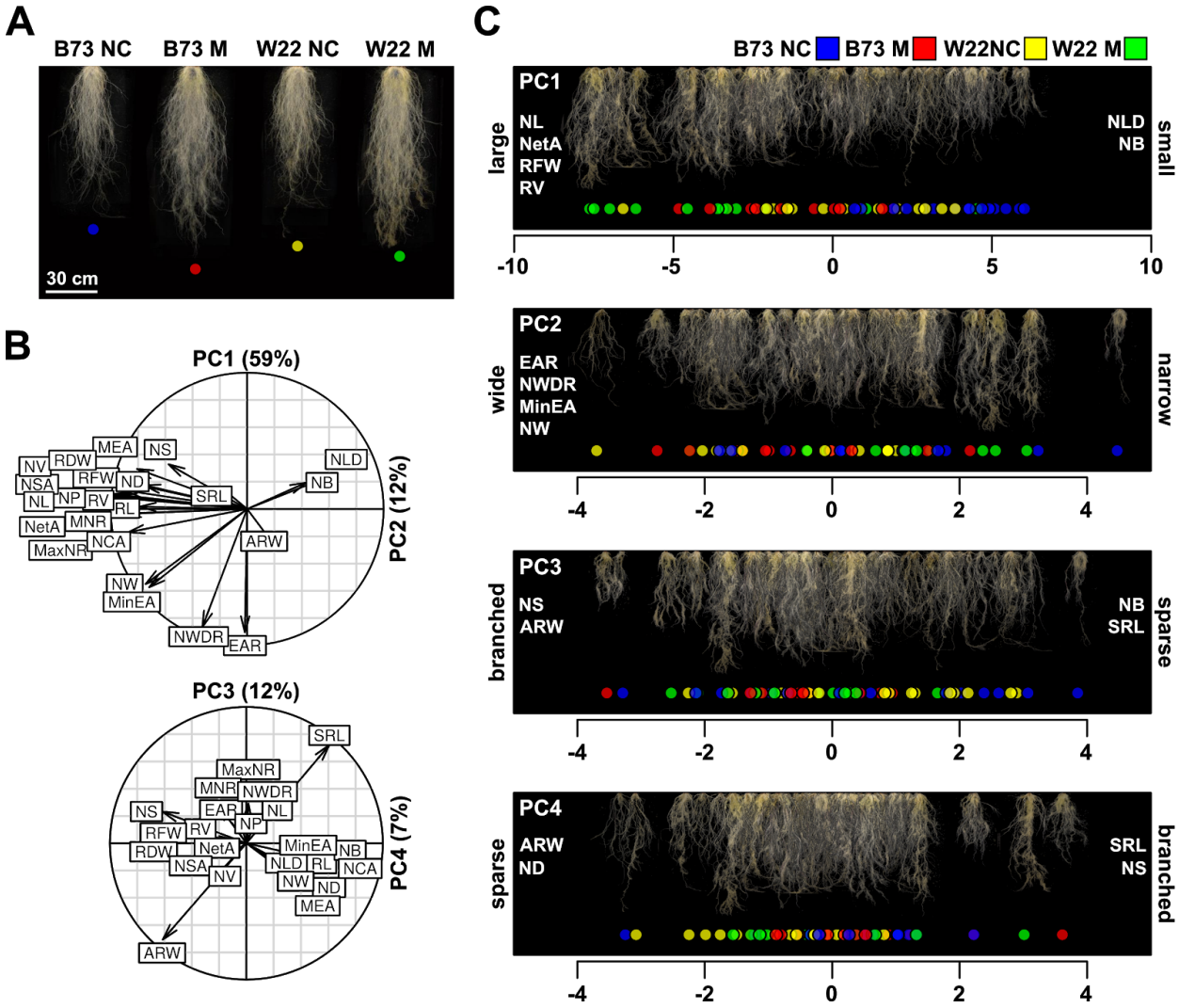

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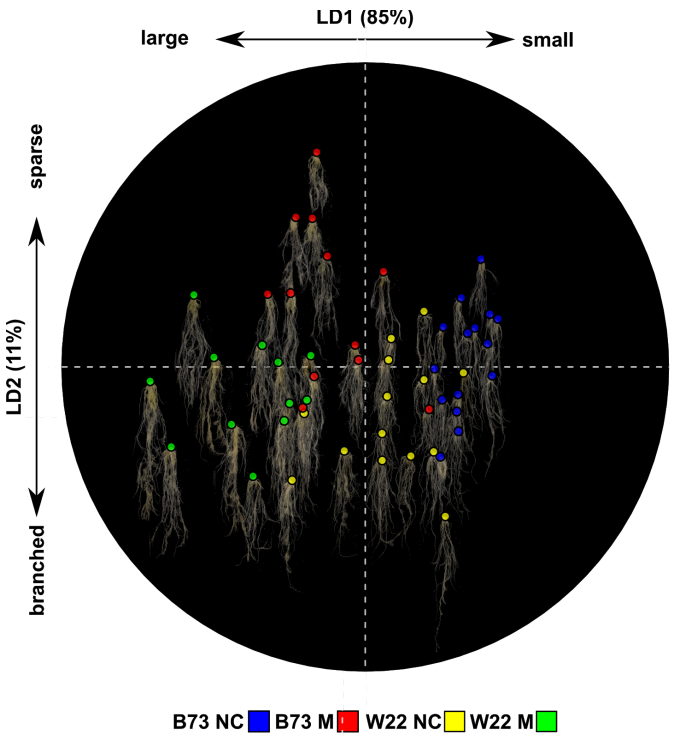

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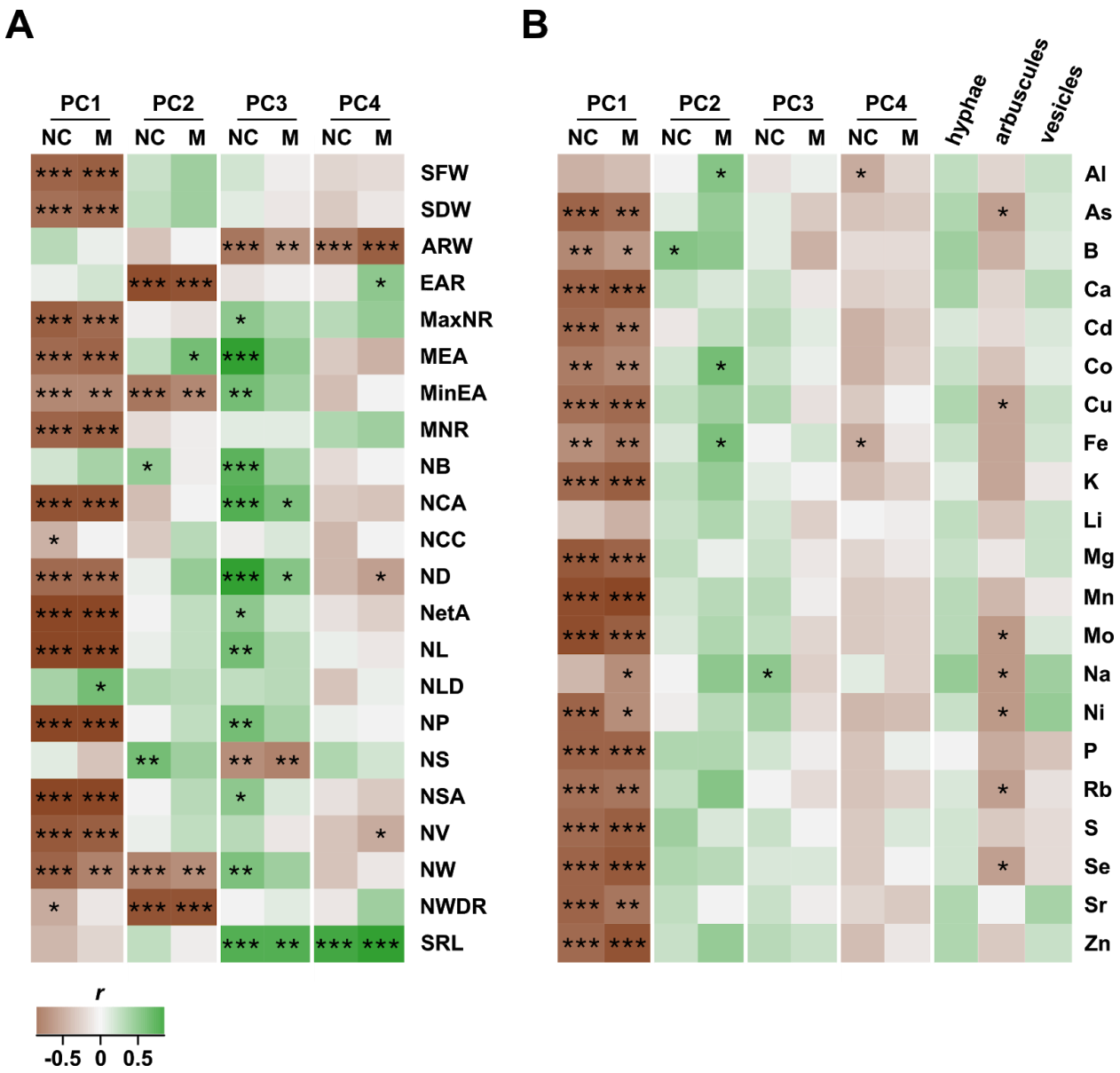

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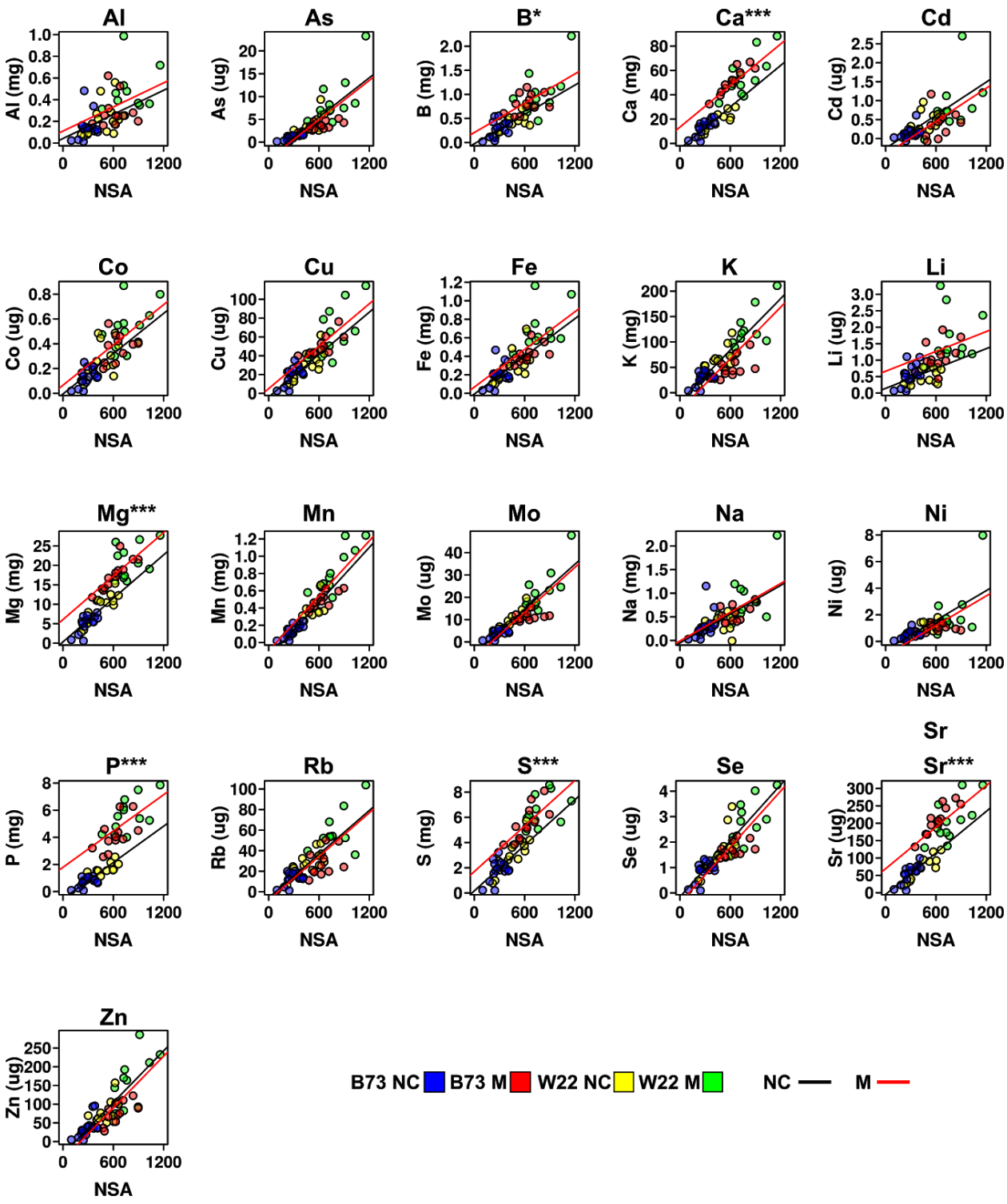

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Figure S1. Boxplots of all traits

Table S1. GiARoots trait descriptions

Table S2. Full data set

Table S3. Results of full model