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Research

Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species

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Summary

- The arbuscular mycorrhizal (AM) symbiosis is key to plant nutrition, and hence is potentially key in sustainable agriculture. Fertilization and other agricultural practices reduce soil AM fungi and root colonization. Such conditions might promote the evolution of low mycorrhizal responsive crops. Therefore, we ask if and how evolution under domestication has altered AM symbioses of crops.
- We measured the effect of domestication on mycorrhizal responsiveness across 27 crop species and their wild progenitors. Additionally, in a subset of 14 crops, we tested if domestication effects differed under contrasting phosphorus (P) availabilities.
- The response of AM symbiosis to domestication varied with P availability. On average, wild progenitors benefited from the AM symbiosis irrespective of P availability, while domesticated crops only profited under P-limited conditions. Magnitudes and directions of response were diverse among the 27 crops, and were unrelated to phylogenetic affinities or to the coordinated evolution with fine root traits.
- Our results indicate disruptions in the efficiency of the AM symbiosis linked to domestication. Under high fertilization, domestication could have altered the regulation of resource trafficking between AM fungi and associated plant hosts. Provided that crops are commonly raised under high fertilization, this result has important implications for sustainable agriculture.

Introduction

The arbuscular mycorrhizal (AM) symbiosis is the most widespread mycorrhizal association (Smith & Read, 2008); 70–80% of land plant species harbor AM fungi in their fine roots, including the vast majority of crops (Hamel, 1996). AM fungi supply mineral nutrients, especially phosphorus (P), and receive carbohydrates from the host plant (Redecker *et al.*, 2000). Moreover, AM fungi provide other benefits to host plants, which are important to agriculture, such as increased protection against pathogens (Newsham *et al.*, 1995). However, despite the global importance of AM in agriculture, we still know little about if and how the AM symbiosis was altered by plant domestication, crop breeding, and agricultural environments. Such knowledge is critical for plant breeding programs aimed at delivering crop genotypes that are less dependent on the input of fertilizers (Wissuwa *et al.*, 2009; Kiers & Denison, 2014; Rillig *et al.*, 2016; Thirkell *et al.*, 2017).

To investigate the shifts in AM interaction experienced by crops, we first need to define how plants and fungi react to the symbiosis. Mycorrhizal response is the intensity of the response of plants to AM colonization. Mycorrhizal growth response (MGR) and mycorrhizal P response (MPR) are the effects of AM on plant growth and P concentration, when compared with plants prevented from establishing the symbiosis. MGR and MPR vary from positive to negative, depending on whether plant performance improves or diminishes in the presence of AM fungi. The intensity and direction of mycorrhizal response are primarily driven by the identities of the host plant and the fungal symbiont, and by soil fertility (Johnson *et al.*, 1997; Klironomos & Hart, 2002; Hoeksema *et al.*, 2010; Johnson, 2010). On the plant side, mycorrhizal response is often associated with root morphological traits that influence nutrient uptake ability, such as root length, root diameter or root hair length (Tawaraya, 2003; Comas & Eissenstat, 2009; Kramer-Walter *et al.*, 2016). Specifically, thick and poorly branched roots, with limited ability to explore the soil, are often more responsive to AM symbiosis (Baylis, 1975; Hetrick *et al.*, 1991; Newsham *et al.*, 1995; Comas *et al.*, 2014). On the fungal side, mycorrhizal response is influenced by the cooperativeness of the AM fungal partner (Chagnon *et al.*, 2013; Werner & Kiers, 2015; Argüello *et al.*, 2016). The cooperativeness of AM fungi is determined by the carbon demands from host plants, P allocation to roots and colonization rates (Hart & Reader, 2002; Chagnon & Bradley, 2013).

Frequently, AM fungi receive more carbohydrates from the host than P transferred by them. Nevertheless, host plants and AM fungi are able to regulate the resources allocated to each other in response to the amount of resources received from the partner (West *et al.*, 2002; Grman, 2012). Thus, several plant species have been reported to reward those AM fungal strains that provide more nutritional benefits to the plant (Bever *et al.*, 2009; Kiers *et al.*, 2011; but see Hoeksema *et al.*, 2010; Werner & Kiers, 2015), and to constrain carbon allocation to AM fungi if the strains are less beneficial (Kiers *et al.*, 2011). Finally, mycorrhizal response is also influenced by environmental variation. In particular, P availability is considered the major driver of plant mycorrhizal response (Johnson, 2010). High soil P availability decreases AM fungal colonization and mycorrhizal benefits (Mäder *et al.*, 2000; Treseder, 2004), sometimes eliciting an antagonistic behaviour of AM fungi (Johnson, 1993). In summary, from a plants' perspective, the symbiosis with AM fungi ranges from beneficial to parasitic relationships, depending on the interplay of involved AM fungi, plant hosts and environmental context (Johnson, 2010).

The diversity and abundance of AM fungi in agricultural soils tend to be low compared with more natural ecosystems (Helgason *et al.*, 1998; Oehl *et al.*, 2010; Verbruggen *et al.*, 2010). Agricultural practices such as tillage, monocropping, or high rates of fertilization hinder the proliferation of hyphae and diminish the functionality of the AM symbiosis (Johnson & Pfleger, 1992; Mäder *et al.*, 2000; Oehl *et al.*, 2003; Tawaraya, 2003; Verbruggen & Kiers, 2010). Moreover, the majority of AM fungi thriving in agricultural soils might have traits that are less beneficial to plants than strains from wild habitats (Verbruggen & Kiers, 2010). For instance, AM fungi typical of agricultural soils tend to show high reproductive output, probably at the expense of providing inputs to the plant host (Chagnon *et al.*, 2013). From an evolutionary perspective, some selective forces that are common in many domestication processes could have promoted less mycorrhizal plants during domestication. For instance, selection for higher yielding plants would have imposed limits on the amount of carbohydrates moved below ground and therefore the resources available for the symbiosis would decrease, discouraging AM fungi from root colonization. Moreover, crops have been raised under high-fertilization agricultural environments, which might deter plants from investing carbon in nutrient acquisition via fungal symbionts (Johnson, 1993; Mäder *et al.*, 2000; Nijjer *et al.*, 2001). Therefore, in light of the lower diversity and abundance, colonization ability, and mutualistic performance of agricultural AM fungi, it might be expected that mycorrhizal response had swung towards a less mutualistic relation during crop domestication (Johnson & Pfleger, 1992; Tawaraya, 2003). In fact, AM colonization tends to decrease in domesticated varieties when compared with landraces or wild varieties in crops such as wheat (*Triticum*) (Hetrick *et al.*, 1993) and sunflower (*Helianthus annuus*) (Turrini *et al.*, 2016). Additionally, in a few crops, mycorrhizal response has been reported to decrease alongside domestication (wheat: Manske, 1989; Hetrick *et al.*, 1993; maize (*Zea mays*): An *et al.*, 2010; tomato (*Solanum lycopersicum*): Bryla & Koide, 1998). However, lower MGR among domesticated plants does not hold in other crops, such as barley (*Hordeum vulgare*) (Baon *et al.*, 1993). Although case studies have provided some useful insights, a broader understanding of the evolution of the AM symbiosis in crops is missing.

In this work, we investigated whether domestication leads to the evolution of reduced mycorrhizal response of crop plants as compared with their wild progenitors across 27 independent domestication events. Specifically, we asked the following questions: have mycorrhizal colonization and mycorrhizal responsiveness decreased during crop domestication; do crops react differently to the presence of AM fungi in P-rich and P-poor environments; and have root morphology and mycorrhizal traits evolved in a coordinated fashion during domestication?

Materials and Methods

We grew 27 plants of domesticated crops and of each of their wild progenitors in sterilized conditions, and provided half of the replicates with a common AM fungi inoculum (*extensive experiment*). We then measured AM colonization, above-ground biomass and leaf P concentration in response to the presence of AM fungi. Additionally, in a subset of 14 crops we fertilized plants with two nutrient solutions differing in P concentration to measure the reaction to P availability (*fertilization experiment*). Methodological details about the fine root trait data are explained in Supporting Information Methods S1. We analysed whether domestication, fertilization, and their interaction changed AM colonization and mycorrhizal response, and whether variation in these changes is explained by shifts in root morphology, using generalized linear mixed-effects models and phylogenetic generalized least-squares models.

Study system and experimental design

We selected 27 herbaceous crops (Table 1) comprising the most important families of agricultural herbaceous crops, and representing a broad range of variability in domestication processes. Representatives of Brassicaceae and Amaranthaceae, known to avoid root colonization by AM fungi (Wang & Qiu, 2006; Brundrett, 2009), were included in the experiment because of their agronomic relevance, and because the presence of AM hyphae in the soil, or adjacent mycorrhizal plants, can affect the performance of 'nonmycorrhizal' plants (Lekberg & Koide, 2005; Veiga *et al.*, 2013). For each crop, we obtained seeds of two accessions: one representative of a domesticated genotype, and another of its recognized wild progenitor (Table 1). Detailed information about the criteria for assigning wild progenitors, or seed accessions identifiers and seed donors are in Table S1.

In order to address our questions, we conducted two glasshouse experiments. To address the first question about generalized domestication effects on mycorrhizal response, we conducted an *extensive experiment* with the whole set of 27 crops (Table 1), including the following treatments in factorial design: domestication status (domesticated and wild progenitor) and presence of AM fungi (inoculated and noninoculated control). To address our second question on the interaction between

Table 1 Common and botanical names of domesticated and wild progenitor taxa of each of the 27 crops used in the *extensive experiment*

Botanic family	Crop name	Domesticated plant	Wild progenitor
Alliaceae	**Leek**	*Allium porrum* L.	*Allium ampeloprasum* L.
Amaranthaceae	**Amaranth**	*Amaranthus cruentus* L.	*Amaranthus hybridus* L.
	Chard	*Beta vulgaris* L.	*Beta vulgaris* L.
	Spinach	*Spinacia oleracea* L.	*Spinacia turkestanica* Iljin
Asteraceae	**Sunflower**	*Helianthus annuus* L.	*Helianthus annuus* L.
	Lettuce	*Lactuca sativa* L.	*Lactuca serriola* L.
	Thistle	*Cynara cardunculus* L.	*Cynara cardunculus* L.
Brassicaceae	**Cabagge**	*Brassica oleracea* L.	*Brassica oleracea* L.
	Rucula	*Eruca vesicaria* L.	*Eruca vesicaria* L.
Cucurbitaceae	**Cucumber**	*Cucumis sativus* L.	*Cucumis sativus* L.
Fabaceae	Chikpea	*Cicer arietinum* L.	*Cicer reticulatum* Ladiz.
	Soya bean	*Glycine max* (L.) Merr.	*Glycine max* subsp. *soja* (Siebold & Zucc.) H. Ohashi.
	Grass pea	*Lathyrus sativus* L.	*Lathyrus cicera* L.
	Lentil	*Lens culinaris* Medik.	Lens culinaris subsp. orientalis (Boiss.) Ponert
	White clover	*Trifolium repens* L.	*Trifolium repens* L.
	Bean	*Vicia faba* L.	*Vicia narbonensis* L.
Linaceae	Flax	*Linum usitatissimum* L.	*Linum usitatissimum* L.
Malvaceae	Cotton	*Gossypium hirsutum* L.	*Gossypium hirsutum* L.
Pedaliaceae	**Sesame**	*Sesamum indicum* L.	*Sesamum indicum* L.
Poaceae	**Oat**	*Avena sativa* L.	*Avena sterilis* L.
	Millet	*Pennisetum glaucum* (L.) R.Br.	*Pennisetum glaucum* (L.) R.Br.
	Rye	*Secale cereale* L.	*Secale cereale* L.
	Sorghum	*Sorghum drummondii* (Nees ex steud.) Millsp. & Chase	*Sorghum arundinaceum* (Desv.) Stapf
	Barley	*Hordeum vulgare* L.	*Hordeum spontaneum* K.Koch
	Wheat	*Triticum durum* Desf.	*Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf.
	Corn	*Zea mays* L.	*Zea mexicana* (Schrad.) Kuntze
Solanaceae	**Tomato**	*Solanum lycopersicum* L.	*Solanum pimpinellifolium* (L.) Mill.

The 14 crops used in the *fertilization experiment* are in bold letters. See Supporting Information Table S1 for more detailed information, particularly on bibliographic references used for assigning wild progenitors for each crop.

fertilization, domestication, and presence of AM fungi, we conducted the *fertilization experiment* with a subset of 14 crops of the *extensive experiment*, selected in order to maximize taxonomical and functional diversity of the complete set of crops (Table 1). The *fertilization experiment* was a full factorial design of domestication status and mycorrhizal treatments, implemented as in the *extensive experiment*, plus a soil P treatment (high and low).

Plant growth, sampling and trait measurements

Both experiments took place at the glasshouse facilities of the Rey Juan Carlos University, located in Móstoles, central Spain (40°18′48″N, 3°52′57″W). All species, except for the legumes, were grown from December 2012 to July 2013. Legume crops were grown separately, from December 2013 to July 2014, because of special microbiological work and conditions to inoculate root nodule bacteria (Methods S2; Table S2).

The AM fungus used in the mycorrhizal treatment was *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler strain EEZ 58 (Gi), a common species abundant in wild and agricultural lands (Oehl *et al.*, 2010). *R. irregularis* was selected for its known ability to rapidly and extensively colonize host roots of multiple herbaceous plants (Hart & Reader, 2002) and because it is frequently used in this type of experiment (i.e. Koide *et al.*, 1988; Gamper *et al.*, 2005; Wright *et al.*, 2005). The mycorrhizal inoculum was multiplied in open pots under bait plants (*Sorghum* and *Trifolium*), filled with sterilized vermiculite, in which the AM fungal inoculum was mixed and cultivated under glasshouse conditions (Estación Experimental del Zaidín CSIC, Granada, Spain). The inoculum (*c.* 60 AM fungal propagules g^{-1}, according to the most probable number test) consisted of soil enriched with AM fungal propagules (infective spores, fresh root fragments with adhering hyphae and hyphal fragments) known to promote fungal colonization of the assigned hosts (Klironomos & Hart, 2002).

All seeds were pregerminated in dark and cold (4°C) growth chambers. Once the radicle emerged, seedlings were individually transplanted to pots (1.8 l volume, 22 × 10.5 × 10.5 cm) filled with a mixture of 80% autoclaved sand and 20% tyndallized soil (93% sand, 5% silt and 1% clay, 0.38% organic matter; pH 8.3). Soil tyndallization is the sterilization of soils by steaming at 100°C for 1 h during three consecutive days. To introduce the AM fungus to the plants, pots were inoculated with 25 g of mycorrhizal inoculum, placed at 5 cm depth in the pot 1 wk before seedling transplantation. Control (noninoculated) plants received a 3 ml aliquot of a microbial wash, to supply nonAM microbes (Koide & Li, 1989). The microbial wash was made by filtering (< 20 μm pore size) 2 l of suspension prepared from 25 g of AM fungal inoculum.

In both experiments, we produced 15–20 replicates per accession for each treatment. We placed all pots of a given accession and treatment in a single tray in the glasshouse, to avoid cross-pot contamination, and the trays were randomly moved once a week. Plants were watered as needed with microbe-free and nutrient-free water, and fertilized once a week with 100 ml of fertilizer solution. The fertilization solution was a modified Hoagland's solution (Hoagland & Arnon, 1950). KH_2PO_4 concentration was 1 mM for the entire *extensive experiment* and also for the low-P treatment of the *fertilization experiment*, and 4 mM for the high-P treatment of the *fertilization experiment*. The base Hoagland's solution consisted of 5 mM Ca $(NO_3)_2$, 5 mM KNO_3, 2 mM $MgSO_4$, 180 μM FeEDTA, 46.2 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.76 μM $ZnSO_4$ and 0.32 μM $CuSO_4$. Finally, KCl was used to maintain constant potassium concentrations across the different fertilization solutions.

Before flowering, *c.* 6–9 wk after sowing depending on the crop species, we randomly harvested five to 10 plants per accession and per treatment. We oven-dried the above-ground biomass of each plant at 60°C for 72 h. Afterwards, we collected green leaves from each plant, which were pooled into three samples per treatment and accession for P analyses. P concentration (% of dry mass) was analysed using vanado-molybdate colorimetry (Allen *et al.*, 1976).

To calculate AM fungal colonization, we removed and washed the fresh roots of harvested plants and randomly selected fine root fragments (*c.* 80 mg). Root samples were cleared with 10% KOH, stained with ink and vinegar solution 5% at 100°C, and rinsed in acidified water for 30 min (Vierheilig *et al.*, 1998). Clearing and staining times varied among species. Once stained, AM fungal colonization was measured using the gridline intersect method, with a magnification of 35× (Giovanetti & Mosse, 1980). AM fungal colonization was quantified as the percentage of intercepts of root colonized by hyphae, vesicles and arbuscules from 250 intercepts per sample.

To address our third question, whether root and mycorrhizal traits have evolved coordinately, we took morphological fine root trait data of the same species from a parallel experiment (methodological details in Methods S1). We used mean root diameter (mm), root tissue density (g ml^{-1}), specific root length (SRL, mg^{-1}), root mass fraction (RMF, %) and root length ratio (RLR, mg^{-1}) as traits highly linked to resource use strategies of roots and to AM fungi colonization. Trait scores come from fine roots grown in deep containers under controlled conditions, and obtained by scanner-based, digital image analyses (WinRHIZO; Regents Instruments, Quebec City, Canada; Arsenault *et al.*, 1995), and computed following general root trait protocols (Pérez-Harguindeguy *et al.*, 2013).

Calculation of mycorrhizal response

The MGR evaluates the effect size of the addition of mycorrhizal inoculum on dry plant biomass (Hetrick *et al.*, 1992). MGR was computed as $MGR = (M_i - M_{ni})/M_{ni}$, where M_i is the above-ground dry mass of inoculated plants and M_{ni} is arithmetic mean of the dry masses of the noninoculated plants (Hetrick *et al.*, 1992). Using the same equation with the P tissue concentration of 'i' and 'ni' plants, we quantified the MPR.

Statistical analyses

Before data analysis, six individuals with extreme trait values, which were randomly distributed across accessions, were excluded from the dataset. All subsequent analyses were conducted with 1014 plants for the *extensive experiment* and 1015 plants for the *fertilization experiment*. All analyses were performed in R v.3.1.2 (R Core Team, 2014).

To assess the domestication and P-fertilization effect on mycorrhizal symbiosis we used mixed-effect models. The differences in the percentage of mycorrhizal colonization among inoculated plants in both experiments were quantified with linear mixed-effect models (GLMMs) with a binomial error distribution; and the differences in plant biomass, P tissue concentration, MGR and MPR were quantified using linear mixed-effect models (LMEs). In all the models, crop identity was included as a random effect over the intercept (random intercept term), and as a random effect over the domestication status parameter (random slope term). Domestication status was the fixed-effects predictor in models, with mycorrhizal colonization as response variable. In models with plant biomass or P tissue concentration as response variables, the fixed effects were domestication status, mycorrhizal treatment and their interaction. Finally, in the models with MGR and MPR as response variables, the fixed effects were domestication status, percentage of AM colonization and their interaction. In the *fertilization experiment*, we added P treatment and its interactions with all other fixed-effect predictors, including the third-level interaction. All other model details were set as in the *extensive experiment*. Generalized linear models used the glmer function of the 'LME4' package (Bates *et al.*, 2007), and linear mixed models used the lme function of the 'NLME' package (Pinheiro *et al.*, 2015).

We tested the significance of the fixed factors of the models with type III analysis of variance, with the mixed function of the 'AFEX' package (Singmann *et al.*, 2015). We estimated pseudo-R^2 values of mixed models using the conditional R^2 (variance explained by random and fixed factors) and marginal R^2 (variance explained by fixed factors) according to Johnson (2014), with the R.SQUAREDGLMM function of the 'MUMIN' package (Barton, 2014). Finally, for the *fertilization experiment*, *post hoc* Tukey test with pairwise comparison among levels of treatment and interactions was conducted using the lsmeans function of the 'LSMEANS' package (Lenth, 2016).

To assess whether mycorrhizal and morphological fine root traits were coordinated along domestication, we calculated the domestication effect and ran phylogenetic generalized least-squares models (PGLSs). We used 24 of the 27 crops in the extensive experiment; *Allium*, *Amaranthus* and *Lactuca* were excluded from the analysis because there were no root trait data available. We calculated the effect size of domestication on AM colonization, MGR and MPR, and on morphological fine root traits with the Hedges G statistic (Hedges *et al.*, 2008), which makes effects comparable among traits and species. The effect size of domestication, which indicates the magnitude of change of a

trait between domesticated and wild progenitor accessions, is positive when domestication increases trait scores, and vice versa. The domestication effect size of mycorrhizal colonization, MGR or MPR was included as response variable in the PGLS models, and the effect size of domestication on each root trait (root diameter, root tissue density, SRL, RMF and RLR) was included as a fixed effects predictor, in separate models for each predictor and response. PGLS models incorporate phylogenetic correlation structure in model residuals to account for phylogenetic nonindependence of species data points (Symonds & Blomberg, 2014). To facilitate the PGLS regressions, a phylogenetic tree with 26 crops was derived from the largest reference tree of the angiosperms (Zanne *et al.*, 2014), with the drop.tip function of the 'PHYTOOLS' package (Revell, 2012). There were no polytomies in the tree. PGLSs were implemented using the gls function of the 'PICANTE' package (Kembel *et al.*, 2010).

Results

Of the noninoculated plants, 2% showed AM colonization and hence were removed from the analysis. Brassicaceae and Amaranthaceae representatives showed negligible AM colonization and mycorrhizal responsiveness (Tables S3, S4).

Domestication effects on mycorrhizal colonization, MGR and MPR under low P availability

The presence of AM fungi increased plant biomass and P tissue concentration in both domesticated and wild progenitor plants (Table 2; Fig. 1a,b). The intensity and direction of domestication effects on AM fungal colonization, MGR and MPR were highly diverse among the 27 crops investigated, as indicated by low R^2m and high R^2c scores (indicative of the percentage of variation explained by fixed-effect and random-effect predictors, respectively; Table 2; Fig. 1a,b). AM fungal colonization increased in some crops (e.g. *Lens*), and decreased in others (e.g. *Linum*), in response to domestication (Table S3; Fig. S1). Therefore, the overall effect of domestication on AM fungal colonization was not significant (domestication effect estimated by LME: −0.01, $P = 0.99$, Fig. 1c). Similarly, certain domestication events increased MGR (as in *Trifolium*) and MPR (as in *Secale*), whereas other domesticated accessions exhibited lower MGR (as in *Vicia*) and MPR (as in *Sesamum*) compared with their wild progenitors (Table S3; Fig. S1). The overall effects of domestication on MGR (domestication effect: 0.03, $P = 0.82$) and MPR (domestication effect: −0.04, $P = 0.62$) were not significant (Table 2; Fig. 1d,e).

Domestication effects on the reaction of AM fungal colonization, MGR and MPR to P availability

In line with the *extensive experiment*, we found diverse responses to the presence of AM fungi and P availability among the 14 crops investigated in the *fertilization experiment*, as indicated by R^2m and R^2c (Tables 3, S4; Fig. S1). However, in the *fertilization experiment*, the growth response to mycorrhizal inoculation (myc) differed between domestication (dom) status and P treatment (dom × myc interaction term, $P = 0.03$ in plant biomass model; and dom × P treatment interaction term in MGR model, $P = 0.01$; Table 3). Specifically, the reaction of MGR to P treatment differed between domesticated plants and wild progenitors, while wild progenitors had similar MGR under the two P availabilities: domesticated plants decreased MGR when P availability increased (Table 3; Fig. 2d). The overall reaction of MPR to P treatment was diverse and independent of the domestication status (Table 3; Fig. 2e).

Arbuscular mycorrhizal fungal colonization decreased with P treatment regardless of domestication status (P effect: −0.475%, $P < 0.001$; Table 3; Fig 2c). However, as indicated by a significant domestication × P treatment interaction (Table 3), domesticated plants reduced AM fungal colonization more strongly than did wild progenitors in response to increased P availability (Fig. 2c). The contribution of AM fungal colonization (col) to MGR was similar in domesticated and wild progenitor species, and was independent of P treatment (dom × AM col interaction term, $P = 0.13$, Table 3). By contrast, the contribution of AM fungal colonization to MPR was bigger in domesticated plants than in wild progenitors (dom × col interaction term, $P = 0.02$, Table 3).

Evolution of mycorrhizal and morphological fine root traits under domestication

Changes in mycorrhizal traits after domestication were poorly associated with shifts in root morphology. Shifts in MGR, AM fungal colonization and MPR were generally unrelated to changes in root morphological traits (Table 4). However, shifts in AM fungal colonization and root tissue density during crop evolution showed a positive relationship (0.248, $P < 0.01$; Fig. S2).

Discussion

In this study we investigated the effects of a large number of independent domestication events on the interaction with a key root symbiont. The strength and direction of the response of AM symbiosis to domestication varied with soil P availability. In P-limited soils, the symbiosis was beneficial to both domesticated plants and their wild progenitors alike, even though the strength and direction of the response to domestication varied depending on the crop species. However, wild progenitors benefited from the AM symbiosis irrespective of P availability, while domesticated species only profited from the AM symbiosis under P-limited conditions (Fig. 3). We have therefore identified a disruption in the efficiency of the AM symbiosis, linked to crop domestication, and taking place under the high nutrient availability conditions typical of agricultural systems. This result might inform much-needed breeding towards optimizing the benefits of mycorrhizal symbionts in agriculture.

Domesticated plants and wild progenitors obtain similar benefits from mycorrhiza under low P availability

In our *extensive experiment* we found multiple patterns of mycorrhizal reactions to domestication (Fig. 1). AM fungal

Table 2 Results of mixed-effect models of data from the *extensive experiment*, where 27 crops were grown under low phosphorus (P) availability

	Plant biomass (g)			P in green leaves (%)			AM colonization (%)			MGR (%)			MPR (%)		
	Estimated values (SE)	$F_{1, 1015}$	P	Estimated values (SE)	$F_{1, 148}$	P	Estimated values (SE)	$F_{1, 1015}$	P	Estimated values (SE)	$F_{1,492}$	P	Estimated values (SE)	$F_{1, 148}$	P
Intercept	2.99 (0.37)		0.00	0.22 (0.02)		0.00	−2.02 (0.68)		0.00	0.10 (0.16)		0.52	0.07 (0.07)		0.32
Domestication	0.63 (0.21)	6.73	**0.01**	−0.01 (0.02)	0.21	0.65	−0.01 (0.38)	0	0.99	0.03 (0.12)	0.05	0.82	−0.04 (0.07)	0.25	0.62
Myc	0.03 (0.08)	5.17	**0.02**	0.02 (0.01)	6.73	**0.01**	–	–	–	–	–	–	–	–	–
AM colonization	–	–	–	–	–	–	–	–	–	0.00 (0.00)	10.3	**0.00**	0.00 (0.00)	0.31	0.58
Dom × Myc	−0.19 (0.11)	2.77	0.1	0.02 (0.01)	3.46	0.06	–	–	–	–	–	–	–	–	–
Dom × AM col	–	–	–	–	–	–	–	–	–	−0.00 (0.00)	0.59	0.44	0.00 (0.00)	3.2	0.08
R^2m	0.018			0.01			0.000			0.028			0.03		
R^2c	0.817			0.745			0.736			0.739			0.847		

The models tested if plant biomass and phosphorus (P) tissue concentration in green leaves were affected by mycorrhizal treatment (Myc) and domestication status (Dom); if arbuscular mycorrhizal (AM) colonization was affected by domestication status; and if mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) were affected by AM colonization and domestication status. The table shows estimated effect values and standard error (SE), and the *F*- and *P*-values of the covariates and interactions. The significant *P*-values are marked in bold. R^2m is the percentage of variance explained by the fixed-effects factors of the models. R^2c is the variance explained by both the fixed and random effects. The dependent variables domestication and AM root colonization were not transformed. Domestication was a factor (domesticated and wild progenitor).

colonization, MGR and MPR decreased in some crops and increased in others during domestication. A meta-analysis on the effects of recent breeding found a signal for domesticates being more mycorrhizal-responsive than landraces (Lehmann *et al.*, 2012). However, in line with our results, case studies that compare wild progenitors with domesticates report diverse mycorrhizal response patterns, depending on the crop species under study (Kapulnik & Kushnir, 1991; Hetrick *et al.*, 1992; Koltai & Kapulnik, 2010; Steinkellner *et al.*, 2012; Xing *et al.*, 2012; Zhu & Zhang, 2013; Turrini *et al.*, 2016). Our broad survey, together with previous case studies, supports the finding that, under the low nutrient availability conditions that are favorable to the AM mutualism, the effect of domestication on mycorrhizal response is diverse.

Given this result, we investigated covariates that might account for the diversity in the size and directionality of domestication effects among crops. In a first step, we asked whether differences in mycorrhizal response and colonization between crops were explained by phylogenic relationships. Taxonomic affinities explain variation in mycorrhizal symbiosis, e.g. Brassicaceae tend to avoid the symbiosis, and the Poaceae family has a low response to mycorrhization (Wang & Qiu, 2006; Brundrett, 2009). We calculated the phylogenetic signal (Blomberg's K, Blomberg *et al.*, 2003) of the domestication effect on mycorrhizal response and colonization. However, domestication effects did not show significant phylogenetic signal (Methods S3; Fig. S3). This is in line with results in Reinhart *et al.* (2012), who analyzed the phylogenetic signal of mycorrhizal response of 95 plant species, and also found no relevant role for phylogenetic affinities.

In a next step we investigated if changes in mycorrhizal response and colonization rates were correlated with shifts in root architecture occurring after domestication. Domestication promoted the evolution of larger plants (Milla & Matesanz, 2017) with thicker fine roots (Methods S1). Species with coarse roots (thick and low-branched) are predicted to be more colonized and responsive to mycorrhiza (Baylis, 1975; Smith & Read, 2008; Kong *et al.*, 2014; Eissenstat *et al.*, 2015). However, we found that domestication effects on MGR, MPR and AM fungal colonization were unrelated to those in root traits (Table 4), with the exception of a loose relationship between AM fungal colonization and root tissue density (Fig. S2). This is surprising, because root structure is reported to influence mycorrhizal colonization and response (Brundrett, 2002; Comas *et al.*, 2014), and previous comparative studies, considering both wild and crop species, support the correlation (Hetrick *et al.*, 1991; Baon *et al.*, 1993; Comas & Eissenstat, 2009). However, the role of fine root thickness as a predictor of plant growth response to AM fungi is debated (Maherali, 2014). One explanation for the independence between root architecture and mycorrhizal traits is that root thickness might change as a result of other pressures. Roots of large plants are usually thicker (Poorter & Ryser, 2015), in order to address biomechanical needs. Therefore, variation in root diameter could be related to increased plant size under domestication, and independent of the mycorrhizal symbiosis. Thus, different selective pressures on mycorrhizal and architectural root traits under domestication might explain such a discrepancy.

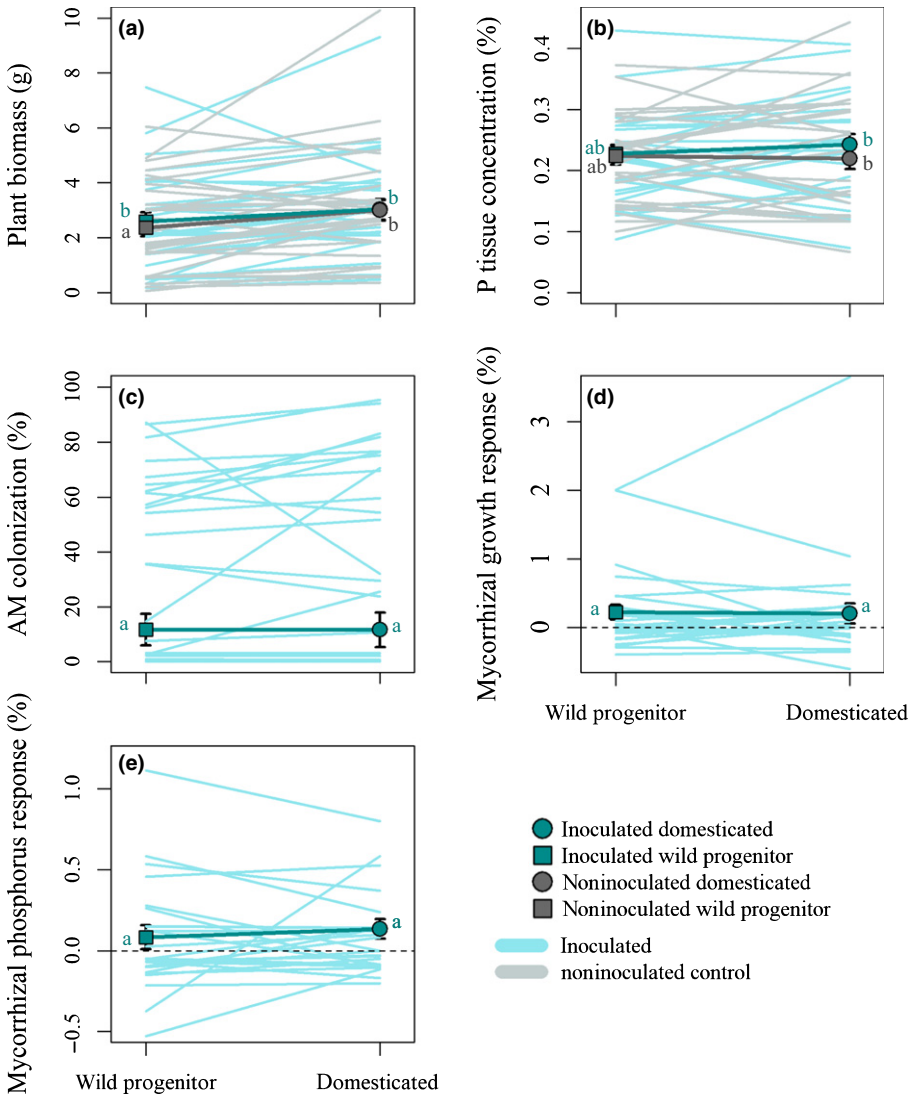

Fig. 1 The reaction of inoculated plants (blue lines) and noninoculated controls (grey lines) to domestication on 27 crops. Arbuscular mycorrhizal (AM) performance was measured as plant biomass (a), leaf phosphorus (P) tissue concentration (b), AM colonization (c), mycorrhizal growth response (d) and mycorrhizal P response (e). The letters and error bars (± SE) are least-squares means and 95% confidence intervals of domesticated plants (circles) and their wild progenitors (squares), estimated from mixed models (Table 2). See Supporting information Table S3 and Fig. S1, for identifying the specific response of each crop.

P fertilization reduced mycorrhizal benefits to domesticated plants

Phosphorous fertilization reduced the mycorrhizal response of domesticated plants. By contrast, wild progenitors kept positive MGRs even under high P supply conditions (Table 3; Fig. 2). The interaction between AM fungi and host plants might change from a strong mutualism to parasitism when P availability increases (Johnson, 1993). Fertilization reduces MGR, even to negative rates (Johnson, 2010), and decreases mycorrhizal colonization (Kaeppler *et al.*, 2000; Nijjer *et al.*, 2001; Treseder, 2004; Konvalinková *et al.*, 2017). Several experiments reporting such a response to fertilization include crops species (Kirk *et al.*, 2011; Aghili *et al.*, 2014). More interestingly, previous studies in maize and wheat reported a negative effect of fertilization in crops, but not in their landraces (Manske, 1989; Wright *et al.*, 2005), suggesting that evolution under cultivation might modulate the mycorrhizal response to fertilization. Our finding extends those reports to a much wider set of crops, and thus raises questions about the mechanism underlying why P fertilization produced negative responses to mycorrhiza only in domesticated plants.

The mechanisms regulating carbon transfer to the fungal partner could explain why fertilization reduced the MGR in domesticated accessions. AM fungi and host plants can regulate their mutual rewards (Kiers *et al.*, 2011). However, plant species differ in their ability to reduce allocation to nonbeneficial AM fungi (Grman, 2012). We speculate that the regulation of resource allocation between partners might be affected by domestication. Selection for higher yield could have changed the biomass allocation pattern in crops, resulting in decreased C translocation towards the roots and hence fungal associates. In such a case, the reduced availability of carbohydrates could lead to decreased AM fungal root colonization, destabilizing the mutual rewards ability and finally destabilizing the cooperativeness of the symbiosis. In fact, Werner & Kiers (2015) theorized that the cultivation history of host plants could affect partner selection, reducing the ability to select high-quality/cooperative AM fungi strains. A parallel line of evidence shows that changes in the symbiotic relationship

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Table 3 Results of mixed-effect models of data from the *fertilization experiment*, where 14 crops were grown under high and low phosphorus (P) availability

	Plant biomass (g)			P in green leaves (%)			AM colonization (%)			MGR (%)			MPR (%)		
	Estimated values (SE)	$F_{1,\ 1015}$	*P*	Estimated values (SE)	$F_{1,\ 148}$	*P*	Estimated values (SE)	$F_{1,498}$	*P*	Estimated values (SE)	$F_{1,492}$	*P*	Estimated values (SE)	$F_{1,\ 148}$	*P*
Intercept	4.56 (0.58)	–	0.00	0.32 (0.03)	–	0.00	−2.34 (0.88)	–	0.01	−0.1 (0.16)	–	0.53	−0.06 (0.08)	–	0.46
Domestication	1.06 (0.30)	9.57	0.005	−0.01 (0.02)	0.01	0.94	−0.07 (0.21)	1.22	0.31	−0.16 (0.09)	0.04	0.84	0.12 (0.08)	1.67	0.22
Myc	−0.19 (0.13)	0.4	0.49	0.01 (0.01)	1.24	0.27	–	–	–	–	–	–	–	–	–
AM colonization	–	–	–	–	–	–	–	–	–	0.003 (0.002)	8.3	**< 0.00**	0.00 (0.00)	0.75	0.4
P treatment	0.73 (0.13)	111.5	**< 0.00**	0.06 (0.01)	113.91	**< 0.00**	−0.47 (0.03)	307.46	**< 0.00**	−0.25 (0.08)	3.44	0.06	−0.02 (0.07)	0.09	0.77
Dom × myc	−0.51 (0.19)	4.81	**0.03**	0.00 (0.02)	0.25	0.62	–	–	–	–	–	–	–	–	–
Dom × AM col	–	–	–	–	–	–	–	–	–	0.00 (0.00)	2.48	0.13	0.00 (0.00)	7.23	**0.02**
Dom × P treatment	0.12 (0.18)	0.62	0.43	−0.01 (0.02)	1.14	0.29	−0.31 (0.04)	77.47	**< 0.00**	0.28 (0.11)	6.24	**0.01**	−0.08 (0.09)	0.66	0.42
P treat × Myc	−0.19 (0.18)	0.06	0.8	0.00 (0.02)	0.44	0.51	–	–	–	–	–	–	–	–	–
P treat × AM col	–	–	–	–	–	–	–	–	–	0.00 (0.00)	0.1	0.75	0.00 (0.00)	0.29	0.59
Dom × P treatment × Myc	−0.44 (0.26)	2.85	0.09	−0.01 (0.03)	0.24	0.63	–	–	–	–	–	–	–	–	–
Dom × P treatment × AM col	–	–	–	–	–	–	–	–	–	0.00 (0.00)	2.1	0.15	0.00 (0.00)	0.47	0.49
R^2m	0.063			0.101			0.005			0.074			0.091		
R^2c	0.778			0.728			0.729			0.612			0.371		

The models tested if plant biomass and P tissue concentration in green leaves were affected by mycorrhizal treatment (Myc), domestication status (Dom) and P treatment; if arbuscular mycorrhizal (AM) colonization was affected by domestication status and P treatment; and if mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) are affected by AM colonization, domestication status and P treatment. The table presents the estimated effect values and standard error (SE), the *F*- and *P*-values of the covariates and interactions. The significant *P*-values are marked in bold. The table also reports the R^2 marginal (R^2m; the variance of the model explained by the fixed effects) and the R^2 conditional (R^2c; the variance explained by both fixed and random effects).The dependent variables were not transformed. Domestication and P fertilization were factors.

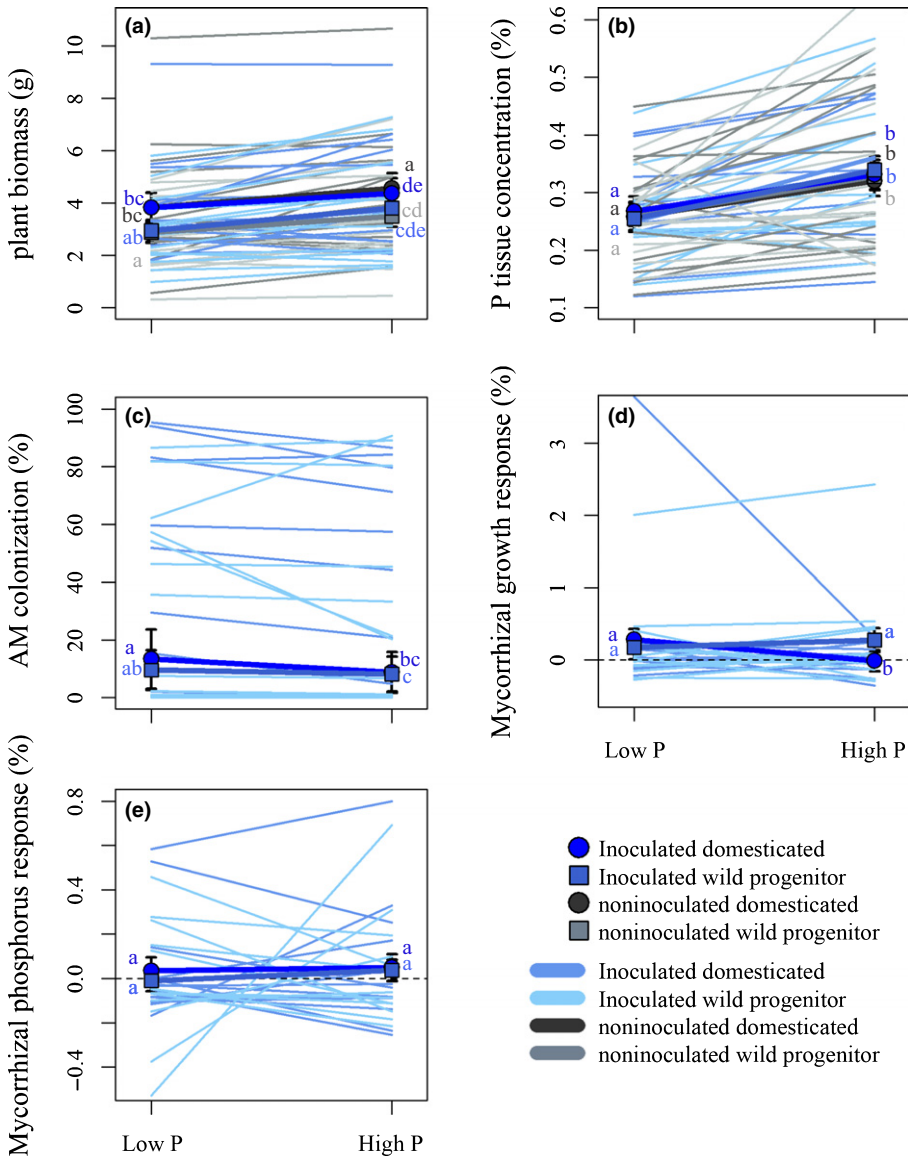

Fig. 2 The reaction of inoculated plants (blue lines) and noninoculated controls (grey lines) to phosphorus (P) treatment in 14 domesticated species (dark lines) and their wild progenitors (light lines). Arbuscular mycorrhizal (AM) symbiosis performance was measured as plant biomass (a), leaf P tissue concentration (b), AM fungal colonization (c), mycorrhizal growth response (d) and mycorrhizal P response (e). The letters and error bars (± SE) are estimated by least-squares means and 95% confidence limits respectively, from mixed models (Table 3). See Supporting Information Table S4 and Fig. S1, for identifying the specific response of each crop.

Table 4 Phylogenetic generalised least-squares models (PGLSs) testing whether the domestication effect (Hedges G) on arbuscular mycorrhizal (AM) colonization, mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) is correlated with the domestication effect on morphological root traits, such as root diameter, root tissue density (RTD), specific root length (SRL), root mass fraction (RMF) and root length ratio (RLR)

	Domestication effect on AM colonization		Domestication effect on MGR		Domestication effect on MPR	
	Estimated values (SE)	*P*	Estimated values (SE)	*P*	Estimated values (SE)	*P*
Domestication effect on root diameter	−0.05 (0.13)	0.71	−0.15 (0.15)	0.33	−0.10 (0.18)	0.59
Domestication effect on RTD	0.25 (0.08)	**0.01**	0.06 (0.12)	0.60	−0.03 (0.13)	0.81
Domestication effect on SRL	−0.31 (0.17)	0.08	0.16 (0.18)	0.37	0.28 (0.22)	0.22
Domestication effect on RMF	0.05 (0.10)	0.62	0.10 (0.10)	0.33	−0.10 (0.11)	0.36
Domestication effect on RLR	−0.59 (0.41)	0.16	−0.21 (0.27)	0.44	0.24 (0.59)	0.22

The table shows the estimated values with standard error (SE) and significance. The significant *P*-values are marked in bold.

might arise in the *Rhizobium* nodules of legumes during domestication (Kiers *et al.*, 2007). Domesticated soybean (*Glycine max*) lacks the ability to spot and reward nodules with cooperative *Rhizobium* strains, and to identify and senesce nodules containing less effective bacteroids (Kiers *et al.*, 2007). The molecular mechanisms for detecting symbionts and establishing the symbiosis between roots and rhizobia, and between roots and AM fungi, are homologous (Ivanov *et al.*, 2012; Tromas *et al.*, 2012).

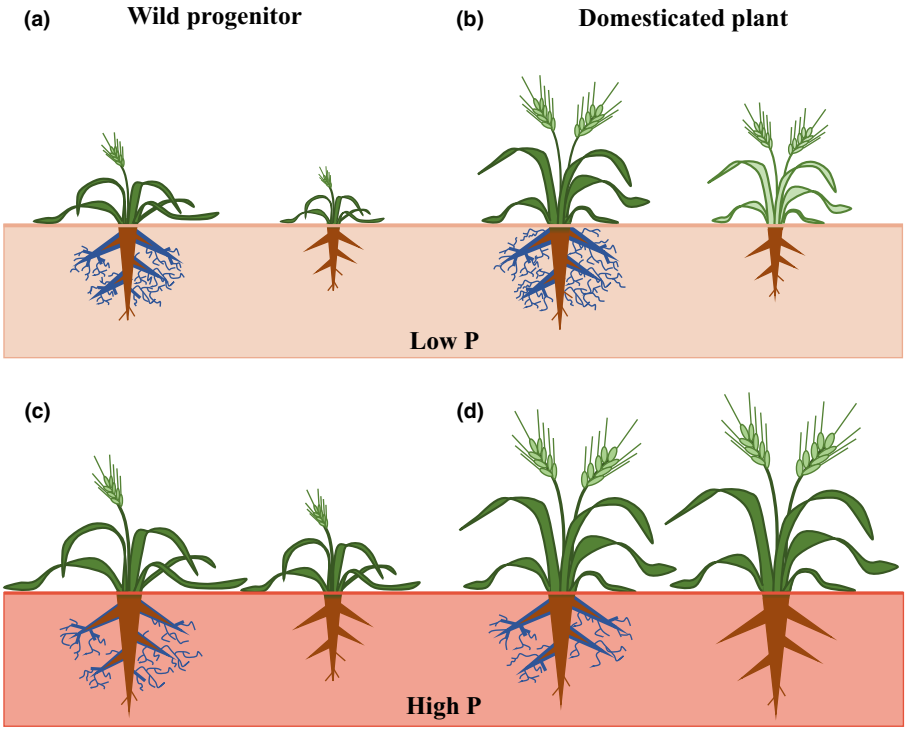

Fig. 3 Conceptual diagram summarizing the main results of this study. Evolution of the plant–mycorrhizal fungi symbiosis under domestication in two scenarios of phosphorus (P) availability. Wild progenitors are represented in (a) and (c), and domesticated plants in (b) and (d). Upper and lower plots are plants grown at low and high P availabilities, respectively. Within each section, the inoculated plant is on the left (arbuscular mycorrhizal (AM) colonization is indicated in blue) and the noninoculated control is on the right. P-limited scenarios promote a mutualistic symbiosis in wild progenitors, where colonized plants grow larger (a), and in domesticated plants, where mycorrhiza enhances plant mass and P concentration (b). However, in P-rich scenarios, progenitors still engage in mutualistic interactions with mycorrhizal fungi, tending to be more responsive (c), while domesticated plants do not benefit from colonization, which might indicate a shift towards a parasitic symbiosis (d).

Therefore, our results are compatible with the hypothesis that the ability to regulate AM fungi might have evolved under domestication in a similar manner to the ability to regulate rhizobia. However, further evidence is needed to test this hypothesis.

Conclusions

Our comparative approach based on 14 crops revealed that domestication reduced mycorrhizal benefits for domesticated crops under high P supply. AM symbiosis provided growth benefits to wild progenitors irrespective of P availability, but the benefits became negligible or costly to domesticated plants when P availability increased. As crop plants are raised under high fertilization in agricultural lands, this result has far-reaching implications. We hypothesize that our finding could be a result of domestication effects on the ability to regulate resource trafficking between AM fungi and associated plant hosts. Further comparative studies are needed to understand whether the ability to regulate host selection and reward the cooperative AM fungi underlie this effect. Our results provide useful information for future plant breeding programs aimed at developing crops that benefit from mycorrhizal fungi effectively. However, to generalize our work, it will be important to analyse the mycorrhizal responsiveness with more AM fungal species, under more diverse experimental conditions and in the field.

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Author contributions

R.M, and N.M-R. conceived this study. Field and laboratory work was done by N.M-R., E.S., A.L. and R.A. Data analyses were performed by N.M-R. and A.L. N.M-R. led the writing with R.M. M.C.R., A.L. and R.A. providing critical reviews of each draft. All authors gave their approval for submission of the final version.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Domestication effect size on mycorrhizal traits at low and high phosphorus treatment of all crops used in the study.

Fig. S2 Correlation plot between domestication effect in arbuscular mycorrhizal (AM) colonization and root tissue density.

Fig. S3 Visualization and phylogenetic signal of the domestication effect on arbuscular mycorrhizal colonization, mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) mapped along the phylogeny of the 27 crops used in the study.

Table S1 Detailed information of each of the 27 domesticated plants and wild progenitors used in this experiment, and reference sources for wild progenitor assignment.

Table S2 List of root nodule bacteria inoculated into the legume crops of the experiment.

Table S3 Mean trait scores of the 27 domesticated plants (D) and their wild ancestors (W) used in the low-phosphorus treatment.

Table S4 Mean trait scores of the 14 domesticated plants (D) and their wild ancestors (W) used in the high-phosphorus treatment.

Methods S1 Root trait data gathering.

Methods S2 Methods of root nodule bacteria inoculation in legume crops.

Methods S3 Analysis of phylogenetic signal on mycorrhizal traits across the 27 crops used in the experiment.

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