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Coordinated 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*

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ABSTRACT

Arbuscular mycorrhizal symbiosis is an ancient interaction between plants and Glomeromycotan fungi. In exchange for photosynthetically fixed carbon, the fungus provides the plant host with greater access to soil nutrients via an extensive network of root-external hyphae. Here, to determine the impact of the symbiosis on the host ionome, the concentration of nineteen elements was determined in the roots and leaves of a panel of thirty maize varieties, grown with, or without, inoculation with the fungus *Funneliformis mosseae*. Although the most recognized benefit of the symbiosis to host plant growth is greater access to soil phosphorus, the concentration of a number of other elements responded significantly to inoculation across the panel as a whole. In addition, variety-specific effects indicated the importance of plant genotype to the response. Clusters of elements were identified that varied in a coordinated manner across genotypes, and that were maintained between non-inoculated and inoculated plants, even if the response itself varied in different varieties.

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INTRODUCTION

Plants require 17 essential mineral elements to complete their lifecycle, namely nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), boron (B), chloride (Cl) and nickel (Ni). Depending on their concentration in the plant, these elements can be classified as macronutrients or micronutrients. In addition, elements can be classified into four major based on their requirement for 1) synthesis of biomolecules, 2) energy transfer, 3) ion balance and 4) electron transport (Kirkby, 2012; Hawkesford *et al.*, 2012; Broadley *et al.*, 2012). As a by-product of nutrient and water acquisition, plants will also take up a number of additional non-essential elements that may, in high concentrations, be toxic, such as Aluminium (Al), Arsenic (As), Cadmium (Ca), Cobalt (Co), Selenium (Se), Strontium (Sr) and Rubidium (Rb) (Marschner, 2012). A deficiency of mineral elements has detrimental consequences on plant fitness, or in the agronomic context, crop yield, and plants have developed a number strategies to promote uptake in nutrient deficient soils and optimize the efficiency of internal use, including modification of the root system architecture, induction of high affinity transporters in roots, re-mobilisation of internal resources, growth arrest, downregulation of photosynthesis and induction of senescence (Lynch, 1995; Aibara and Miwa, 2014; Whitcomb et al., 2014). In addition, plants form mutualistic associations with rhizosphere organisms, such as nitrogen-fixing rhizobia or arbuscular mycorrhizal (AM) fungi (Bucher, 2007).

AM symbiosis is a mutualistic interaction established between soil fungi belonging to the phylum Glomeromycota and the majority (70-90%) of land plant species (Schübler *et al.*, 2001; Smith and Read, 2008). One of the major benefits of AM symbiosis to the plant host is enhanced nutrient

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uptake as the result of enhanced soil foraging by an extensive network of root-external fungal hyphae (Bago *et al.*, 2003; Finlay, 2008). An increase in P uptake in P limiting soils is well established as the primary physiological consequence of AM symbiosis on the plant host (Bucher, 2007). In addition, however, AM symbiosis may impact also the uptake of other elements, potentially increasing the uptake of essential nutrients such as N, Cu, Fe, Mn and Zn or by limiting the uptake of potentially toxic elements such as Cd, Pb, Hg and As (Göhre and Paszkowski, 2006; Smith and Read, 2008). These effects may be mediated through direct transport by the fungi, or by alterations in the root physiology that leads to alterations in the uptake of multiple elements. There is evidence for AM having effects on non-P elemental pathways in symbiotic roots. *LjSultr1;2*, a sulfate transporter, is induced in the cells surrounding the arbuscules, which suggests a possible specific role during the symbiosis and is linked to the fungal presence and not to sulfate starvation (Giovannetti *et al.*, 2014). The expression of three known Na transporters, *OsNHX3,OsSOS1* and *OsHKT2;1,* is increased in AM plants under high saline levels compared to non-AM plants. suggesting that the movement of Na out of the cytosol to apoplastic or vacuole locations is enhanced by AM (Porcel *et al.*, 2016). To better understand the impact of AM symbiosis on plant nutrition, it may be informative to consider the ionome - the total element composition - as a whole, investigating the relationships that exist between elements as a result of their interaction in soil chemistry, common uptake machinery, and the mechanisms of plant internal homeostasis (Baxter *et al*., 2008; Baxter, 2015).

Here, the concentration of nineteen elements was determined in the leaves and roots of a diverse panel of thirty maize lines grown in the greenhouse under phosphorus deficient conditions, with or without inoculation with the AM fungus *Funneliformis mosseae.* The host ionome responded broadly to AM symbiosis, reinforcing the idea that chemical elements behave as a coordinated system, rather than individual entities, when growth conditions are altered. Given the interest in the potential

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agronomic application of AM symbiosis to increase nutrient uptake, improve nutritional value, and maintain the concentrations of toxic metals at safe levels, these results provide a valuable reference dataset for further characterization of the effect of mycorrhizal colonization on the maize ionome under field conditions.

MATERIALS AND METHODS

Growth of maize diversity panel inoculated with *Funneliformis mosseae*

As described previously (Sawers *et al*., 2017), a panel of 30 diverse maize lines, comprising the 26 diverse inbred founders of the maize NAM population (McMullen *et al.*, 2009), Pa36 (a line tolerant of low P availability; Kaeppler *et al.*, 2000), and the broadly used reference lines B73 and W22, and W64A (a line used previously for study of AM symbiosis; Paszkowski *et al.*, 2006), was evaluated with (M) or without (NC) inoculation with *F. mosseae* (isolate number 12, European Bank of Glomales, http://www.kent.ac.uk/bio/beg/), as previously described (Sawers *et al*., 2017). Briefly, plants were grown in 1 L pots, in sand/clay (9:1 vol/vol), fertilized three times per week with 100 ml of modified Hoagland solution (Hoagland and Broyer, 1936) containing 10% (100µM) of the standard concentration of KH_2PO_4, the potassium concentration being maintained by addition of KCl. A total of 1200 plants (30 genotypes x 2 treatments x 6 replicates) were grown in a complete block design, over five separate plantings, at the University of Lausanne, Switzerland. Plants were harvested after 8 weeks and shoot dry weight measured (SDW). For two plantings (corresponding to six complete blocks) roots were collected also, and stained to confirm efficacy of the fungal inoculum as previously described (Gutjahr *et al.*, 2008).

Determination of elemental concentration by ICP-MS analysis

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Root and shoot samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) to determine the concentration of twenty metal ions. Weighed tissue samples were digested in 2.5mL concentrated nitric acid (AR Select Grade, VWR) with an added internal standard (20 ppb In, BDH Aristar Plus). Sample digestion and dilution was carried out as described previously (Ziegler *et al.*, 2013). Concentration of the elements B11, Na23, Mg25, Al27, P31, S34, K39, Ca43, Mn55, Fe57, Co59, Ni60, Cu65, Zn66, As75, Se82, Rb85, Sr88, Mo98 and Cd111 was measured using an Elan 6000 DRC-e mass spectrometer (Perkin-Elmer SCIEX) connected to a PFA microflow nebulizer (Elemental Scientific) and Apex HF desolvator (Elemental Scientific). A control solution was run every tenth sample to correct for machine drift both during a single run and between runs. Measurements for B11 were not considered further as concentrations were apparently below the level of reliable detection.

Statistical analysis

Least squares (LS) means (lsmeans::lsmeans; Lenth, 2016) for concentrations were obtained based on fixed effect model for each genotype x ion x inoculation x tissue (root or shoot) combination. Differences in measured traits between treatments were investigated by Wilcoxon test (stats::wilcox.test; R Core Team, 2016) for paired comparisons, and by ANOVA (stats::lm) and *post hoc* Tukey HSD test (agricolae::test.HSD, de Mendiburu, 2016) for multiple comparisons. Percentage root length colonization data was square root transformed prior to analysis. Ion concentration LS means were used to calculate separate correlation matrices for root and shoot (Hmisc::rcorr; Harrel, 2016) that were visualized as heatmaps (gplots::heatmap.2, Warnes *et al*., 2016), using the default hierarchical clustering. Principal component analysis (PCA) was performed separately for root and leaf samples, using all ion concentrations, with R statistics ade4::dudi.pca (Dray & Dufour, 2007) using centered and scaled data, and the results visualized with ade4::scatter. Only selected ions were included in the biplot.

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RESULTS

The maize ionome responds to inoculation with *Funneliformis mosseae*

To assess the impact of mycorrhizal colonization on the host ionome, root and shoot samples collected from a previously reported maize evaluation (Sawers *et al*., 2017) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). In this experiment, 30 maize inbred lines, selected to maximize genetic diversity (McMullen *et al.*, 2009), were grown with (M) or without (NC) inoculation with *Funneliformis mosseae*, under phosphorus (P) limiting conditions (Sawers *et al*., 2017). It was reported previously that plants were well colonized (about 60% of the total root length contained fungal structures, and about 30% of the total root length contained arbuscules) with an associated increase in shoot dry weight (SDW) of approximately two-fold (Sawers *et al.,* 2017). Here, in addition to the concentration of P included in the initial report, concentrations of a further 18 elements are presented. Initially, all genotypes were considered together, to generalize as to the main effect of fungal inoculation on the maize ionome (Fig.1; Table 1). Inoculation with *F mosseae* was associated with a significant increase in P concentration in both roots (Wilcoxon test, $p < 0.05$) and leaves ($p < 0.01$). In addition, in roots, a significant increase of Na ($p < 0.001$) and S ($p < 0.05$) was observed, along with a decrease of Cd, Co, Mn and Ni (all, $p < 0.001$). In leaves, there were significant increases in the concentration of Al ($p < 0.001$), Fe ($p < 0.001$) and a decrease of Zn ($p < 0.05$) concentration.

Patterns in ion concentration shift following inoculation with *F. mosseae*

To characterize relationships among ions, pairwise correlations were calculated among all 38 ion-inoculation combinations, separately for roots and leaves, across the thirty varieties evaluated (Fig. 2, 3). Correlation in the concentration of any given ion between NC and M plants ranged from 0.05 to

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0.71 in roots and 0.03 to 0.74 in leaves (Table 1). Clustering of the correlation matrix revealed covariation among ions and between M and NC treatments (Fig. 2, 3). Considering the treatments, for certain ions M and NC grouped together (e.g. K in both roots and leaves), indicating that patterns of variation among lines were maintained, while for other ions, M and NC treatments were not close in the clustering (e.g. P in both roots and leaves), indicating patterns of relative accumulation among lines to be changing under colonization. Clustering revealed also relationships among ions. The largest cluster was seen in the roots, consisting of Al, As, Co, Fe, Ni and Rb (Fig 2). Interestingly, this cluster is maintained under both NC and M treatments, although the correlations of the ions between NC and M treatments are low: *i.e.* the impact of AM symbioses on the concentration of these ions was genotype specific, such that level in NC plants did not well predict the level in M plants; and yet, whatever response did occur in a given variety was coordinated across the clustered ions.

To further investigate patterns of covariance in ion concentration, a principal component (PC) analysis was performed. Root and leaf data were analyzed separately (Fig. 4; Table 2). In both tissues, NC and M plants were partially separated on the basis of the first two PCs (representing 48% and 40% of the total variance in roots and leaves, respectively). In roots, the ions with greatest representation in PC1 were Al, As, Co, Fe, Ni and Rb (combined contribution of 71%; Fig. 5). Al, As, Co, Fe, Ni and Rb were clustered in the covariance matrix, and, as is consistent, contributed similarly to the PCs (Fig. 4; Table 2), their concentration generally decreasing in M plants. A significant reduction in Co and Ni was observed also in the single ion analysis, while median root concentrations of Al, As and Fe were reduced in M plants (Fig. 1; Table 1). Ca, Na, Mo, S, Sr and Rb contributed most to root PC2 (79% total; Fig.5), largely increasing in concentration in M plants, again consistent with the single ion analysis (Fig. 1; Table 1). Rb contributed equally to both PC1 and PC2 (7.8% and 5.9%, respectively) and showed no clear pattern with respect to inoculation (Fig. 1, Fig. 5). In leaves, PC1 and PC2 were

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predominantly represented by Ca, Mg, Mn, Mo, Sr and S (70%), and Al, As, Co, Fe, Ni and P (74%), respectively (Fig. 5; Table 2). NC and M treatments were best distinguished by PC2, generalized by an increase in the leaf concentration of Al, As, Co, Fe, Ni and P in M plants that was consistent with the single ion analysis (Fig. 1; Table 1). The contribution of P was notably low, and in a direction opposite to the ions making the greatest contributions (Fig. 4), reflecting the negative correlations observed between concentrations of P and these ions, not only between treatments, but among genotypes within a single treatment (Fig. 3, S1, S2).

Differences in the ionome response to AM inoculation among maize varieties indicates the importance of host genotype

To investigate further the importance of plant genotype on the response to inoculation with *F. mosseae*, the change in ionomic PC scores between NC and M treatments was compared (Fig. 5). Reaction norm plots illustrated the main effect of inoculation in roots and shoots, but revealed also evidence of genotype specific differences (indicated by non-parallel lines in Fig. 5). As observed in PC biplots (Fig. 4), the difference between NC and M treatments was best captured by PC2, in both roots and leaves. In roots, there was a clear trend towards an increased PC2 score in inoculated plants, related to increasing concentrations of Ca, Na, Mo, S, Sr and Rb; in leaves the trend was towards a lower score in PC2, related to increasing concentrations of Al, As, Co, Fe, Ni and P. A number of lines, however, did not follow these general trends: in roots CML247, Ki3, M162W, M37W, Oh7b and Pa36 showed a reduction in PC2 when inoculated; in leaves, the lines B97, CML52, CML103 and CML247 showed an increase in PC2 when inoculated. These lines were not found to be exceptional with regard to growth response in the previous analysis (Sawers *et al*., 2017), although it should be noted that P, the limiting nutrient, made only minor contributions to these PCs. As would be predicted by the clustering results

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(Fig. 2, 3), there were instances in which genotype specific effects were correlated among ions, well illustrated by the behavior of Al and Fe in the roots (Fig. 6)

DISCUSSION

Measurement of the concentration of nineteen ions in the leaves and roots of maize seedlings grown with or without inoculation with the AM fungus *Funneliformis mosseae* revealed coordinated changes in response to AM colonization. By using a panel of maize varieties designed to maximize genetic diversity, it was possible to both make meaningful generalizations and to examine patterns of covariation in ion concentration. Analysis of single ions and PCA indicated AM colonization to be associated with an increase in the root concentration of Ca, Na, Mo, P, Rb, S and Sr, and a decrease in Cd, Co, Cu, Mn , Ni and Zn (Fig. 1, 4, 5; Table 1). In the leaves, similar analyses revealed an increase in Al, As, Co, Fe, Na, Ni and P, and decrease of Mn and Zn in colonized plants (Fig. 1, 4, 5; Table 1). Our analysis cannot distinguish between elements in the root and elements stuck to the surface of the roots, however, since there was a large overlap between the elements changing in the root and shoot, it is likely that the changes observed in the root reflect meaningful alterations in the uptake of those elements. In single ion analysis, the concentrations of Mn, Na, Ni, P and Zn responded ($p < 0.05$) similarly to AM colonization in both roots and leaves, with Ni the only ion for which the sign of the response differed between the two tissues (Fig. 1; Table 1). Although P was limiting in the experiment, and plant concentrations were at deficient levels (Reuter and Robinson, 1997), the P response to AM inoculation was far from the most significant change to the ionome, nor did P contribute greatly to PCs (Fig. 1, 5; Table 1).

One of the most significant ($p < 0.001$) changes in the ionome of AM plants was the reduction in root concentration of Co and the potentially toxic, non-essential heavy metal Cd (Fig. 1; Table1).

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Although Co and Cd concentrations were relatively low in both NC and M plants in this experiment, these data are consistent with the previously reported role of AM fungi in protecting the host plant from accumulation of toxic elements (Göhre and Paszkowski, 2006). Mn concentration was also lower, although non-limiting, in M plants, both in roots and leaves (Fig. 1; Table 1). Reduced Mn accumulation in M plants has been reported previously in maize and other plants, and attributed to reduced plant production of P-mobilizing carboxylates (e.g. Kothari et al., 1991; Posta et al., 1994; Nazeri et al., 2013; Gerlach et al., 2015). Genotypes varied in Mn accumulation, and a negative correlation ($r = -0.35$, $p = 0.06$; Fig. S1) was observed between P and Mn accumulation in the leaves of M plants. Measurement of the leaf Mn concentration has been proposed as a method of distinguishing different strategies of P acquisition at higher taxonomic levels (Lambers et al., 2015). Species that favor mycorrhizal associations will tend to accumulate less Mn in the leaves while species favoring carboxylate exudation as an strategy for P acquisition will tend to accumulate more Mn in the shoots due increased, carboxylate-induced mobilization of Mn from the rhizosphere that facilitating Mn uptake and shoot translocation. Our data indicate that there can also be intraspecific correlations between Mn and P concentrations in M plants and that this variation could be used for further mapping strategies to identify P deficiency strategies in maize. A similar, although more marked, negative correlation ($r = -0.62$, $p < 0.01$; Fig. S2) was observed between P and Mg in the leaves of M plants. Notably, when all genotypes were considered together, Mg concentration was not responsive to AM colonization (Fig. 1; Table 1). No correlation was observed between P and Mg in the leaves of NC plants (Fig. S1). A positive correlation ($r = 0.47$, p , 0.01; Fig. S3) between P and Mg in the roots was specific also to M plants (Fig. S3). Such relationships among ions illustrate more subtle effects of AM symbiosis on the host ionome beyond increase or decrease in the median concentration. Cluster analysis of pairwise correlations revealed further groups that varied together across genotypes (Fig. 2,

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3). A number of clusters were common to NC and M plants, although the correlations between the two treatments were not strong, indicating a coordinated but genotype specific response: *i.e.* responses differed, but for a given genotype, the ions in a cluster responded in a similar way, a pattern well illustrated by concentrations of Al and Fe in the roots (Fig. 6).

In contrast to controlled environment, experimental systems, field soils present a far more complex range of physicochemical properties, promoting correlation and interaction in the availability of nutrients to plants. For example, P deficiency is often accompanied by insufficiency of other nutrients, such as Ca, Mg and Zn in acid soils, Fe and Zn in alkaline conditions (Calderón-Vázquez *et al.*, 2009; Hinsinger, 2001; Osaki *et al.*, 1999; Uexküll and Mutert, 1995). Water content will impact also nutrient uptake, the uptake of K and P being strongly affected, while that of other nutrients, such as Ca and Mg, less so, which may even be increased (Talha *et al.*, 1979). Although best characterized for an effect on P nutrition, it is clear from results presented here and elsewhere that AM symbiosis has a broad impact on the host ionome. Although the quantity of a given element delivered directly by the fungus to the host is not quantified here, genotypic specific responses are consistent with variation in symbiotic function, in a form analogous to that reported previously for P uptake in this same panel of maize varieties (Sawers *et al*., 2017). Similarly, genotype specific responses to AM colonization in the absence of a panel-wide change in the average concentration of certain elements (e.g. Al and Fe in roots; Fig. 6) suggest a "hidden" impact on the host ionome, whereby although concentrations may not change, the contribution of AM symbiosis may be significant - as has been shown previously with respect to P acquisition (Smith *et al*., 2003).

These data also demonstrate the utility of considering the total elemental composition, the ionome, as a readout of the interaction between the plant, coded by its genome, and its environment. Although we only used a single AMF inoculant, we observed dramatic differences in the nutrient

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uptake response between genotypes. While our experiments did not have the power to disentangle the underlying physiological parameters, they indicate that the ionome contains a signal of the different responses that AM symbiosis induces in different genotypes. The root ionome as well as the characterization of the extent and type of AMF colonization are difficult phenotypes to measure in the field, but the leaf ionome is both relatively stable and relatively inexpensive to measure. Our results suggest that the leaf ionome could be a useful phenotype for understanding the complicated interaction between AMF and their host plants by providing readouts indicative of the biological processes occurring in the roots as it has been suggested by others (Lambers *et al.* 2015).

In summary, we provide evidence of AM symbiosis induced changes in the ionome of shoots and roots across a wide range of maize inbreds. AM symbiosis can be considered as a particular environmental modification that affects root function including the ability of roots to take up both beneficial and/or toxic elements.

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Table 1. Concentration of twenty ions in the roots and leaves of maize plants with and without inoculation with *Funneliformis mosseae.* Marginal mean (Conc; ppm) and standard error (SE) for the concentration of twenty ions in the roots and leaves of non-inoculated (NC) and inoculated (M) plants, calculated across thirty genotypes. r, Pearson correlation coefficient between NC and M plants. p, P-value from Mann-Whitney test for equivalent concentration in NC and M plants.

	Root						Leaf					
	NC		M				NC		M			
Ion	Conc (ppm)	SE	Conc (ppm)	SE	r	p	Conc (ppm)	SE	Conc (ppm)	SE	r	p
Al27	1500	100	1300	72	0.09	0.317	36	1.7	48	2.3	-0.27	0.001
As75	2.4	0.13	2.2	0.085	0.14	0.263	0.35	0.011	0.36	0.0094	0.46	0.539
Ca43	6300	320	7000	380	0.12	0.112	5500	350	5100	290	0.6	0.615
Cd111	0.68	0.02	0.56	0.014	0.36	0.000	0.36	0.01	0.35	0.0086	0.28	0.226
Co59	3.9	0.15	2.7	0.099	0.7	0.000	0.085	0.0059	0.091	0.0059	0.5	0.160
Cu65	24	0.97	21	1	0.53	0.036	15	0.83	18	1.6	0.32	0.173
Fe57	3300	240	3000	150	0.14	0.684	110	4	140	6	0.08	0.001
K39	21000	840	22000	670	0.56	0.260	28000	1400	26000	1100	0.49	0.343
Mg25	3200	130	3100	110	0.71	0.976	3200	160	3000	120	0.57	0.491
Mn55	240	17	140	6.8	0.42	0.000	82	6.3	64	3.6	0.7	0.047
Mo98	4.1	0.31	4.1	0.21	0.6	0.796	4.9	0.49	4.5	0.44	0.8	0.631
Na23	1400	91	1900	82	0.6	0.000	260	27	330	29	0.35	0.043
Ni60	8.8	0.32	7.5	0.22	0.37	0.005	0.58	0.03	0.71	0.049	0.02	0.006
P31	300	13	340	13	0.05	0.041	390	16	450	16	0.09	0.005
Rb85	3.2	0.12	3.3	0.092	0.13	0.296	1.7	0.074	1.8	0.089	0.58	0.277
S34	5500	250	6400	270	0.55	0.011	2100	53	2200	42	0.29	0.361
Se82	0.59	0.24	0.48	0.15	0.19	0.906	0.31	0.089	0.018	0.11	0.22	0.086
Sr88	40	1.2	43	1.6	0.22	0.042	30	2.2	26	1.6	0.74	0.407
Zn66	85	3.8	74	3.1	0.21	0.032	46	1.9	39	1.2	0.63	0.017

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Table 2. Principal component analysis of the concentration of nineteen ions in roots and leaves. Scores of ions on the first three principal components (PCs) in root and leaf analysis. Coordinates were scaled x10 and rounded to two decimal places. Var, the percentage of variance associated with each PC.

	Root			Leaf		
	PC1	PC2	PC3	PC1	PC2	PC3
Var	31%	17%	12%	26%	14%	10%
Al27	-9.12	1.48	-2.6	-1.28	-8.01	-0.47
As75	-8.99	1.71	-1.83	-6	-3.88	1.27
Ca43	-1.97	8.21	-0.66	-8.32	2.5	-0.94
Cd111	-5.3	-1.65	7.17	-2.53	-2.06	-2.79
Co59	-8.16	-2.26	3.5	-4.05	-5.48	0.78
Cu65	-4.16	-3.04	-0.53	-3.74	-3.16	-2.43
Fe57	-8.93	1.56	-3.19	-2.97	-7.47	0.38
K39	2.61	2.74	2.54	0.81	3.44	7.28
Mg25	-1.87	3.24	2.86	-8.41	0.95	2.04
Mn55	-6	-1.47	5.84	-7.96	2.48	-0.67
Mo98	1.33	4.67	5.95	-6.35	0.63	-0.71
Na23	4.71	5.73	-1.88	-3.69	-1.69	5.23
Ni60	-9.02	0.58	0.7	1.2	-4.84	-0.97
P31	-3.13	2.8	-1.27	3.36	-3.59	1.75
Rb85	-6.85	4.42	-3.9	3.59	-0.04	6.23
S34	2.35	6.91	4.04	-5.88	-1.79	5.81
Se82	-1.37	1.73	3.88	0.47	-0.15	1.38
Sr88	-1.14	8.45	-0.41	-7.58	3.08	-1.57
Zn66	-4.42	-3.64	-0.01	-6.03	3.4	-0.43

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Fig. 1. Element concentration responds to inoculation with *Funneliformis mosseae*. Concentration (ppm) of nineteen elements in the roots of non-colonized plants (yellow), the roots of colonized plants (brown), the leaves of non-colonized plants (pale green) and the leaves of colonized plants (dark green) determined by inductively coupled plasma mass spectrometry. 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 not shown. Ions for which accumulation differed significantly (Wilcoxon test) between NC and M plants indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

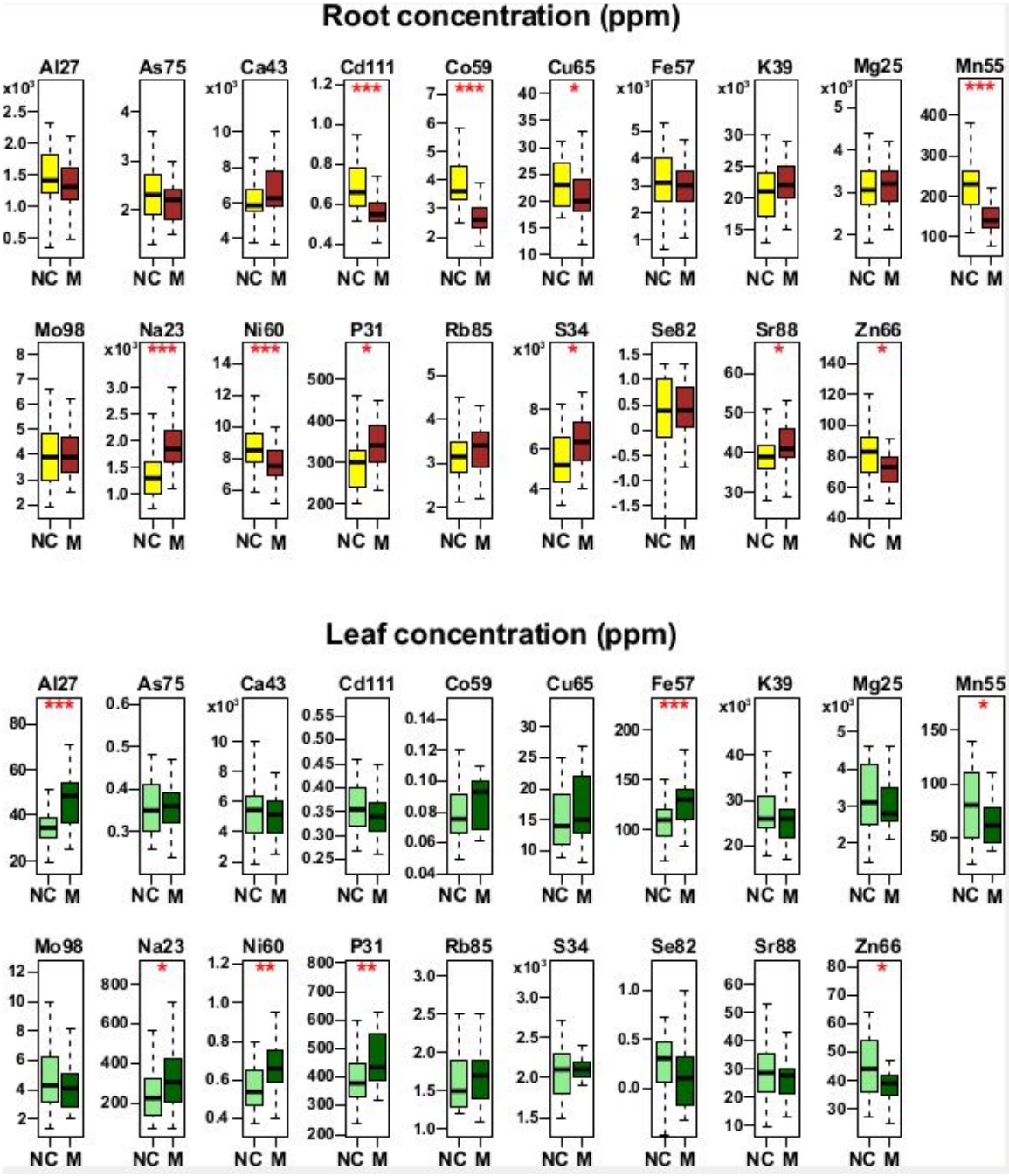

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Fig. 2. Patterns of ion concentration in roots shift following inoculation with *Funneliformis mosseae.* Pairwise correlation of ion concentration (colour-coded square) in the roots of thirty maize varieties grown with (indicated by red point adjacent to ion name) or without (indicated by blue point) inoculation with *F. mosseae* . The forty ion x inoculation combinations are clustered hierarchically. Nodes are marked with a red or blue point to indicate all adjoining lower order nodes to share the same inoculation status.

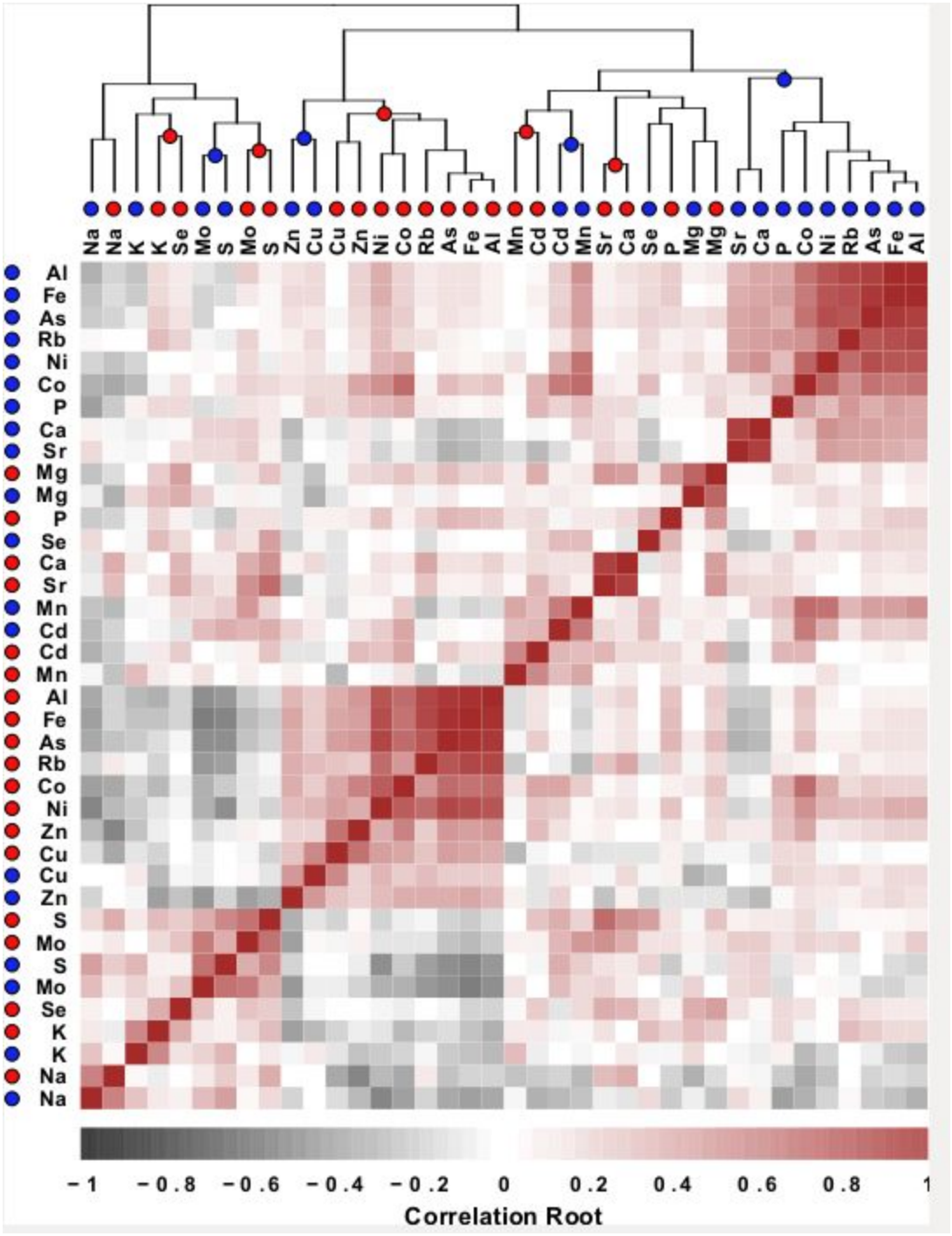

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Fig. 3. Patterns of ion concentration in leaves shift following inoculation with *Funneliformis mosseae.* Pairwise correlation of ion concentration in the leaves of thirty maize varieties grown with or without inoculation with *F. mosseae* . Data represented as Fig. 2.

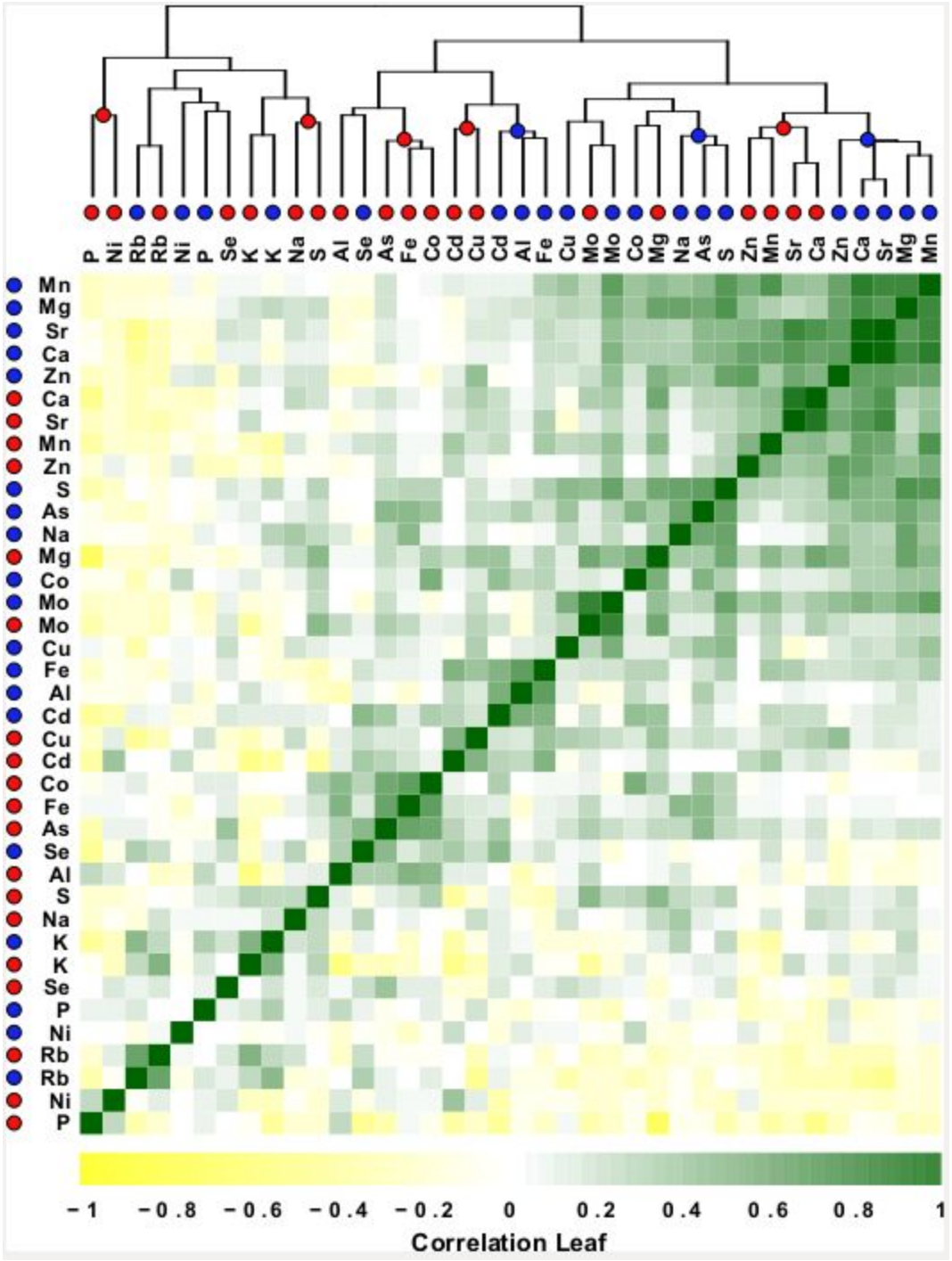

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Fig. 4. Inoculation with *Funneliformis mosseae* impacts the root and leaf ionomes. Differentiation of the mycorrhizal and non-mycorrhizal ionome. Principal component (PC) analysis of the concentration of nineteen ions in the roots and leaves of the thirty maize varieties (points) grown with (M) or without (NC) inoculation with *F. mosseae.* Biplot showing scores in the first two principal components (PC1: x-axis, PC2: y-axis). The sign and magnitude of the contribution of selected ions is shown by arrows. Ions are shown using conventional element coloring.

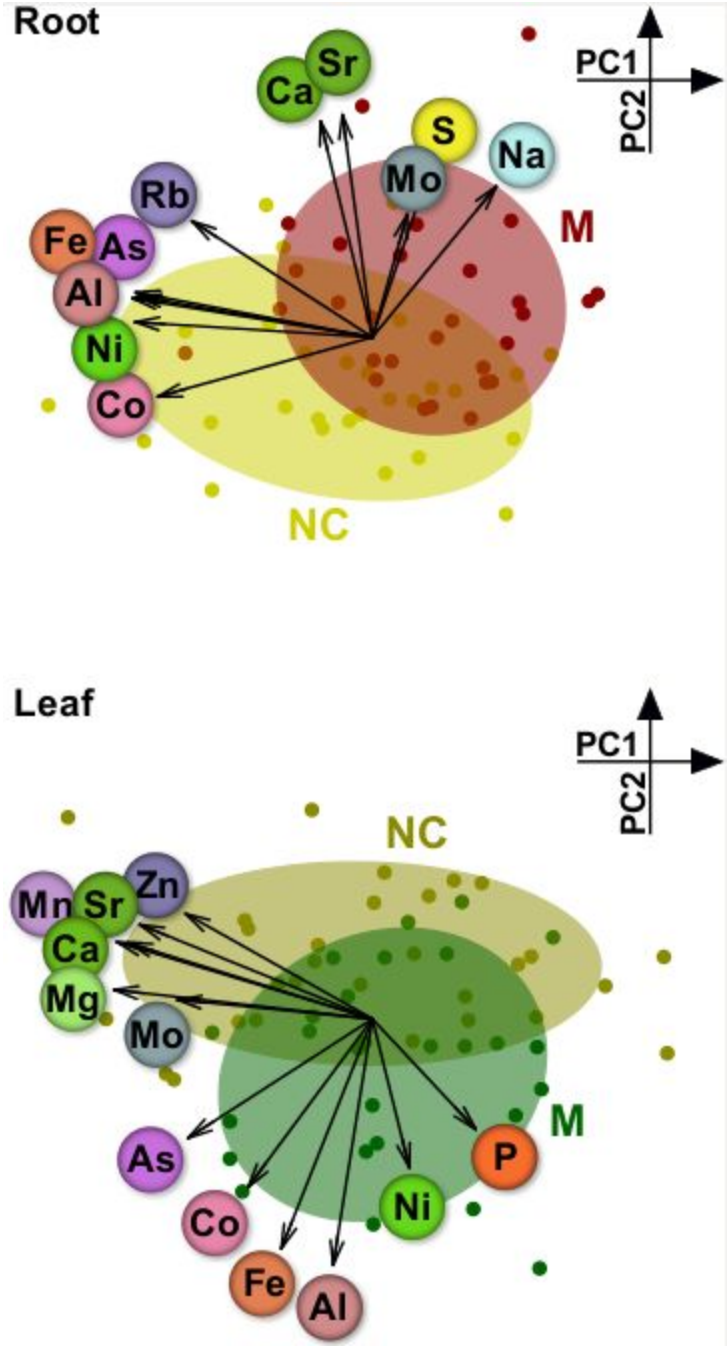

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Fig. 5. Ionome responses to inoculation with *Funneliformis mosseae* are dependent on plant genotype. Principal component (PC) coordinates for thirty maize inbred lines, for the first two PCs in analysis of the root and leaf ionomes. For each PC, the upper panel indicates the total contribution of the PC, along with the contributions of the six most important ions to that PC. Bars are filled using conventional coloring. For each genotype, the coordinates in a given PC are shown for non-inoculated (NC, blue points) and inoculated (M, red points) plants, linked by a line segment indicating the reaction norm (a plot of phenotype against environment, here contrasting NC and M). Coordinate units are arbitrary and scaled differently in the four panels, zero indicated by a red dashed line. Arrowheads in the top left of each coordinate panel indicate the direction of increasing concentration of the associated ions with reference to the y axis. Lower case letters indicate tightly clustered groups of genotypes that could not be clearly labeled and that are consequently presented in the corners of the relevant panels.

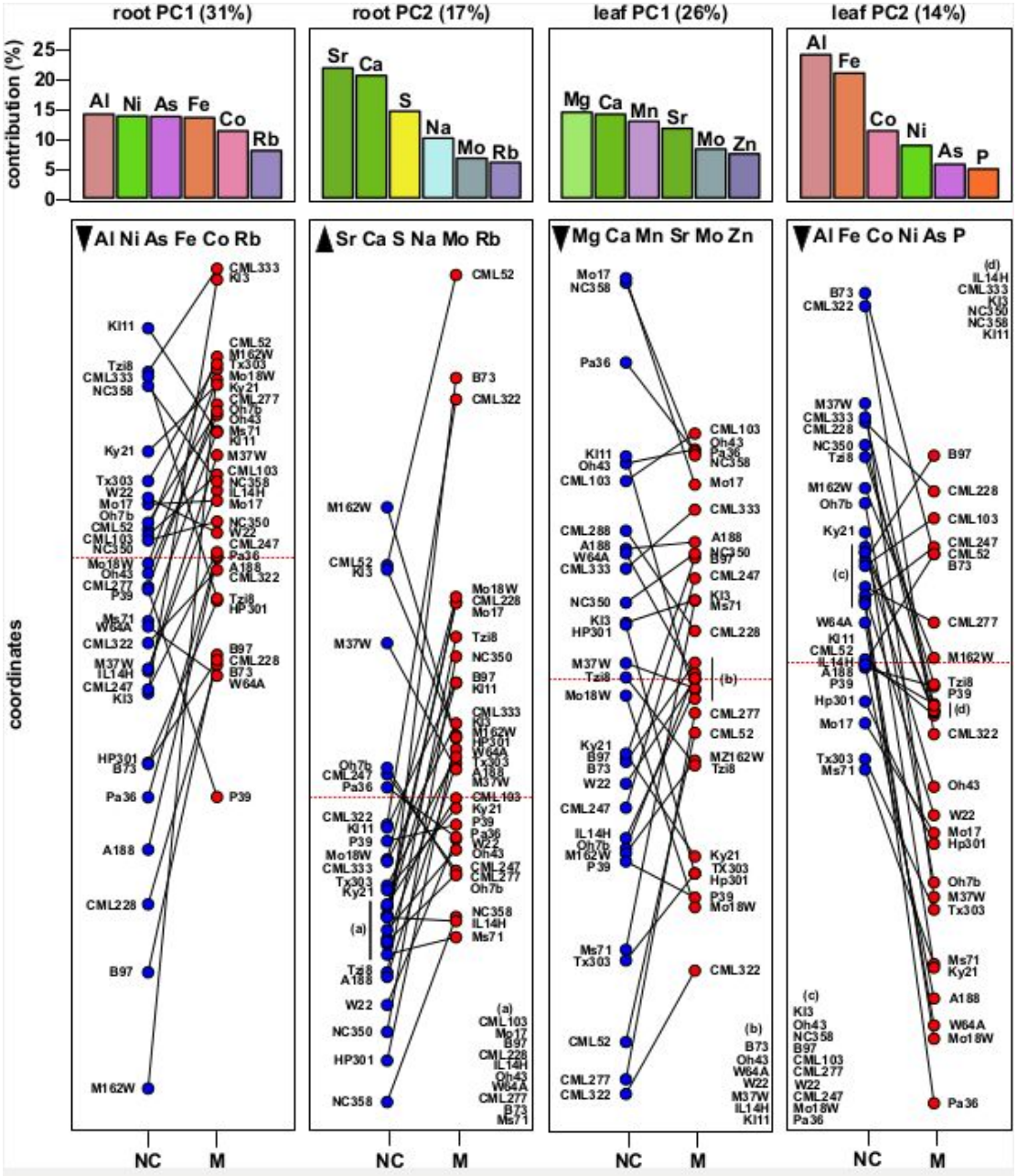

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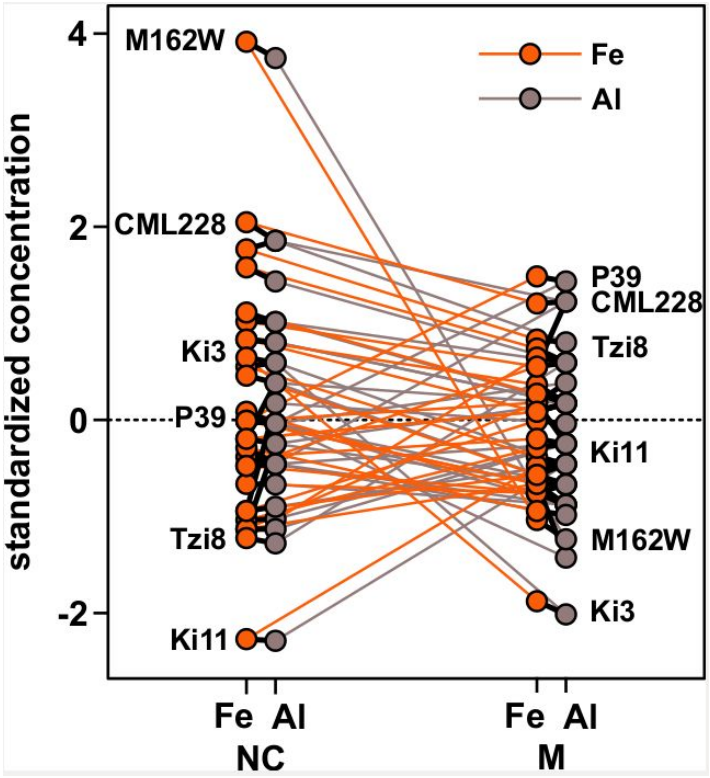
Fig. 6. Root Fe and Al responses to AM colonization are correlated across genotypes. Standardized concentrations (Z score) of Fe and Al in the roots of thirty maize varieties, grown with (M) or without (NC) inoculation with *Funneliformis mosseae.* The reaction norm (plot of phenotype against environment, here contrasting NC and M) for each ion-genotype combination is shown by a colored line. Concentrations of the two ions for each genotype within a treatment are connected by a black line. Selected varieties are labelled.



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Supplementary Figures

Fig. S1. P and Mn concentrations in plants with and without inoculation with *Funneliformis mosseae.* A - D concentrations (ppm) of P31 and Mn55 in the roots and leaves of non-colonized (NC) plants and plants inoculated with *F. mosseae*. Points represent mean values for each of thirty maize varieties.

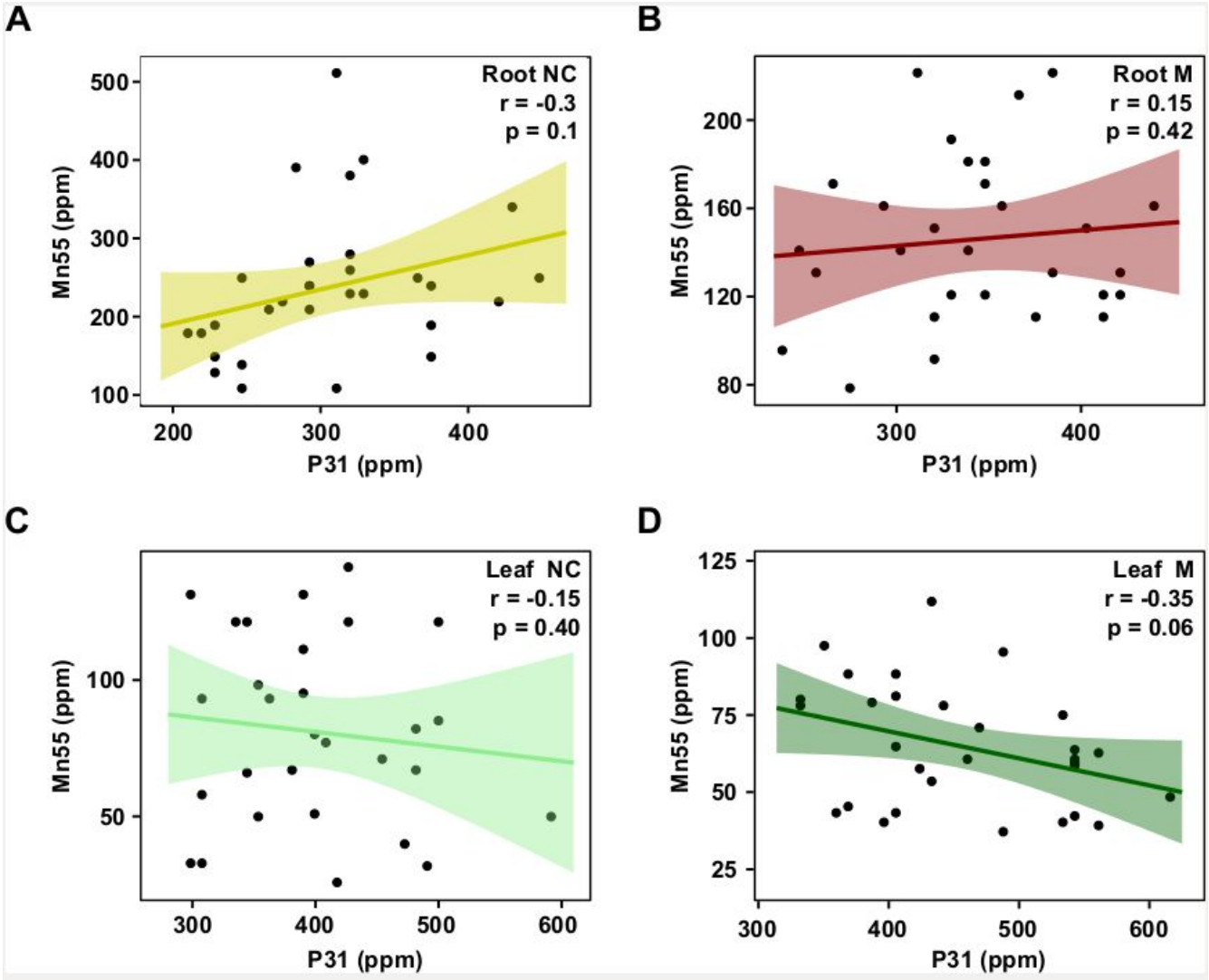

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Fig. S2. P and Mg concentrations in plants with and without inoculation with *Funneliformis mosseae.* A - D concentrations (ppm) of P31 and Mg25 in the roots and leaves of non-colonized (NC) plants and plants inoculated with *F. mosseae*. Points represent mean values for each of thirty maize varieties.