Universidad Rey Juan Carlos

Rubén Milla Gutiérrez,

profesor titular del Departamento de Biología y Geología,

Física y Química Inorgánica de la Universidad Rey Juan Carlos,

CERTIFICA:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: "Cambios en la morfología radicular e interacciones planta-microorganismos del suelo en respuesta a la domesticación " han sido realizados bajo su supervisión y son aptos para ser presentados por la licenciada Nieves Martín Robles ante el tribunal que en su día se consigne para aspirar al grado de Doctora por la Universidad Rey Juan Carlos.

V.o B.o Director de Tesis

Universidad Rey Juan Carlos

TESIS DOCTORAL

Cambios en la morfología radicular e interacciones planta-microorganismos del suelo en respuesta a la domesticación

Autora:

Nieves Martín Robles

Director:

Rubén Milla Gutiérrez

Programa de Doctorado en Conservación de Recursos Naturales
Escuela Internacional de Doctorado

2018

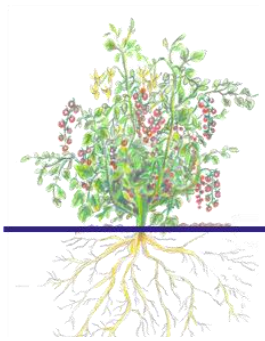
"La ciencia será siempre una búsqueda,

jamás un descubrimiento real.

Es un viaje, nunca una llegada."

Karl Popper

Indice



Resumen abreviado

Antecedentes

La domesticación es un proceso selectivo de adaptación de las plantas a las condiciones agroecológicas y preferencias impuestas por los humanos. Surge de la relación entre humanos y plantas durante un largo periodo de tiempo con la influencia de factores socio-culturales, biológicos y ecológicos (Gepts, 2004). El resultado de ese proceso continuado de uso y selección de las plantas desencadenó cambios a nivel morfológico y fisiológico en los organismos, que los diferenció de sus ancestros silvestres. Denominamos ancestro a la especie silvestre actual más relacionada filogenéticamente a cada cultivo. Los principales cambios comúnmente asociados a la selección artificial son: el aumento del tamaño de frutos y semillas; pérdida de los mecanismos de dormancia y dispersión de semillas, sincronía en la germinación y dominancia apical de la planta (Gepts, 2004). Sin embargo, los rasgos de las plantas domesticadas son el producto de la selección artificial y selección natural, fruto de nuevas presiones ambientales para adaptarse a las nuevas condiciones de cultivo (Milla *et al.*, 2015). Durante la domesticación, los cambios en las plantas dirigidos por la selección consciente fueron acompañados por cambios progresivos y favorables en las condiciones de crecimiento de las plantas. Es esperable que, como consecuencia de estos cambios en las condiciones ambientales, los rasgos de las plantas hayan evolucionado para adaptarse a estas nuevas condiciones.

La ecología basada en rasgos funcionales predice la aparición de plantas con un conjunto de rasgos distintos dependiendo si están en un ambiente rico o pobre en recursos (Craine, 2009). Esta teoría, resalta el equilibrio existente entre rasgos que promueven la adquisición rápida de recursos del medio

con otros rasgos que confieren tolerancia al estrés y escasez de recursos (Craine, 2009). Siguiendo esta línea de razonamiento, el aumento en la disponibilidad de recursos asociado a la domesticación habrá favorecido la selección de plantas con estrategias adquisitivas de los recursos, en comparación con la estrategia de los ancestros silvestres (Chapin, 1980; McKey *et al.*, 2012; Milla *et al.*, 2015). Estudios previos indican una evolución de las plantas domesticadas hacia estrategias más adquisitivas en comparación con sus ancestros (Chen & Welter, 2007; Turcotte *et al.*, 2014; Whitehead *et al.*, 2016). Sin embargo, estas evidencias se han centrado en el estudio de rasgos aéreos, mientras que las consecuencias de la domesticación en los rasgos radiculares y su interacción con los microorganismos del suelo permanecen poco exploradas hasta la fecha.

Las raíces y las interacciones con los microorganismos del suelo son esenciales en el crecimiento, desarrollo y salud de las plantas. De entre los componentes de esta rizosfera, destaca el papel positivo de las micorrizas y otros organismos que mejoran la adquisición de recursos de la planta, la resistencia a estreses abióticos y protección frente a patógenos. Nos basamos en la teoría ecológica de estrategias para predecir determinados cambios en la morfología de las raíces, sus interacciones organismos del suelo con la domesticación y el legado biótico que dejan las plantas en el suelo. Conocer estos cambios ayudará a proponer estrategias de mejora de cultivos que optimicen los rasgos de las plantas encargados de la adquisición de los recursos del suelo, con el fin de poder reducir la aplicación de fertilizantes y pesticidas en agricultura (Schmidt *et al.*, 2016).

Objetivos

El objetivo general es analizar el efecto que la domesticación de los cultivos ha tenido sobre los rasgos radiculares de las plantas y sus interacciones con

los microorganismos del suelo, mediante el estudio comparado de plantas domesticadas y sus ancestros silvestres empleando un amplio conjunto de cultivos. Basamos nuestros objetivos e hipótesis específicas en la hipótesis general de que la domesticación de los cultivos ha supuesto la evolución de las estrategias de uso de los recursos hacia estrategias más adquisitivas.

Metodología

En todos los experimentos realizados en esta tesis, trabajamos con un amplio y diverso conjunto de cultivos anuales de uso alimenticio. El número exacto de cultivos varió de 10 a 30 según el experimento. La selección de los cultivos de cada experimento se realizó para incluir la mayor diversidad posible de procesos de domesticación: diferentes órganos de selección (como hoja o semilla), orígenes diversos y antigüedad variable (desde los 600 a los 10.000 años). Para estudiar el efecto de la domesticación, comparamos cada especie domesticada con su ancestro silvestre. De manera que, para cada cultivo analizado obtuvimos semillas procedentes de dos accesiones: una representativa de la especie domesticada y otra representativa de su ancestro silvestre.

Realizamos tres experimentos de jardín común para medir los rasgos de la raíz (capítulo 1) y las interacciones con los hongos micorrícicos (capítulo 2 y 3) y los nemátodos radiculares (capítulo 3). Brevemente: en el **capítulo 1** cultivamos 30 especies domesticadas y sus ancestros silvestres para obtener diámetro medio de la raíz, densidad del tejido de raíz, longitud de raíz específica (SRL), fracción de biomasa de raíz (RMF). Además, compilamos datos de otras especies herbáceas silvestres, crecidas en condiciones similares a nuestro experimento, para ubicar los rasgos de la raíz de nuestras parejas domesticadas-ancestros en el contexto de la variación dibujada por especies silvestres. En el **capítulo 2**, cultivamos 27 especies domesticadas

y sus ancestros en condiciones esterilizadas y proporcionamos la mitad de las réplicas con un inóculo común de hongos micorrízico arbuscular (AM). Luego medimos la colonización AM, la biomasa aérea y la concentración de fósforo foliar en respuesta a la presencia de hongos AM. Además, para medir la reacción a la disponibilidad de fósforo (P), fertilizamos plantas con dos soluciones de nutrientes que difirieron en la concentración de P en un subconjunto de 14 cultivos. En el **capítulo 3**, realizamos un experimento clásico de retroalimentación de suelos de plantas (plant soil feedback) que consta de dos fases. En una primera fase, cultivamos especies domesticadas y sus ancestros en macetas independientes para acondicionar los suelos. En la segunda fase, cultivamos los mismos genotipos de plantas en los suelos previamente acondicionados por ellos mismos o por la pareja domesticada o ancestro, para examinar los efectos del acondicionamiento del suelo sobre la micorriza y la colonización de raíces de nemátodos y la biomasa aérea. Medimos la biomasa aérea, la micorrización y la colonización de nemátodos.

Resultados

En el **capítulo 1**, vimos que ninguno de los rasgos raíz evolucionó consistentemente hacia una estrategia adquisitiva de los recursos. Sin embargo, encontramos que los ancestros tenían raíces de mayor diámetro y menor densidad. Las raíces más gruesas y menos densas son indicativas de suelos fértiles, lo que sugiere que los ancestros podrían estar pre-adaptados a las condiciones agrícolas. En el **capítulo 2**, vimos que la respuesta de la simbiosis AM a la domesticación varió según la disponibilidad de P. En general, los ancestros se beneficiaron de la simbiosis AM independientemente de la disponibilidad de P, mientras que las plantas domesticados solo se beneficiaron en condiciones de P limitadas. Finalmente, en el **capítulo 3** encontramos que las plantas en suelos

condicionados por plantas domesticadas mostraron menor colonización micorrícica y más infección por nemátodos que en suelos acondicionados por sus ancestros. Además, las plantas domesticadas mostraron en ambos suelos menor colonización de micorrizas y mayor infección de nemátodos que sus ancestros. Sin embargo, la repuesta de la biomasa vegetal varió entre los cultivos y los suelos y no estuvieron relacionadas con la colonización de micorrizas ni nemátodos.

Conclusiones

Nuestros resultados indicaron que el fenotipo raíz de los ancestros silvestres estaría adaptado a los hábitats fértiles y en consonancia con las estrategias adquisitivas de los recursos. Esto nos lleva a sugerir que la adaptación de los fenotipos de las raíces de los cultivos a las condiciones fértiles de los campos agrícolas podría estar determinada en gran parte por elecciones tempranas de especies silvestres, en lugar de por una mayor evolución bajo la domesticación.

De acuerdo la evolución hacia estrategias adquisitivas esperada con la domesticación, encontramos una reducción en la simbiosis con la micorriza y la resistencia a los herbívoros que se alimentan de las raíces en respuesta a la domesticación. Identificamos una interrupción en la eficiencia de la simbiosis de AM, vinculada a la domesticación de cultivos, y que tiene lugar bajo las condiciones de alta disponibilidad de nutrientes típicas de los sistemas agrícolas. Además, encontramos una reducción en la resistencia de las plantas a los herbívoros que se alimentan de las raíces en respuesta a la domesticación. La domesticación de plantas habría afectado al reclutamiento de organismos de la rizosfera a través de un efecto global negativo sobre el mutualismo con la micorriza y la resistencia frente a herbívoros. Por lo tanto, la domesticación de los cultivos alteraría el legado

del suelo y promovería la aparición de efectos negativos en las plantas que ocupan esos suelos. Este conocimiento resulta útil para elaborar estrategias de mejora de plantas dirigidas a optimizar las funciones de las plantas con los microorganismos del suelo, necesarias para una agricultura más sostenible.

Summary

Introduction

Plant domestication involves selection, modification and long-term use of wild plant species with traits regarded as favorable by humans (Evans 1996; Gepts 2004). Major changes commonly associated with artificial selection include increased yield in the organs of interest (e.g. seeds or fruits), stronger apical dominance, and loss of seed dispersal and seed dormancy mechanisms (Evans 1996; Gepts 2004; Abbo *et al.* 2014). However, crop plants have not only been shaped by artificial selection but also by natural selection pressures (Denison, Kiers & West 2003; Zohary 2004; Milla *et al.* 2015). Natural selection under agricultural lands, which are different from wild habitats in the availability of resources, or the intensity and frequency of disturbance, might have led to adaptations in above and belowground traits (Milla *et al.* 2015). Aboveground, the consequences of natural selection include decreased herbivore defense (Turcotte, Turley & Johnson 2014), higher stomatal densities at the upper side of leaves (Milla, Diego-vico & Martín-robles 2013) and increased nitrogen and phosphorus concentration in leaves (Delgado-Baquerizo *et al.* 2016). While the consequences of crop evolution on the aboveground traits are well described for a few traits (Meyer, DuVal & Jensen 2012; García-Palacios *et al.* 2013; Meyer & Purugganan 2013; Preece *et al.* 2016; Whitehead, Turcotte & Poveda 2016; Kluyver *et al.* 2017; Milla & Matesanz 2017), knowledge on the consequences belowground is still scarce (Lynch & Brown 2012; Bishopp & Lynch 2015).

The consequences of crop evolution aboveground might guide us in making predictions for belowground traits. Ecologists have identified the existence of fundamental trade-offs associated with resource uptake rate

and life-history strategies (Grime *et al.* 1997; Craine 2009; McCormack *et al.* 2015; Diaz *et al.* 2016). In this context, some of the aboveground consequences of crop evolution (high growth rates and leaf nitrogen content, Turcotte *et al.* 2014; Delgado-Baquerizo *et al.* 2016) would be typical of fast-growing resource-acquisitive strategies (Lambers & Poorter 1992; Craine, 2009 Reich 2014). Theoretical and empirical evidence based on aboveground traits suggest that domesticated species have fast acquisitive strategies, either as a consequence of pre-adaptions to the agricultural environment and/or as evolution under cultivation. While our knowledge is pretty substantial when it comes to how domestication affected above-ground plant traits, we have only very limited insight into what happened below-ground (Bishopp & Lynch, 2015; Lynch & Brown, 2012).

Objective

We investigated the evolution of root traits and the interactions with soil biota in response to plant domestication. According with ecological theories, domesticated plants would show root traits and interactions of fast acquisitive strategists. Specifically, we analyzed root morphology, the interactions with mycorrhizal fungi and soil herbivores and the soil legacy of domesticated species in comparison with their wild progenitors.

Methodological details

To maximize the generality of our results, we worked with phylogenetically diverse set of herbaceous crop species and their most likely wild progenitors. The number of crops ranged from 10 to 30 depending on the experiment. The choice of study species was made to include a wide range of variability in the domestication process, such as different target organs of selection (leaves, seeds and fruits), diverse origins and antiquity of

domestication ranging from 10.000 to 600 years (Sauer, 1993). We obtained two seed lots for each crop: one belonging to an accession of a common domesticated cultivar and another from the most likely wild progenitor.

We set up three common garden experiments to measure root traits (chapter 1) and the interactions with mycorrhizal fungi (chapter 2 and 3) and root feeding nematodes (chapter 3). Briefly: in **chapter 1** we grew 30 crop species and their wild progenitors to obtain root thickness, root tissue density, specific root length (SRL), root mass fraction (RMF) and root length ratio. In addition, we compiled data from other wild herbaceous species, growth in similar conditions to our experiment, to place the root traits of our crops in the context of wider botanical variation. In **chapter 2**, we grew the domesticated and wild progenitors in sterilized conditions and provided half of the replicates with a common arbuscular mycorrhizal (AM) fungi inoculum. We then measured AM colonization, aboveground biomass and leaf phosphorus 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 phosphorus (P) concentration to measure the reaction to P availability. In **chapter 3**, we stablished a classical plant soil feedback experiment consisting in two phases. In a first phase, we grew domesticated species and each of their wild progenitors in separate containers to condition the soils. In the second phase, we examined the effects of the soil conditioning on mycorrhizal and nematodes root colonization and aboveground biomass by growing the same plant genotypes on soils previously conditioned by themselves or by the domesticated or progenitor partner. We measured the aboveground biomass, mycorrhizal and nematode colonization.

Results

In **chapter 1**, we found that none of the root traits evolved consistently towards a more resource acquisitive strategy. Nevertheless, we found that wild progenitors had thicker and less dense roots. Thicker and less dense roots are indicative of fertile soils, which suggests that wild progenitors could already have been adapted to agricultural conditions. In **chapter 2,** we found that 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 unrelated to phylogenetic affinities, or to the coordinated evolution with fine root traits. Finally, in **chapter 3** we found that plants grown in soils conditioned by domesticated plants showed less mycorrhizal colonization and more nematode infection. Moreover, domesticated plants were less colonized by mycorrhiza but more infected by nematodes than theirs wild progenitors. However, magnitudes and directions of plant biomass and PSF were diverse among the crops, and unrelated with mycorrhizal and nematodes colonization.

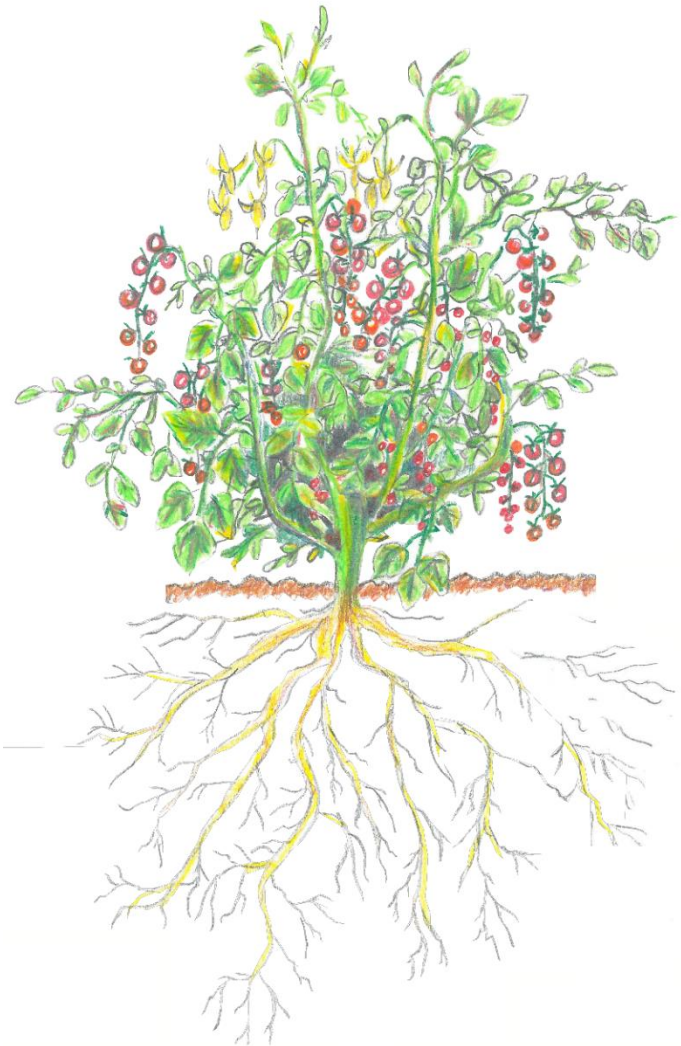
Conclusions

Our results indicated that the root phenotype of wild progenitors would be already adapted to fertile habitats and would be in accordance with fast acquisitive strategies. Thus, the good adaptation of crop root phenotypes to the fertile conditions of agricultural fields might be largely determined by early choices of wild species, rather than by further evolution under domestication.

According with an evolution towards acquisitive strategies, we found a reduction in mycorrhizal symbiosis and resistance to root feeding

herbivores in response to plant domestication. We 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. Moreover, we found a reduction on plant resistance to root feeding herbivores in response to domestication. Plant domestication would have impacted the recruitment of rhizosphere organisms through an overall negative effect on mycorrhizal mutualism and plant resistance to herbivores. Thus, crop domestication would alter soil legacy promoting negative feedbacks. This knowledge highlights the importance to undertake plant breeding strategies to optimize the profitable functions from the plant-soil interactions towards a sustainable agriculture.

Introducción general



Antecedentes

La domesticación de las plantas y los animales es uno de los eventos culturales y evolutivos más relevantes de los últimos 20.000 años de historia de la humanidad (Diamond, 2002). La domesticación es un proceso complejo que surge de la relación entre humanos y plantas durante un largo periodo de tiempo con la influencia de factores socio-culturales, biológicos y ecológicos (Gepts, 2004). La influencia de factores tan diversos en la domesticación impulsa su estudio desde múltiples ángulos y perspectivas desde disciplinas como la arqueología, la agronomía, la ecología o la biología evolutiva. En esta tesis abordamos la domesticación desde una perspectiva ecológica y evolutiva, en la que analizamos la influencia de los factores ecológicos en las plantas durante el proceso de domesticación. Concretamente, analizamos la evolución de los rasgos de las plantas cultivadas involucrados en la adquisición de recursos en respuesta a las condiciones ambientales agrícolas. Estudiar la evolución de estos rasgos ayuda a identificar caracteres poco eficientes en el uso y adquisición de los recursos y sugerir estrategias de producción vegetal orientadas hacia la sostenibilidad.

Origen, causa y consecuencias de la domesticación

La domesticación de las plantas surge al final de la última glaciación, entre el Holoceno temprano y Medio (12.000 a 4.000 años A.C.), en varios puntos del planeta. Vavilov *et al.*, (1992) enunciaron que la domesticación se desarrolló en ocho puntos geográficos independientes, y posteriores estudios han ido agregando nuevas ubicaciones hasta llegar a doce localizaciones geográficas (Brown *et al.*, 2009; Meyer *et al.*, 2012; Larson *et al.*, 2014). Los primeros, desarrollados en el Holoceno temprano (12.000 a 9.000 años A.C.) fueron Oriente medio con el cultivo del Trigo y la cebada

(*Tritricum* sp. y *Hordeum vulgare*), China con la domesticación del arroz (*Oryza sativa*) y centro-América con el maíz (*Zea mays*) y los frijoles (*Phaseolus* sp.). Durante el Holoceno medio (7.000 a 4.000 años A.C.) apareció en dos puntos del continente africano con cultivos como el sorgo (*Sorghum sudanense*), en Nueva Guinea la caña de azúcar (*Saccharum officinarum*) y en el sureste de Asia (Khoury & Achicanoy, 2016). Las causas de la domesticación pueden haber diferido entre los distintos orígenes geográficos y se encuentran actualmente en debate (Larson *et al.*, 2014). No obstante, uno de los argumentos más aceptados establece que fue la diversificación de la dieta la que propició los inicios del cultivo y domesticación de plantas. La disminución de presas de gran tamaño por motivos climáticos llevó al ser humano a diversificar y ampliar su dieta a pequeños animales y plantas. Durante el transporte de semillas y plantas, algunas caerían accidentalmente en las proximidades de los asentamientos humanos, lo que contribuyó a modificar la composición vegetal en esas áreas e inició un cultivo inconsciente, que condujo finalmente a la domesticación (Willcox *et al.*, 2008; Smith, 2011; Weiss *et al.*, 2017).

La domesticación de las plantas aumentó la disponibilidad de alimentos (Diamond, 2002). Este hecho junto a la aparición de la agricultura, desencadenaron profundos cambios sociales como el cambio de hábito de cazador-recolector a agricultor, la sociedad se volvió sedentaria y surgieron las primeras formas de organización social los oficios y las ciudades (Diamond, 2002). En la actualidad, el cultivo de plantas se ha extendido por todo el planeta y los alimentos procedentes de especies domesticadas conforman la práctica totalidad de nuestra dieta (www.fao.org). Aunque hemos obtenido más de 2.500 especies cultivadas pertenecientes a 160 familias botánicas, la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) estima que en torno a un 75% de la

producción mundial de alimentos pertenecen tan solo a una docena de plantas y cinco especies animales. La domesticación y la agricultura han tenido consecuencias muy favorables para el desarrollo de la humanidad, pero también ha supuesto la expansión y uso de unos pocos cultivos por todo el planeta, disparando así el éxito reproductor de esas especies. De manera que humanidad y plantas son mutuamente favorecidas, lo que lleva a sugerir que la domesticación es la interacción mutualista más extendida y exitosa del planeta (Purugganan & Fuller, 2009).

El proceso de la domesticación: los rasgos de las plantas domesticadas

La domesticación es un proceso selectivo de adaptación de las plantas a las condiciones agroecológicas y preferencias impuestas por los humanos. Los ancestros silvestres debieron de poseer pues el potencial de vivir en condiciones antrópicas y expresar rasgos favorables para su uso, cosecha y consumo (Larson *et al.*, 2014). El resultado de ese proceso continuado de uso y selección de las plantas desencadenó cambios a nivel morfológico y fisiológico en los organismos, que los diferenció de sus ancestros silvestres. La identidad de la mayoría de ancestros silvestres, su origen geográfico y duración de la domesticación se conocen para la mayoría de cultivos, gracias a los avances genéticos y arqueológicos de las últimas décadas. Estudios comparativos de las plantas domesticadas con sus ancestros silvestres, hace que nuestro conocimiento sobre el proceso de domesticación avance.

Fruto de la observación detallada de la diversidad de cultivos, es posible reconocer un conjunto de rasgos comunes entre cultivos al que se ha denominado síndrome de domesticación (Hawkes, 1983; Harlan, 1992; Gepts, 2004; Fuller, 2007). Este síndrome es definido por una amplia variedad de rasgos, que dependiendo del cultivo incluye algunos como: el

aumento del tamaño de frutos y semillas; pérdida de los mecanismos de dormancia y dispersión de semillas, sincronía en la germinación, reducción de los sistemas de defensa físicos y químicos, dominancia apical de la planta y reducción de la ramificación lateral, adelantamiento, sincronía de la floración y mayor número y tamaño de las inflorescencias (Gepts, 2004). El síndrome de domesticación agrupa los rasgos más comunes y extendidos a todos los cultivos, aunque no todos los rasgos aparecen en todos los cultivos (ver revisión reciente Meyer *et al.*, 2012).

Charles Darwin fue el primero en hablar de los mecanismos que subyacen al proceso de domesticación. En el libro "La variación de las plantas y los animales con la domesticación" publicado en 1868, Darwin sentó las bases de la selección artificial como motor de cambio en los procesos de domesticación de plantas y animales. Además, identificó y diferenció dos tipos de selección operantes en la domesticación: una consciente, aquella en la que los humanos directamente seleccionan determinados rasgos deseados, y otra inconsciente, donde los rasgos son seleccionados de manera inconsciente (Darwin, 1859). El concepto de selección inconsciente que ha llegado a nuestros días ha sido reformulado, ya que aunque Darwin identificó la existencia de algún tipo de selección ajena a la intención del domesticador, no explicó que mecanismo operaba (Zohary, 2004). La selección inconsciente supone la selección de otros rasgos ligados o de selección natural para adaptarse a las nuevas condiciones ambientales y de manejo impuestas en la agricultura (Rindos, 1984; Harlan, 1992; Zohary, 2004). Por ejemplo, es probable que la pérdida de mecanismos de dispersión de las semillas surgiría como consecuencia de la forma de cosechar, los recolectores seleccionarían los tallos con semillas que no se han caído del golpe de la hoz con el tallo, este rasgo sería fruto de la selección inconsciente (Zohary, 2004). Otros rasgos como pérdida de

mecanismos de dormancia, sincronía de germinación y dominancia apical pueden ser el resultado de la selección inconsciente (Purugganan & Fuller, 2009). En la actualidad, se establece que los rasgos que presentan las especies domesticadas son fruto de una selección consciente (selección artificial), dirigida a obtener unas características deseadas, y otros rasgos que han evolucionado de manera inconsciente (selección natural) fruto de nuevas presiones ambientales para adaptarse a las nuevas condiciones de cultivo (Figura 1) (Gepts, 2004; Zohary, 2004; McKey *et al.*, 2012; Milla *et al.*, 2015).

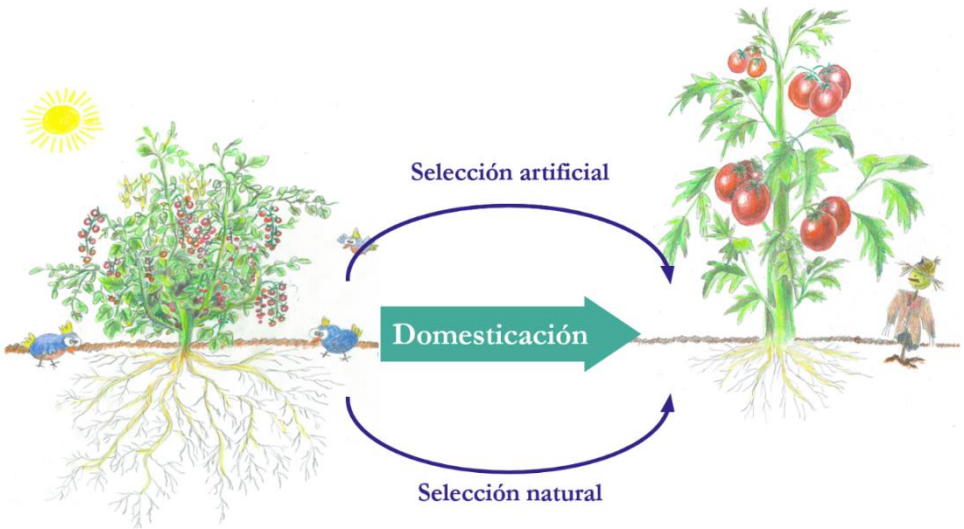


Figura 1. Proceso de domesticación de las plantas de cultivo.

Aplicación de la teoría de estrategias ecológicas al estudio de la domesticación

Durante la domesticación, los cambios en las plantas dirigidos por la selección consciente fueron acompañados por cambios progresivos en el medio. Los agricultores tratan de cultivar las plantas en condiciones de crecimiento óptimas, suministrando los recursos normalmente limitantes como el agua o nutrientes minerales, y protegiendo a los cultivos de

patógenos y herbívoros. De manera que los ambientes agrícolas resultantes presentan fuertes contrastes ecológicos con los ambientes donde crecen los ancestros (Denison *et al.*, 2003). Es esperable que, como consecuencia de estos cambios en las condiciones ambientales, los rasgos de las plantas hayan evolucionado para adaptarse a estas nuevas condiciones.

Inspirados por la línea de conocimiento basada en rasgos funcionales y estrategia del uso de los recursos, en esta tesis analizamos la respuesta de las plantas a la domesticación basándonos en predicciones creadas por esta teoría. La ecología basada en rasgos funcionales predice la aparición de plantas con un conjunto de rasgos distintos dependiendo si están en un ambiente rico o pobre en recursos (Craine, 2009). La teoría, desarrollada en plantas silvestres, resalta el equilibrio existente entre rasgos que promueven la adquisición rápida de recursos del medio con otros rasgos que confieren tolerancia al estrés y escasez de recursos (Grime *et al.*, 1997; Craine, 2009; Reich, 2014; Diaz *et al.*, 2016). Las plantas con estrategia conservadora de recursos muestran rasgos fisiológicos asociados a aumentar la adquisición de recursos y disminuir en toda la planta los requerimientos nutricionales y la pérdida la pérdida de nutrientes; mientras que la estrategia adquisitiva está asociada a rasgos para aumentar el crecimiento de la planta. Así, rasgos que facilitan la adquisición de recursos serán favorecidos en ambientes ricos, mientras que rasgos que favorezcan la conservación de los recursos serán promovidos en los ambientes pobres. Siguiendo esta línea de razonamiento, el aumento en la disponibilidad de recursos asociado a la domesticación habrá favorecido la selección de plantas con estrategias adquisitivas de los recursos, en comparación con la estrategia de los ancestros silvestres (Chapin, 1980; Craine, 2009; McKey *et al.*, 2012; Milla *et al.*, 2015).

Estudios previos, que comparan los rasgos de especies domesticadas con sus ancestros silvestres, indican una evolución hacia estrategias más adquisitivas. Por ejemplo, las plantas domesticadas disminuyen la resistencia frente a herbívoros en múltiples cultivos (Chen & Welter, 2007; Turcotte *et al.*, 2014; Whitehead *et al.*, 2016). Delgado-Baquerizo *et al.*, 2016 encontraron mayor contenido de Nitrógeno en hojas de plantas domesticadas en comparación con sus ancestros, en un estudio con 24 cultivos. Roucou *et al.*, (2018) también encuentran mayor contenido de Nitrógeno en hojas, así como mayores tasas fotosintéticas y menor longevidad en un estudio con 39 genotipos de trigo representativos de varios estadios de domesticación (*Tritricum* sp.). Los cambios en la expresión de los rasgos mencionados arriba coinciden con una evolución hacia estrategias más adquisitivas de recursos (Reich, 2014; Diaz *et al.*, 2016). Sin embargo, estas evidencias se han centrado en el estudio de rasgos aéreos, mientras que las consecuencias de la domesticación en los rasgos radiculares y su interacción con los microorganismos del suelo permanecen poco exploradas hasta la fecha.

Las raíces y las interacciones con los microorganismos del suelo son esenciales en el crecimiento, desarrollo y salud de las plantas. De entre los componentes de esta rizosfera, destaca el papel positivo de las micorrizas y otros organismos que mejoran la adquisición de recursos de la planta, la resistencia a estreses abióticos y protección frente a patógenos. Los consorcios de microorganismos del suelo pueden suprimir y proteger a la planta de enfermedades. Por tanto, las plantas dependen en gran medida del microbioma formado en la rizosfera, este microbioma es fruto de la interacción planta y microorganismos y está sujeto a las presiones selectivas del entorno. Cabe preguntarse, si las presiones selectivas se han visto

modificadas en los ambientes agrícolas y si el reclutamiento de organismos radiculares específicos se altera con la domesticación (Wissuwa, 2009).

Varios argumentos predicen cambios en la morfología de las raíces y sus interacciones organismos del suelo con la domesticación: (a) La teoría ecológica de estrategias establece que las raíces de plantas con estrategia adquisitiva invierten menos biomasa en las raíces, que se traduce en un ratio raíces:planta bajo, además tienen poca inversión estructural, son raíces con menor densidad tisular, menos longevas y con alto contenido de nutrientes, alta tasa de actividad y poca dependencia de la simbiosis con las micorrizas(Craine, 2009; Reich, 2014; Diaz *et al.*, 2016; Kramer-Walter *et al.*, 2016). (b) Como citábamos en párrafos anteriores, las plantas con estrategia adquisitiva de recursos invierten menos en defensa (Craine, 2009). Como citábamos en párrafos anteriores, las tasas de herbivoría en hojas son mayores en plantas domesticadas que en sus ancestros silvestres (por ejemplo Whitehead *et al.*, 2016). La reducción en el sistema de defensa de la planta también tendrá consecuencias aumentando la herbivoría causada por organismos del suelo en las raíces como los nemátodos, y aumentando otros patógenos causantes de enfermedades como hongos. Como consecuencia de esta reducción en el sistema de defensa, las interacciones negativas con microorganismos del suelo se habrán visto alteradas. (c) Las prácticas agrícolas alteran la diversidad y composición de microorganismos del suelo (Thiele-Bruhn *et al.*, 2012; Bell & Tylianakis, 2016; Pieterse *et al.*, 2016). El arado, la fertilización o las rotaciones de cultivo afectan negativamente a los hongos formadores de micorrizas (Mäder *et al.*, 2000; Oehl *et al.*, 2003), además impulsan la aparición de hongos micorrícicos con características menos beneficiosas para la simbiosis con la planta (Verbruggen & Toby Kiers, 2010). Como consecuencia, se habrán alterado las interacciones con los organismos del suelo, especialmente la interacción beneficiosa con los

hongos micorrícicos. En conclusión, existen varios argumentos para esperar que la domesticación de los cultivos haya alterado los rasgos radiculares y sus interacciones con microorganismos del suelo. Conocer estos cambios ayudará a proponer estrategias de mejora de cultivos que optimicen los rasgos de las plantas encargados de la adquisición de los recursos del suelo, con el fin de poder reducir la aplicación de fertilizantes y pesticidas en agricultura (Schmidt *et al.*, 2016).

Objetivos

El objetivo general de esta tesis doctoral es analizar el efecto que la domesticación de los cultivos ha tenido sobre los rasgos radiculares de las plantas y sus interacciones con los microorganismos del suelo, mediante el estudio comparado de plantas domesticadas y sus ancestros silvestres empleando un amplio conjunto de cultivos. Basamos nuestros objetivos e hipótesis específicas en la hipótesis general de que la domesticación de los cultivos ha supuesto la evolución de las estrategias de uso de los recursos hacia estrategias más adquisitivas. Esta evolución habría provocado cambios en la morfología de las raíces y sus interacciones con microorganismos del suelo, lo cual nos llevó a plantear los siguientes objetivos específicos:

1. Determinar si los rasgos radiculares morfológicos y de inversión de biomasa de las plantas de cultivo son característicos de plantas con estrategias adquisitivas de recursos, y si surgieron como consecuencia de una evolución de estos rasgos con la domesticación de los cultivos; o si influyó la selección de progenitores silvestres, mediante la pre-selección de especies con rasgos favorables a medios antrópicos (**Capítulo 1**).

2. Analizar si la domesticación ha afectado a la relación simbiótica establecida con hongos formadores de micorrizas vesículo-arbusculares. Concretamente, cuantificar si el beneficio aportado por la relación simbiótica a la planta cambia con la domesticación, y si esta respuesta es dependiente de la disponibilidad de nutrientes (**Capítulo 2**).

3. Evaluar si el legado biótico que dejan las plantas en el suelo (diversidad y abundancia de microorganismos) y la influencia que éste ejerce en el

crecimiento de cohortes sub-siguientes de plantas son afectados por la domesticación (**Capítulo 3**).

Listado de manuscritos

Esta tesis está basada en 3 artículos escritos en inglés para su publicación en revistas científicas de ámbito internacional. A continuación, se detalla el título, la lista de coautores y el estado de publicación de cada capítulo.

Capítulo 1. Martín-Robles, N., Morente-López, J., Freschet G.T., Poorter, H., Roumet, C., Milla, R. Root traits of herbaceous crops: pre-adaptation to cultivation or evolution under domestication?. Manuscrito en revisión en *Functional Ecology*.

Capítulo 2. Martín-Robles, N., Lehmann, A., Seco, E., Aroca, R., Rillig, M.C., Milla, R. (2018) Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. *New Phytologist*, **218**(1), 322-334.

Capítulo 3. Martín-Robles, N., García-Palacios, P., Rodríguez, M., Rico, D., Vigo, R., De Deyn, G.B., Milla, R. Crops and their wild progenitors recruit beneficial and detrimental root-associated biota in opposing ways. Manuscrito en preparación.

Afiliación de los coautores

Rubén Milla (Director de tesis): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain

Catherine Roumet (Capítulo 1): CEFE, CNRS, Université de Montpellier, Université Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

Grégoire T. Freschet (Capítulo 1): CEFE, CNRS, Université de Montpellier, Université Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

Hendrik Poorter (Capítulo 1): Plant Sciences (IBG-2), Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

Javier Morente-López (Capítulo 1): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain.

Anika Lehmann (Capítulo 2): Institut für Biologie, Dahlem Center of Plant Sciences, Freie Universität Berlin, Altensteinstr. 6, 14195 Berlin, Germany.

Erica Seco (Capítulo 2): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain

Matthias C. Rillig (Capítulo 2): Institut für Biologie, Dahlem Center of Plant Sciences, Freie Universität Berlin, Altensteinstr. 6, 14195 Berlin, Germany.

Ricardo Aroca (Capítulo 2): Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación experimental del Zaidín, CSIC, C/Profesor Albareda 1, 18008, Granada, Spain

Daniel Rico (Capítulo 3): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain

Gerlinde B. De Deyn (Capítulo 3): Soil Biology and Biological Soil Quality, Wageningen University and Research, PO Box 47, 6700 AA, Wageningen, The Netherlands.

Marta Rodríguez (Capítulo 3): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain.

Pablo García-Palacios (Capítulo 3): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain.

Rocio Vigo (Capítulo 3): Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain.

Sara Sánchez Moreno (capítulo 3): Departmento Unidad de Productos Fitosanitarios; Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. de La Coruña, km 7,5, 28040 Madrid, Spain

Metodología general

En este apartado se describe el sistema de estudio, así como la metodología general de los experimentos y los análisis estadísticos empleados. No obstante, la metodología más específica para la consecución de cada objetivo se describe detalladamente en el apartado "Material y Métodos" de cada capítulo.

Sistema de estudio

Para estudiar el efecto de la domesticación en los rasgos radiculares y sus interacciones con los microorganismos del suelo, comparamos cada especie domesticada con su ancestro silvestre. Denominamos ancestro a la especie silvestre actual más relacionada filogenéticamente a cada cultivo, asumiendo que compartieron un origen común previo a la domesticación, momento en que sus caminos evolutivos se separaron. De manera que para cada cultivo abordado en nuestros estudios obtuvimos semillas procedentes de dos accesiones: una representativa de la especie domesticada y otra representativa de su ancestro (Tabla 1, Figura 2).

Todos los experimentos de esta tesis tuvieron un enfoque extensivo (con muchas especies), tratando de abarcar un amplio espectro de cultivos. En total, incluimos 35 cultivos anuales de porte herbáceo, pertenecientes a 34 géneros y a 11 familias botánicas (Tabla 1). El número de cultivos varió entre experimentos, siendo 30 cultivos en el primer experimento (primer capítulo), 28 en el segundo experimento (segundo capítulo) y 10 cultivos en el último (tercer capítulo). Los experimentos compartieron la mayoría de cultivos (Tabla 1), para optimizar la comparación de resultados y el uso de datos entre experimentos.

Familia	Cultivo	Especie domesticada	Especie progenitora	Cap. I	Cap. II	Cap. III
Alliaceae	Puerro	*Allium porrum* L.	*Allium ampeloprasum* L.		■	■
Amaranthaceae	Acelga	*Beta vulgaris* L.	*Beta vulgaris* L.	■	■	■
	Amaranto	*Amaranthus cruentus* L.	*Amaranthus hybridus* L.		■	■
	Espinaca	*Spinacia oleracea* L.	*Spinacia turkestanica* Iljin		■	
Asteraceae	Lechuga	*Lactuca sativa* L.	*Lactuca serriola* L.		■	
	Cardo	*Cynara cardunculus* L.	*Cynara cardunculus* L.	■	■	■
	Girasol	*Helianthus annuus* L.	*Helianthus annuus* L.	■	■	
Brassicaceae	Col	*Brassica oleracea* L.	*Brassica oleracea* L.	■	■	
	Rucula	*Eruca vesicaria* L.	*Eruca vesicaria* L.	■	■	
Cucurbitaceae	Pepino	*Cucumis sativus* L.	*Cucumis sativus* L.	■	■	
Fabaceae	Alfalfa	*Medicago lupulina* L.	*Medicago lupulina* L.	■		
	Almorta	*Lathyrus sativus* L.	*Lathyrus cicera* L.	■	■	
	Altramuz	*Lupinus luteus* L.	*Lupinus luteus* L.	■		
	Alubia	*Vicia faba* L.	*Vicia narbonensis* L.	■	■	
	Carilla	*Vigna unguiculata* (L.) Walp.	*Vigna unguiculata* (L.) Walp.	■		
	Garbanzo	*Cicer arietinum* L.	*Cicer reticulatum* Ladiz.	■	■	
	Guisante	*Pisum sativum* L.	*Pisum sativum* subsp. *elatius* (M.Bieb.) Asch. & Graebn	■		
	Judía	*Phaseolus lunatus* L.	*Phaseolus lunatus* L.	■		
	Lenteja	*Lens culinaris* Medik.	*Lens culinaris* subsp. orientalis (Boiss.) Ponert	■	■	
	Soja	*Glycine max* (L.) Merr.	*Glycine max* subsp. *soja* (Siebold & Zucc.) H.Ohashi	■	■	
	Trébol	*Trifolium repens* L.	*Trifolium repens* L.	■	■	■
Linaceae	Lino	*Linum usitatissimum* L.	*Linum usitatissimum* L.	■	■	
Malvaceae	Algodón	*Gossypium hirsutum* L.	*Gossypium hirsutum* L.	■	■	
Pedaliaceae	Sesamo	*Sesamum indicum* L.	*Sesamum indicum* L.		■	
Poaceae	Sorgo	*Sorghum drummondii* (Nees ex Steud.) Millsp. & Chase	*Sorghum arundinaceum* (Desv.) Stapf	■	■	■
	Arroz	*Oryza sativa* L.	*Oryza rufipogon* Griff.	■		
	Avena	*Avena sativa* L.	*Avena sterilis* L.	■	■	
	Cebada	*Hordeum vulgare* L.	*Hordeum spontaneum* K.Koch	■	■	■
	Centeno	*Secale cereale* L.	*Secale cereale* L.	■	■	
	Maiz	*Zea mays* L.	*Zea mexicana* (Schrad.) Kuntze	■	■	■
	Mijo	*Pennisetum glaucum* (L.) R.Br.	*Pennisetum glaucum* (L.) R.Br.	■	■	
	Trigo	*Triticum durum* Desf.	*Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf.	■	■	
Solanaceae	Chile	*Capsicum baccatum* L.	*Capsicum baccatum* var. *pendulum* (Willd.) Eshbaugh	■		
	Pimiento	*Capsicum annuum* L.	*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill	■		■
	Tomate	*Solanum esculentum* Dunal	*Solanum pimpinellifolium* L.	■	■	■

Tabla 1. Cultivos abordados en los experimentos que componen esta tesis. La tabla muestra la familia botánica, nombre de la especie domesticada y su ancestro de cada cultivo, así como su representación en cada capítulo (color indica su uso en el capítulo).

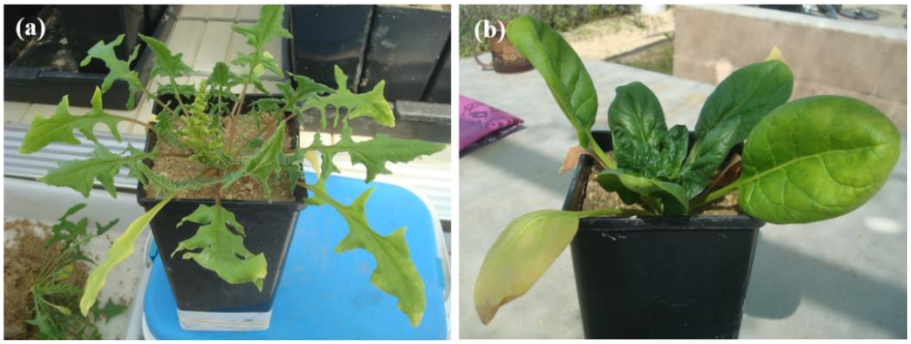


Figura 2. Ejemplo de pareja de cultivo en la espinaca, formada por el ancestro (a) y la planta domesticada (b).

El proceso de domesticación de un cultivo posee características propias que lo distinguen del proceso de domesticación de otros cultivos. Peculiaridades como: antigüedad de la domesticación, eventos de hibridación, órgano de selección, continente y bioma original del ancestro, o intensidad de selección influyen en los procesos de domesticación. Con nuestra selección de cultivos tratamos de representar esta variabilidad. Por ejemplo, incluimos cultivos que comprenden un rango amplio de periodos de domesticación, desde cultivos antiguos con más de 10.000 años, como la Avena o Lentejas, a cultivos jóvenes como la Rúcula o el Cardo. También abordamos cultivos con distintos órganos de selección, por ejemplo los frutos en el Tomate, las hojas en la Lechuga, las semillas en el Girasol, o los peciolos en el Cardo. Además, la domesticación no siempre es un proceso lineal de acumulación de diferencias con respecto al ancestro, sino que frecuentemente comprende entre una y múltiples hibridaciones con especies silvestres, como en el caso del Arroz o el Trigo. Esta gran

variabilidad entre procesos de domesticación justifica el uso de múltiples cultivos para abordar nuestros objetivos y nos permite además identificar patrones de cambio ante la domesticación.

Metodología general de los experimentos

Todos los experimentos llevados a cabo en esta tesis fueron experimentos de jardín común realizados en las instalaciones de la Universidad Rey Juan Carlos entre los años 2011 a 2014 (Figura 3). Los experimentos de jardín común consisten en cultivar plantas en un único ambiente para controlar el efecto de los factores ambientales en la expresión de caracteres de interés, de manera que sea posible asegurar que cualquier diferencia observada entre los tratamientos experimentales tiene base genética. En cada experimento, las parejas de cultivo (ancestro y especie domesticada) crecieron simultáneamente para minimizar diferencias ambientales, adecuando en lo posible el momento de siembra a la fecha recomendada para cada cultivo.

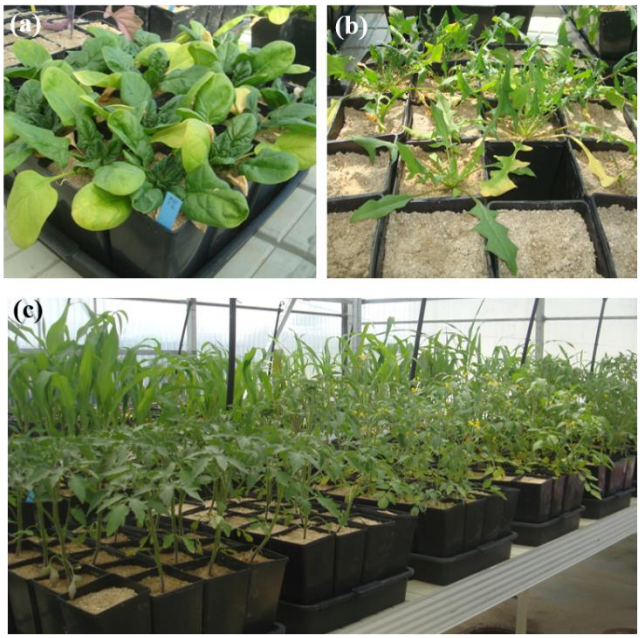


Figura 3. Pareja de planta domesticada (a) y su ancestro (b) de la espinaca durante uno de los experimentos de jardín común llevados a cabo (c).

El protocolo de cada experimento varió según los objetivos específicos, aunque mantuvieron algunas similitudes. En líneas generales, las semillas se pregerminaron en condiciones de humedad y baja temperatura durante aproximadamente una semana. Transcurrido ese tiempo, las semillas se sembraron en macetas individuales, de volumen variable según experimento, rellenas con una mezcla de arena y suelo. Además, el sustrato con el que se llenaba la maceta se completó con los tratamientos de inoculación de micorrizas (capítulo 2); o tratamiento de adición de suelo donde previamente crecieron ancestros o especies cultivadas (capítulo 3). El sustrato arenoso fue escogido por dos motivos: facilita el muestreo de raíces y, dada su pobreza de nutrientes, facilita el control de la fertilidad del suelo. La composición y concentración de nutrientes varió según el experimento: un fertilizante completo para permitir desarrollo normal de las plantas (capítulo 1, 3 y un tratamiento del 2) y un fertilizante sin fósforo para testar la colonización de micorrizas (capítulo 2). Las plantas se

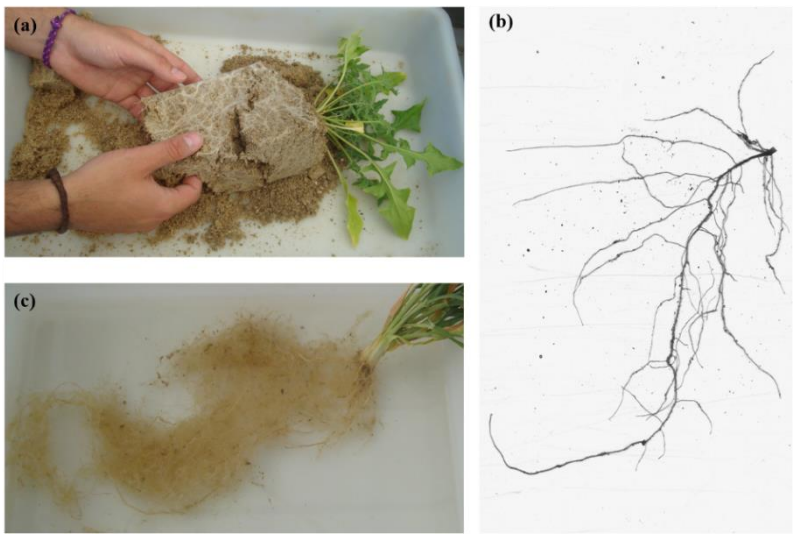


Figura 4. Muestreo de raíces en los experimentos. Desentierro y limpiado del sistema radicular (a). Imagen escaneada de una raíz para obtener los parámetros morfológicos (b). Limpieza de raíces para seleccionar fragmentos para tinción y cuantificación de colonizaciones de micorriza y nemátodos (c).

mantuvieron en condiciones de humedad óptimas para asegurar un desarrollo normal; y se movieron al azar dentro del invernadero dos veces al mes, para minimizar los efectos de la ubicación en el invernadero en el crecimiento de la planta.

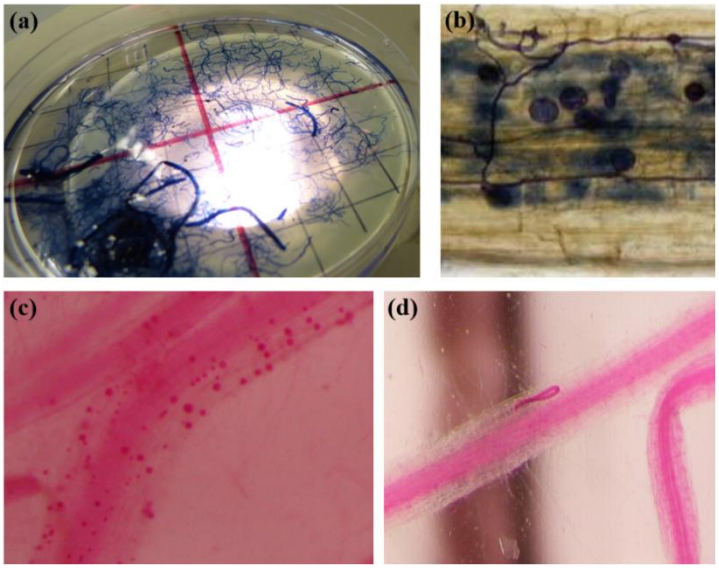


Figura 5. Estimación de colonizaciones de micorrizas (b y c) y nemátodos (d) en el experimento del capítulo 2 (a, b) y 3 (c, d,). Cuantificación del porcentaje de colonización de micorrizas en las raíces, previamente teñidas (a). Vesículas e hifas de micorrizas del capítulo 2 (b) y 3 (c). Adulto de nemátodo del capítulo 3 (d).

Aproximadamente 60 días tras la siembra, las plantas fueron sacrificadas para el muestreo, excepto en el experimento del primer capítulo, que las plantas se sacrificaron antes de que la raíz llegara al final de la maceta para evitar deformaciones. Seleccionamos de ocho a diez plantas bien desarrolladas de cada accesión. De cada planta se recogió su parte aérea para estimar biomasa; y se desenterró y limpió su raíz (Figura 4). Ésta fue escaneada para obtener los parámetros morfológicos (capítulo 1, Figura 4b); se tiñó para cuantificar colonización de micorrizas (capítulo 2 y 3) y nemátodos (capítulo 3). El método de tinción seleccionado fue diferente

entre experimentos según el objetivo fuera estimar solo micorrizas o micorrizas y nemátodos en la misma muestra. Por último, la cuantificación de la colonización de micorrizas y nemátodos se hizo por el método del entrecruzamiento de líneas (*line intersect method*) en placas Petri (Figura 5a) mediante inspección visual de presencia de estos organismos a la lupa (Figura 5b-d).

Análisis estadístico

Los experimentos con múltiples especies, como los desarrollados en esta tesis, son poderosas herramientas en la búsqueda de patrones generales (van Kleunen *et al.* 2014). Los datos que se extraen de estos experimentos presentan una gran diversidad filogenética que debe ser tratada apropiadamente en los análisis estadísticos. Por tanto, para abordar los objetivos de esta tesis, todas las técnicas empleadas controlaron la variabilidad filogenética entre los distintos cultivos incluidos en cada experimento. La más usada fueron los modelos mixtos, lineales o generalizables según la naturaleza de la variable respuesta. En este caso, la variabilidad de cultivos fue incluida en la estructura aleatoria de los modelos. Estos análisis se emplearon en todos los capítulos con el objetivo de explicar la relación directa entre diferentes variables dependientes y su variable explicativa (Tabla 2). Además, en los capítulos 1 y 2 usamos análisis filogenéticos, donde la estructura de relaciones filogenéticas entre los cultivos se incluyó en los residuos del modelo. Para ello construimos los árboles filogenéticos del conjunto de cultivos empleados en cada análisis. Usamos modelos filogenéticos generalizables por mínimos cuadrados (PGLS) para analizar la influencia de las peculiaridades de cada proceso de domesticación (órgano de selección y duración del proceso de domesticación) en el efecto de cada variable (Capítulo 1 y 2). Finalmente, en el capítulo 1 usamos análisis filogenético de ruta (*phylogenetic path analysis*)

para analizar las relaciones directas e indirectas entre variables radiculares con el tamaño de la planta y su respuesta a la domesticación.

Todos los análisis estadísticos se han llevado a cabo en el entorno y lenguaje de programación R (R Foundation for Statistical Computing, Vienna, Austria), incluyendo paquetes específicos como nlme (Pinheiro et al., 2015), MuMIn (Barton, 2014), lsmeans (Lenth, 2016), Picante (Kembel et al., 2010) and phytools (Revell, 2012).

Variable respuesta	Capítulo I	Capítulo II	Capítulo III
Biomasa de la planta	■	■	■
Diámetro medio de raíces	■	■	
Densidad tisular de raíces	■	■	
Longitud específica de raíces	■	■	
Fracción de biomasa radicular	■	■	
Ratio de longitud de raíces	■	■	
Colonización radicular de micorrizas		■	■
Concentración de fósforo		■	
Respuesta en biomasa a la micorrización		■	
Respuesta en fósforo a la micorrización		■	
Colonización radicular de nemátodos			■
Respuesta en biomasa al tipo de suelo			■
Materia orgánica del suelo			■
Biomasa microbiana suelo			■

Tabla 2. Variables respuesta empleadas en cada capítulo.

Referencias de la introducción

Barton K. 2014. MuMIn: multi-model inference.–R package ver. 1.10. 0.
Bell T, Tylianakis JM. **2016**. Microbes in the Anthropocene: spillover of agriculturally selected bacteria and their impact on natural ecosystems. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20160896.

Brown TA, Jones MK, Powell W, Allaby RG. **2009**. The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology and Evolution* **24**: 103–109.

Chen YH, Welter SC. **2007**. Crop domestication creates a refuge from parasitism for a native moth. *Journal of Applied Ecology* **44**: 238–245.

Darwin, C. 1859. On the origin of species by means of natural selection. J. Murray, London.

Darwin, C. 1868. The variation of plants and animals under domestication. J. Murray, London.

Delgado-Baquerizo M, Reich PB, García-Palacios P, Milla R. **2016**. Biogeographic bases for a shift in crop C: N: P stoichiometries during domestication. *Ecology Letters* **19**: 564–575.

Denison RF, Kiers ET, West S a. **2003**. The Quarterly Review of Biology Solutions beyond the reach of natural selection? *Review Literature And Arts Of The Americas* **78**: 145–168.

Diamond J. **2002**. Evolution, consequences and future of plant and animal domestication. *Nature* **418**: 700–707.

Diaz S, Kattge J, Cornelissen JH, Wright IJ, Lavorel S, Dray S, Reu B, … Violle C. 2016. The global spectrum of plant form and function. *Nature* **529**: 167–171.

Fuller DQ. **2007**. Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the old world. *Annals of Botany* **100**: 903–924.

Gepts P. **2004**. Crop Domestication as a Long-term Selection Experiment. *Plant Breeding* **24**: 1–44.

Grime JP, Thompson K, Hunt R, Hodgson JG, Cornelissen JHC, Rorison IH, … Whitehouse, H. 1997. Integrated Screening Validates Primary Axes of Specialisation in Plants. *Oikos* **79**: 259.

Harlan, JR. 1992. *Crops and man*. 2nd ed. Am. Soc. Agronomy, Madison, WI.

Hawkes, JG. 1983. *The diversity of crop plants*. Harvard Univ. Press, Cambridge, MA.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly

DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463-1464.

Khoury CK, Achicanoy HA. 2016. Origins of food crops connect countries worldwide. *Proc. R. Soc. B* **283**: 468–74.

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC, Mommer L. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *Journal of Ecology* **104**: 1299–1310.

Larson G, Piperno DR, Allaby RG, Purugganan MD, Andersson L, Arroyo-Kalin M, Barton L, … Fuller, D.Q. 2014. Current perspectives and the future of domestication studies. *Proceedings of the National Academy of Sciences* **111**: 6139–6146.

Lenth RV. 2016. Least-squares means: the R package lsmeans. *J Stat Softw* 69: 1-33.

Mäder P, Edenhofer S, Boller T, Wiemken A, Niggli U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic , biological) and high-input (conventional) farming systems in a crop rotation. : 150–156.

McKey DDBD, Elias M, Pujol B, Duputié A. 2012. Ecological Approaches to Crop Domestication. *Biodiversity in Agriculture: Domestication, Evolution and Sustainability.*: 377–406.

Meyer RS, DuVal AE, Jensen HR. 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *The New Phytologist* **196**: 29–48.

Milla R, Osborne CP, Turcotte MM, Violle C. 2015. Plant domestication through an ecological lens. *Trends in Ecology and Evolution* **30**: 463–469.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A, Ma P. 2003. Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. *Applied and Environmental Microbiology* **69**: 2816–2824.

Pieterse CMJ, de Jonge R, Berendsen RL. 2016. The Soil-Borne Supremacy. *Trends in Plant Science* **21**: 171–173.

Pinheiro J, Bates D, DebRoy S, Sarkar D. 2015. nlme: Linear and Nonlinear Mixed Effects Models R package version 3.1–117.

Purugganan MD, Fuller DQ. 2009. The nature of selection during plant domestication. *Nature* **457**: 843–848.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Reich PB. **2014**. The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology* **102**: 275–301.

Revell LJ. 2012. phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3: 217–223.

Rindos, D. 1984. *The origins of agriculture*. Academic Press, San Diego, CA.

Roucou A, Violle C, Fort F, Roumet P, Ecarnot M, Vile D. **2018**. Shifts in plant functional strategies over the course of wheat domestication. *Journal of Applied Ecology* **55**: 25–37.

Schmidt JE, Bowles TM, Gaudin ACM. **2016**. Using Ancient Traits to Convert Soil Health into Crop Yield: Impact of Selection on Maize Root and Rhizosphere Function. *Frontiers in plant science* **7**: 373.

Smith BD. **2011**. General patterns of niche construction and the management of 'wild' plant and animal resources by small-scale pre-industrial societies. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**: 836–848.

Thiele-Bruhn S, Bloem J, Vries FT de, Kalbitz K, Wagg C. **2012**. Author â€™ s personal copy Linking soil biodiversity and agricultural soil management § So. *Environmental Sustainability* **4**: 523–528.

Turcotte MM, Turley NE, Johnson MTJ. **2014**. The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytologist* **204**: 671–681.

Verbruggen E, Toby Kiers E. **2010**. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evolutionary Applications* **3**: 547–560.

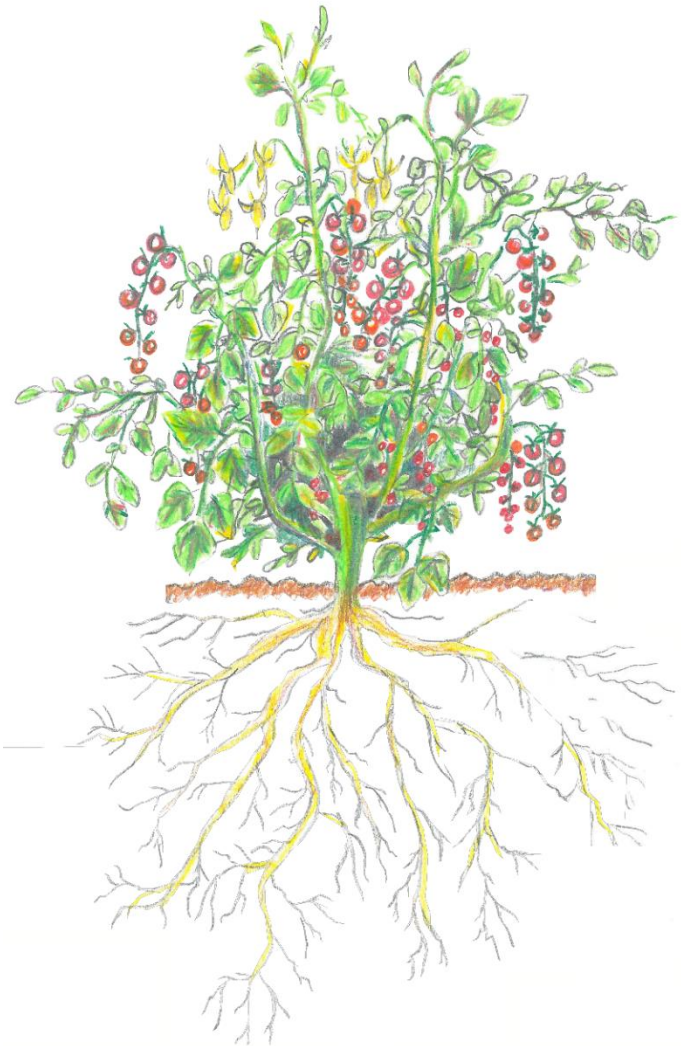
Weiss E, Kislev ME, Hartmann A, Weiss E, Kislev ME, Hartmann A. **2017**. Autonomous Cultivation before Domestication Linked references are available on JSTOR for this article : Autonomous Cultivation Before Domestication. **312**: 1608–1610.

Whitehead SR, Turcotte M, Poveda K. **2016**. Domestication impacts on plant-herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B*.

Willcox G, Fornite S, Herveux L. **2008**. Early Holocene cultivation before domestication in northern Syria. *Vegetation History and Archaeobotany* **17**: 313–325.

Zohary D. **2004**. Unconcious selection and the evolution of domesticated plants. *Economic Botany* **58**: 5–10.

Capítulos / Chapters



Capítulo 1 / Chapter 1

Root traits of herbaceous crops: pre-adaptation to cultivation or evolution under domestication?

Nieves Martín-Robles, Javier Morente-López, Grégoire T. Freschet, Hendrik Poorter, Catherine Roumet, Rubén Milla

Manuscript submitted in *Functional Ecology*

Summary

Agricultural fields are commonly characterized by high nutrient and water availabilities, which are favorable for plant growth. Plants with resource acquisitive strategies are typically the best performers under such conditions. We asked whether crop plants show root traits typical of resource acquisitive strategies and whether this strategy is primarily a result of their evolution under domestication or of the early selection of successful candidates for domestication.

We studied a set of 30 crop species and their wild progenitors. We set up a greenhouse experiment to measure five root traits: root thickness, root tissue density, specific root length (SRL), root mass fraction (RMF) and root length ratio. In addition, we compiled data from other wild herbaceous species, growth in similar conditions to our experiment, to place the root traits of our crops in the context of wider botanical variation.

Wild progenitors had thicker and less dense roots, with higher RMF and lower SRL, than other wild herbs. Thicker and less dense roots are indicative of fertile soils, which suggests that wild progenitors could already have been adapted to agricultural conditions. Additionally, we found that domestication generally increased total plant dry mass, but none of the root traits evolved consistently towards a more resource-acquisitive strategy after domestication across all species. Root trait values differed between progenitors and crop species for most pairs surveyed, but this occurred in diverse directions depending on crop species.-Such differences were independent of phylogeny, functional group or variability in the domestication processes, such as timing of the domestication event or organ under focal artificial selection.

Our comparative study revealed that the root phenotype exhibited by wild progenitors (thick roots with low density and SRL), when compared with other wild herbs, was in accordance with plants typical from fertile habitats. However, none of the root traits reacted to domestication in accordance with evolution

towards fast-growth strategies. Thus, the good adaptation of crop root phenotypes to the fertile conditions of agricultural fields might be largely determined by early choices of wild species, rather than by further evolution under domestication.

Key words

Crop progenitors, domestication, functional traits, origins of agriculture, plant resource economics, root economics spectrum, root tissue density, specific root length.

Introduction

Plant domestication involves selection for, and modification as well as long-term use of traits regarded as favorable by humans in wild species (Evans, 1996; Gepts, 2004). Major changes commonly associated with artificial selection include increased yield for the organs of interest (*e.g.* seeds or fruits), strong apical dominance, and loss of seed dispersal and seed dormancy mechanisms (Evans, 1996; Gepts, 2004; Abbo et al., 2014). The consequences of crop domestication on plant traits also include decreased herbivore defense (Turcotte, Turley & Johnson, 2014; Whitehead, Turcotte & Poveda, 2016), higher stomatal densities at the upper side of leaves (Milla, Diego-vico & Martín-Robles, 2013) or increased nitrogen and phosphorus concentration in leaves (Delgado-Baquerizo, Reich, García-Palacios, & Milla, 2016). Some consequences of crop domestication, such as higher leaf nitrogen contents (Delgado-Baquerizo, Reich, García-Palacios, & Milla, 2016) and higher relative growth rates (Preece et al., 2017), would be typical of fast-growing resource-acquisitive strategies (Lambers & Poorter, 1992; Craine, 2009; Reich, 2014). Thus, domestication might have led to the evolution towards fast-growing plants with resource-acquisitive strategies in response to agricultural conditions (Chapin, 1980; Craine, 2009; Milla, Osborne, Turcotte, & Violle, 2015).

One obvious cause of these domestication effects is recurrent natural selection by the farmers (Denison Kiers & West, 2003; Zohary, 2004; Milla, Osborne, Turcotte, & Violle, 2015). Such selection in agricultural fields might have led to adaptations in above and belowground traits, because croplands differ from wild habitats in the availability of resources (nutrients and water), or in the intensity and frequency of disturbances (Mckey, Elias, Pujol, & Duputié, 2012; Milla, Osborne, Turcotte, & Violle, 2015).

Nevertheless, an alternative would be that wild progenitors may also have shown acquisitive strategies before domestication started. Human society has been shaping the ecosystems around their settlements before agriculture started changing the environment (Smith, 2007). The new environmental conditions would be characterized by high fertility and increase of disturbance (fires, selective plant culling) and would therefore have led to the modification of diversity, enhancing the short-term productivity of herbaceous plants (Smith, 2011). The 'Dump Heap' hypothesis suggests that early domestication started with species growing near human settlements (Sauer, 1952; Zeven, 1973; Hawkes, 1983). If so, successful candidates of domesticated species would be pre-adapted to cultivation conditions (Hawkes, 1983) with ruderal, generalist and fast-growing characteristics (Mercuri, Fornaciari, Gallinaro, Vanin, & di Lernia, 2018). In support of this idea, a few studies have shown higher seed mass, faster growth rates, higher specific leaf areas, and higher nitrogen concentrations in wild progenitors than in other wild species, which fits with fast-growing strategies for crops´ ancestry (Cunniff et al., 2014; Milla, Osborne, Turcotte, & Violle; Preece et al., 2015). Thus, theoretical and empirical evidence based on aboveground traits suggest that domesticated species have fast acquisitive strategies, either as a consequence of pre-adaptions to the agricultural environment and/or as evolution under cultivation. While our knowledge is pretty substantial when it comes to how domestication affected above-ground plant traits, we have only very limited insight into what happened below-ground. (Bishopp & Lynch, 2015; Lynch & Brown, 2012).

A diversity of physiological and morphological root traits has been put forward as indicative of root resource acquisitive strategies (see Freschet & Roumet, 2017 for a review). The root length ratio (RLR; see Table 1 for

abbreviations and definitions) and its determinants – root mass fraction (RMF), specific root length (SRL), mean root diameter (MRD) and root tissue density (RTD) – are among the most important morphological and allocational traits determining root nutrient acquisition capacity (Ryser & Lambers, 1995). Fast acquisitive strategies are generally characterized by low structural investment (Ryser 1996) in roots (low RMF, MRD and RTD). Poorter and Ryser (2015) suggested a general model of root trait coordination (Fig. 1a) where increasing soil fertility would result in larger plants, with thicker and less dense roots, with contrasting effects on SRL (see also Freschet, Swart, & Cornelissen, 2015a). Overall, as fertility would decrease the proportion of biomass allocated belowground (RMF), the model predicts that, via indirect effects, RLR would generally decrease with fertility. Since agricultural and pre-agricultural environments are mostly fertile ecosystems (Denison, Kiers & West, 2003; Mckey, Elias, Pujol, & Duputié, 2012), one could therefore expect that crop evolution should have followed the pathway of phenotypic adjustments proposed by Poorter and Ryser (2015). If correct, novel agricultural conditions would trigger larger plants with higher MRD and lower RTD, implying contrasting impacts on SRL and, together with the lower RMF would determine the RLR.

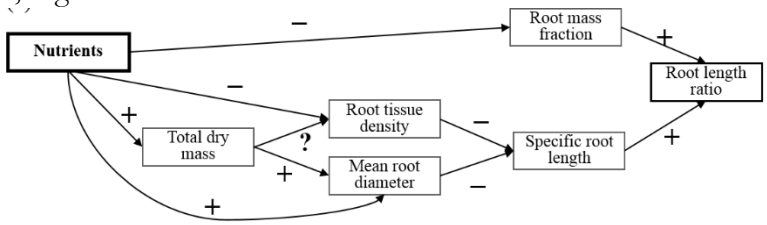


Figure 1. Conceptual model of Poorter and Ryser (2015) for root inter-trait relationships, and the effect of nutrient availability. The predicted direction of each effect is indicated with + or –. Nutrient availability increases total dry mass and reduces root mass fraction. Larger plants generally have a higher mean root diameter, but their effect on root tissue density is less pronounced. Through the predicted increase of mean root diameter, the specific root length decreases. As a consequence, the root length ratio decreases, achieving less root length per unit of total plant mass.

In this study we investigated whether domesticated plants show root trait values typical of resource acquisitive strategies and whether this strategy is primarily a result of their evolution under domestication or of the early selection of successful candidates (or wild progenitors) for domestication. These questions will be arisen by: i) comparing root traits of 30 domesticated species and their wild progenitors with root traits of other wild herbaceous species taken in global data bases, ii) examining the domestication effect on plant biomass and root traits and iii) testing whether the response of root traits to domestication is consistent with the causal model of Poorter and Ryser (2015). We hypothesized that i) wild progenitors already show trait values typical of plants adapted to fertile habitats, ii) domestication has a similar effect on root traits as fertility. Domestication would thus have selected larger plants with higher MRD, but lower RTD, lower SRL, and RMF values as compared to their progenitors which are expected to show more acquisitive root traits than other wild species.

Materials and Methods

Study system

To maximize the generality of our results, we worked with a phylogenetically diverse set of 30 herbaceous crop species and their most likely wild progenitors (Table 2). Our set includes seven grasses, 11 legumes and 12 non-leguminous forbs, with different domestication geographies and histories. We obtained seed lots for each of these 30 crops: one belonging to an accession of a common domesticated cultivar and another from the most likely wild progenitor. More information about the species and accessions (references of domesticated cultivar and wild progenitor assignment, seed donor banks accession identifier, time under

domestication and organ under selection) can be found in Table S1. In addition, to place the root traits of wild progenitors and domesticated plants in the context of global herbaceous variation, we compiled root data from taxonomically

Trait	Abbreviation	Definition	Units
Total dry mass	TDM	plant mass	mg
Mean root diameter	MRD	root thickness	mm
Root tissue density	RTD	root mass/root volume	g/cm^3
Specific root length	SRL	root length/root mass	m/g
Root mass fraction	RMF	root mass/plant mass	g/g
Root length ratio	RLR	root length/plant mass	m/g

Table 1. Abbreviations, definitions and units of the traits measured in the experiment.

Growth conditions

For logistical reasons, the 30 crop pairs were grown staggered from January to June 2012, matching the most appropriate time of the year for the performance of each crop. The two accessions (domesticated plant and wild progenitor) belonging to each pair were simultaneously grown at the same spatial location within the greenhouse (located in Móstoles, central Spain, 40º18´48´´N, 3º52´57´´W). To avoid plants from becoming severely pot-bound (Poorter, Bühler, Van Dusschoten, Climent, & Postma, 2012), we built special long containers to allow the growth of root systems for several weeks before reaching the bottom of the container. A round plastic cylinder (42 cm deep, 8 cm diameter) was embedded inside, and down to the bottom end of a 25 cm long Jumbo Rootrainer (Haxnicks Ltd., Wiltshire, UK), resulting in a final container of 42 cm depth x 50 cm^2 area (2.1 L, Fig. S1). The bottom of this final container was removable without root or substrate disturbance, to analyze the depth of the deepest root (Fig. S1). Containers

were filled with pure sand to facilitate recovery of the complete root system. Finally, plants were fertilized twice a week with 50 mL of a complete nutrient solution to allow normal development in the sandy substrate and watered through regular automatic water sprinkling as needed to maintain plants under optimal growth conditions.

Plant root harvest and trait measurements

Every second day we checked the depth of the roots in the container by opening the removable bottom. As soon as the roots of a given species reached the bottom of the container, the complete set of individuals belonging to a species pair were harvested. At that time, plants were 30 - 40 days old after germination, the exact time depending on the crop pair. We harvested 5-10 (median 9) healthy and well developed plants per accession (wild progenitor and domesticated plant), and carefully cleaned the whole root system. The whole root system of each individual was transferred to a transparent tray filled with water, where the root branches were carefully spread out to avoid overlapping and was scanned as greyscale images at a resolution of 400 dpi (Epson scan GT 15000). Total root length (m), root mean diameter (mm), and root volume (cm^3) were determined for the whole root system using a scanner-based, digital image analysis system (WinRHIZO; Regents Instruments, Quebec City, Canada; Arsenault, Poulcour, Messier & Guay, 1995). Following root scanning, roots and the aboveground part of each plant were oven dried (60ºC) and weighed to estimate: total plant dry mass (mg), root tissue density (RTD, g root cm^{-3}root), specific root length (SRL, m root g^{-1} root), root mass fraction (RMF, g root g^{-1} plant) and root length ratio (RLR, m root g^{-1} plant) (Table 1). A total of 527 plants were phenotyped.

Functional group	Family	Crop identity	Domesticated species	Progenitor species
Forb	Amaranthaceae	Chard	*Beta vulgaris* L.	*Beta vulgaris* L.
	Asteraceae	Cardoon	*Cynara cardunculus* L.	*Cynara cardunculus* L.
		Sunflower	*Helianthus annuus* L.	*Helianthus annuus* L.
	Brassicaceae	Cabbage	*Brassica oleracea* L.	*Brassica oleracea* L.
		Rucola	*Eruca vesicaria* (L.) Cav.	*Eruca vesicaria* (L.) Cav.
	Cucurbitaceae	Cucumber	*Cucumis sativus* L.	*Cucumissativus* L.
	Linaceae	Flax	*Linum usitatissimum* L.	*Linum usitatissimum* L.
	Malvaceae	Cotton	*Gossypium hirsutum* L.	*Gossypium hirsutum* L.
	Solanaceae	Chillipepper	*Capsicum baccatum* L.	*Capsicum baccatum var. pendulum (Willd.) Eshbaugh*
		Pepper	*Capsicum annuum* L.	*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill
		Tomato	*Solanum esculentum* Dunal	*Solanum pimpinellifolium* (L.) Mill
Grass	Poaceae	Barley	*Hordeum vulgare* L.	*Hordeum spontaneum* K.Koch
		Corn	*Zea mays* L.	*Zea mexicana* (Schrad.) Kuntze
		Milllet	*Pennisetum glaucum* (L.) R.Br.	*Pennisetum glaucum* (L.) R.Br.
		Oat	*Avena sativa* L.	*Avena sterilis* L.
		Rye	*Secale cereale* L.	*Secale cereale* L.
		Sorghum	*Sorghum sudanense* (Piper) Stapf	*Sorghum bicolor* (L.) Moench
		Wheat	*Triticum durum* Desf.	*Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf.
		Rice	*Oryza sativa* L.	*Oryza rufipogon Griff.*
Legume	Fabaceae	Bean	*Phaseolus lunatus* L.	*Phaseolus lunatus* L.
		Chickpea	*Cicer arietinum* L.	*Cicer reticulatum* Ladiz.
		Cowpea	*Vigna unguiculata* (L.) Walp.	*Vigna unguiculata* (L.) Walp.
		Lentil	*Lens culinaris* Medik.	*Lens culinaris* (Boiss.) Ponert
		Lupin	*Lupinus luteus* L.	*Lupinus luteus* L.
		Pea	*Pisum sativum* L.	*Pisum sativum* subsp. *elatius* (M.Bieb.) Asch. & Graebn
		Soybean	*Glycine max* (L.) Merr.	*Glycine max* subsp. *soja* (Siebold & Zucc.) H.Ohashi
		White clover	*Trifolium repens* L.	*Trifolium repens* L.
		Faba bean	*Vicia faba* L.	*Vicia narbonensis* L.
		Lucerne	*Medicago lupulin*a L.	*Medicago lupulina* L.
		Vetch	*Lathyrus sativus* L.	*Lathyrus cicera* L.

Table 2. Functional group, botanical family, common and botanical names of each of the 30 domesticated species and wild progenitors used in this experiment.

Data gathering

To test whether the roots of domesticated plants and wild progenitors were different to those of other wild herbaceous species, we compiled root data from to two global databases of root traits of wild herbaceous species. Data from plants growth in pots in controlled conditions (indoors or outdoors), were carefully selected to ensure the comparability with the data of the 30 crops. The Rhizopolis-db, a global database of fine root traits (details in Freschet et al., 2017) was used for comparisons of MRD (145 species; 53% forbs, 30% grasses and 17% legumes), RTD (141 species; 54% forbs, 30% grasses and 16% legumes) and SRL (99 species; 43% forbs, 36% grasses and 20 % legumes). The RMF database (398 species; 49% forbs, 42% grasses and 9% legumes) was taken from Poorter et al. (2015).

Statistical analyses

Prior to hypotheses testing we imputed missing values (1.6%), which were randomly distributed along the data, using multivariate imputations with chained equations (Nakagawa & Freckleton, 2008; Penone et al., 2014) with the R package "mice" (Buuren & Groothuis-Oudshoorn, 2011). In addition, five individuals with extreme trait values were excluded from the data. Finally, all subsequent analyses were ran with 522 individuals, and trait data were $\log_{10}$-transformed to meet normality assumptions and homogeneity of variance of models´ residuals. All statistical analyses were performed with the R software (R Core Team, 2013).

To test whether the roots of domesticated plants and wild progenitors were different to those of other wild herbaceous species, we performed phylogenetic generalized least squares models (PGLSs) comparing wild progenitors and domesticated species with databases of root traits of wild herbaceous species. The root traits: MRD, RTD, SRL and RMF were

included as response variables in the PGLS models. Plant type (wild progenitor, domesticated plant or other wild species) was included as explanatory variable. Additionally, we analyzed whether differences in the root traits along plant types varied for grasses, legumes and forbs (functional group). For doing so, we included functional group and the interaction with plant type as explanatories in the models. PGLS models incorporate phylogenetic correlation structure in model residuals to account for phylogenetic non-independence of species data points (Symonds & Blomberg, 2014). To run the PGLS regressions, we built a phylogenetic tree for each root trait containing the species of each database and the 30 crops pairs. To do so, each phylogenetic tree was derived from a largest reference tree of the angiosperms (Zanne et al., 2014), with the drop.tip function of 'phytools' package (Revell, 2012). Species not represented in the reference tree were replaced by other species of the same genus presented in the reference tree, only when there was one or two species representatives of the genus in the data set; or removed from the data sets when there were more species representing the genus. The resulting trees did not have polytomies. PGLSs were implemented using the gls function of the 'picante' package (Kembel et al., 2010). Finally, post hoc test with pairwise comparison among levels of the fixed effects factors and the interaction were conducted using the phylANOVA function of the 'phytools' package (Revell, 2012).

To assess the effect of domestication on each root trait separately, we used linear mixed effect models. The dependent variables were the five root traits and total plant dry mass (TDM). In all models, domestication status (domesticated plant or wild progenitor) was included as fixed factor. Crop identity (30 crops, Table 2) was included as a random effect over the intercept of the model, and as a random effect over domestication status

(random slope effect, analogous to an interaction term in fixed effects models). In addition, we analyzed whether domestication effects were different for grasses, legumes, and forbs. For doing so, we included functional group and its interaction with domestication status as fixed effect terms in the models. All models were run with the lme function of the "nlme" R package (Pinheiro et al., 2015). The significance of the fixed factors was tested with type III analysis of variance, with the mixed function of the 'afex' package (Singmann, Bolker, & Westfall, 2015). The mixed function fits the complete model and creates reduced versions removing a single effect, then compares the reduced model to the complete model. In order to assess goodness of fit, we obtained the conditional R^2 (variance explained by random and fixed factors) and marginal R^2 (the variance explained by fixed factors) of the models following Johnson (2014), using the R package "MuMIn" (Bartón, 2013).

Finally, to test how domestication changed root traits and the consequences thereof for the whole root phenotype, we used the multivariate model proposed by Poorter and Ryser (2015), and tested it using path analyses (Shipley, 2009). The original model predicts the response of root traits to nutrient availability (Fig.1a), but since we hypothesized that evolution under domestication occurs in high fertility habitats (Denison, Kiers & West, 2003), we replaced "nutrient availability" by "domestication status" (Fig. 1b). To test whether our data fit the Poorter and Ryser (2015) model, we conducted a phylogenetic confirmatory path analysis. Phylogenetic analysis was selected to account for non-independence of data due to phylogenetic relatedness of the crop species (González-Voyer & Von Hardenberg, 2014). In phylogenetic path analysis, the predicted relationships between the variables are translated into models and analyzed using PGLS with phylogenetic signal (Pagel's lambda) estimated with maximum likelihood.

To conduct the PGLS we pruned the large dated angiosperm phylogeny tree (Zanne et al., 2014), to our set of genera using the "phytools" R package (Revell, 2012). The significance of the paths was calculated using a d-sep approach (Shipley, 2009), based on an acyclic graph that depicts the hypothetical relationships and independence claims between variables. The d-seps are translated into models and analyzed using PGLS. Likewise, we assessed the goodness of fit of the data to the path model using the associated p-values with the Fisher's C statistic (Shipley, 2009). The standardized path coefficients were obtained from PGLS (Grace & Bollen, 2005). In addition, we estimated the coefficients and significance of indirect and total effects of domestication on each trait in the path diagram (Grace & Bollen, 2005). The indirect effects were calculated by multiplying all the path coefficients that link the domestication variable with each variable of the model, and total effects were computed as the sum of direct and indirect effects (Grace & Bollen, 2005). The significance of the total effects of domestication on each root trait was calculated with the sum of the variance associated to each direct and indirect effect.

Results

Root functional differences between domesticated species, wild progenitors and other wild herbaceous species

To see how wild progenitors and domesticated plants were relative to wild plants in other experiments, we compared our data with large compilations from the literature. Wild progenitors and domesticated plants significantly differed from other wild herbaceous species for the four root traits analysed (plant type: $P < 0.05$, Fig. 2), irrespective of functional group (functional group: $P > 0.05$, Table S2). The wild progenitors and domesticated

accessions of this experiment had thicker and less dense roots in comparison with the data from wild herbaceous species, with lower SRL scores, and higher allocation to root biomass (Fig. 2).

Domestication and crop identity effects on TDM and root traits

We found a general increase of total dry mass after domestication (Table 3, Fig. 3a). TDM ranged from 20-400 mg for wild progenitors and 50-800 mg for domesticated accessions (Table S3). The response to domestication varied among crops, as indicated by the variance associated with crop identity (Table 3). In addition, the response to domestication was more positively pronounced for larger wild progenitors (correlation term: crop identity x domestication status = 0.63), such as bean or cucumber; and was even slightly negative for smaller progenitors, such as white clover or Rucola (Fig.3a). We also found a stronger response to domestication in legumes, which increased TDM after domestication more than grasses and forbs (Fig. 4a).

None of the five root traits showed a significant response to domestication across species (Domestication effect $P > 0.15$, Table 3). The variance associated with the random structure indicated a wide variability in the response to domestication among the 30 pairs of crops (Figs. 3b-f). For example, RMF increased with domestication in crops such as soybean or chickpea, but decreased in others such as cabbage or oat (Table S3, Fig. 3e). MRD, SRL, RMF and RLR was significantly affected by functional group (Table 3, Fig. 3b, d, e and f). Similarly, the response to domestication of each functional group was insignificant for the five root traits (interaction domestication status x functional group, Table 3, Figs. 4b-f).

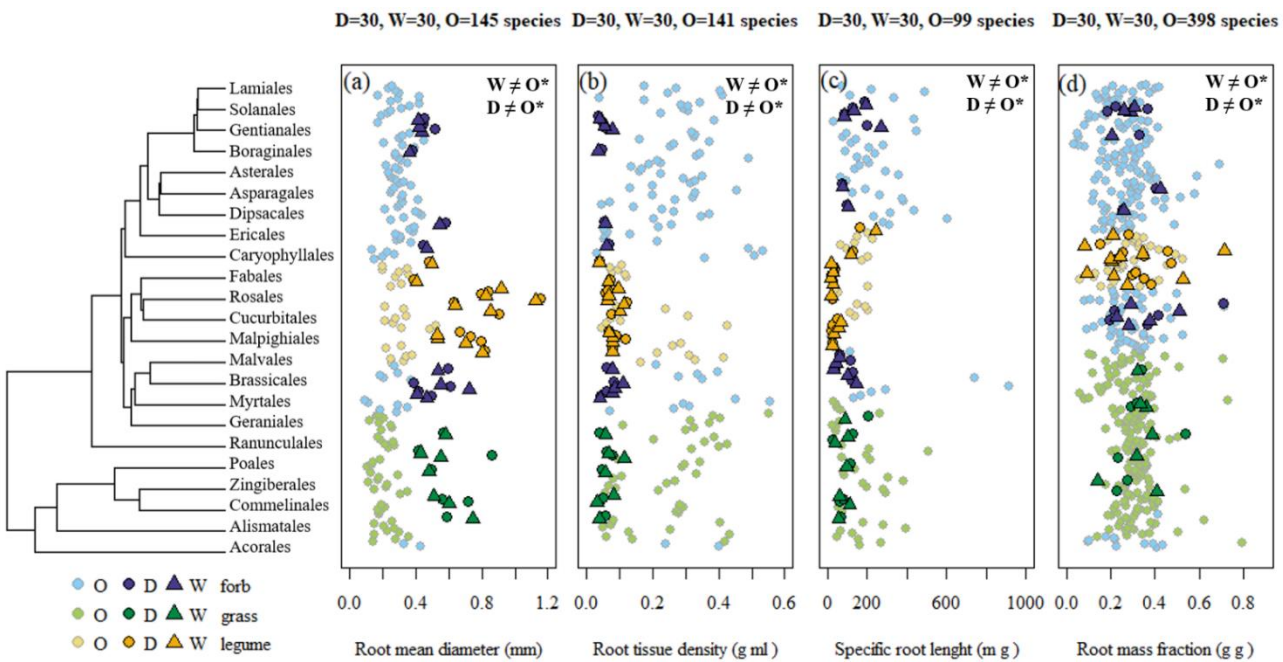


Figure 2. The domesticated and wild progenitor species of this study in the context of botanical diversity of four root traits: mean root diameter w(a), root tissue density (b), specific root length (c) and root mass fraction (d). The symbols represent the mean score of a given species: domesticated (squares), wild progenitors (triangles) and global database (circles), sorted by phylogeny (phylogenetic tree on the left side). Colors of the symbols correspond to functional group ascriptions: forbs (blue), grasses (green) and legumes (yellow). The total number of species is indicated on top of each plot. Statistically significant differences (*, $P<0.05$; ns, $P>0.05$) among domesticated (D), wild progenitor (W) and other wild species (O), extracted from post hoc test, are displayed in the upper left corner.

	Fixed effects				Random effects			
	Domestication effect (Dom)	**Functional group (FG)**	**Dom x FG**	**R^2m**	**Crop identity (Crop)**	**Crop x Dom**	**Residual**	**R^2c**
	F	F	F		variance	variance	variance	
TDM (mg)	11.15**	1.82	1.84	0.14	0.33	0.28	0.14	0.85
MRD (mm)	2.63	7.72**	0.67	0.29	0.04	0.02	0.01	0.91
RTD (g/cm^3)	2.69	1.63	0.28	0.07	0.01	0.01	0.01	0.73
SRL (m/g)	0.04	8.60**	0.45	0.28	0.25	0.20	0.13	0.85
RMF (g/g)	0.08	4.94*	0.22	0.15	0.02	0.03	0.02	0.73
RLR (m/g)	0.00	7.97*	0.29	0.27	0.27	0.22	0.13	0.87

Table 3. Effect of domestication on total plant dry mass (TDM) and root traits: mean root diameter (MRD), root tissue density (RTD), specific root length (SRL), root mass fraction (RMF) and root length ratio (RLR), resulted from the linear mixed-effect models. The table shows the *F* value and significance (*, $P<0.05$; **, $P<0.01$) of domestication effect, functional group and the interaction domestication status x functional group. The variance of the model explained by the fixed effects is indicated by R^2marginal (R^2m). The variances associated with the random effects are indicated by the terms: crop identity, the effect of crop identity on the response of domestication (i.e. random effect on the slope) and the residual variance. Finally, the variance explained by both: random and fixed effects are presented with the R^2conditional (R^2c).

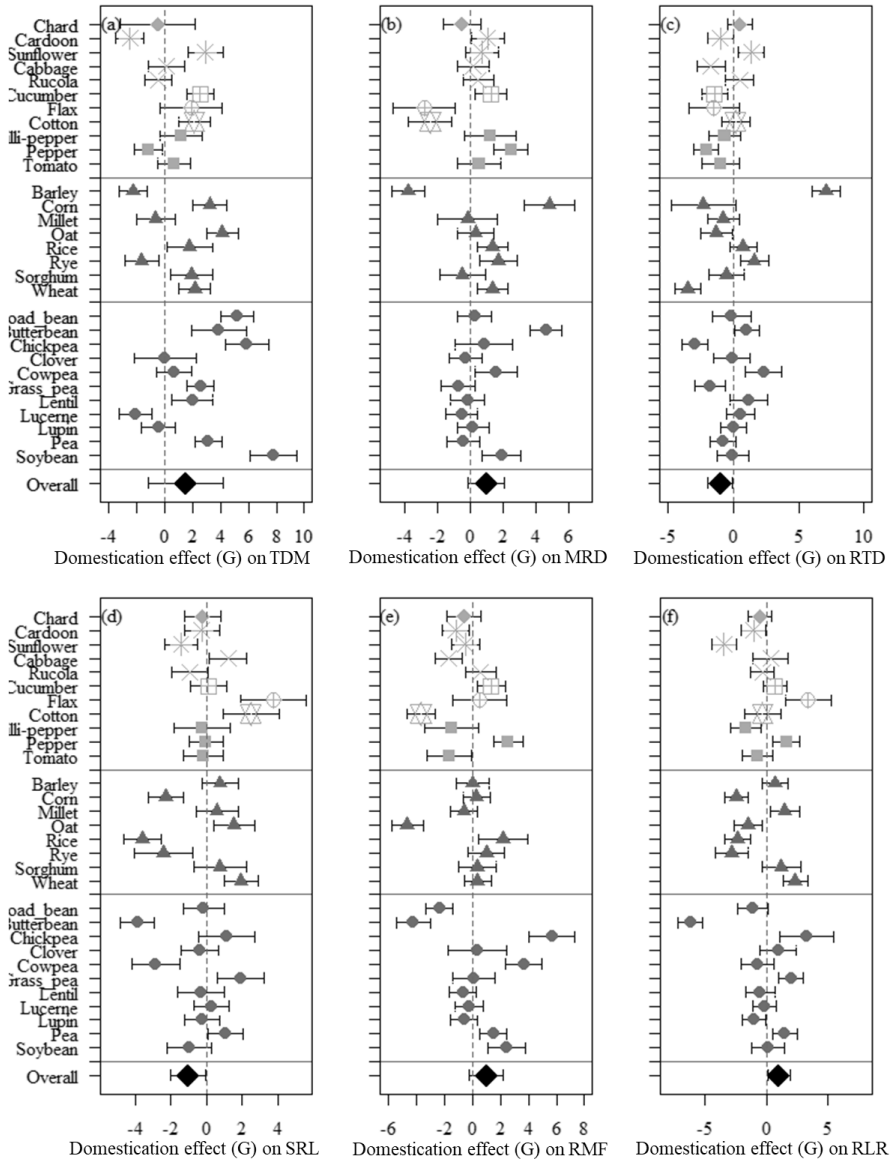


Figure 3. Effect of domestication on on total dry mass (TDM, a), mean root diameter (MRD, b), root tissue density (RTD, c), specific root legth (SRL, d), root mass fraction (RMF, e) and root length ratio (RLR, f). The symbols show the domestication effect size estimated by Hedges´G and 95% confidence intervals for each crop. The overall effect of domestication on each trait, taken from results of mixed models (Table 3), is indicated in each graph with a black diamond. Colors of the points correspond to functional group: forb (blue), grass (green) and legume (yellow) and the shapes indicate the botanical families.

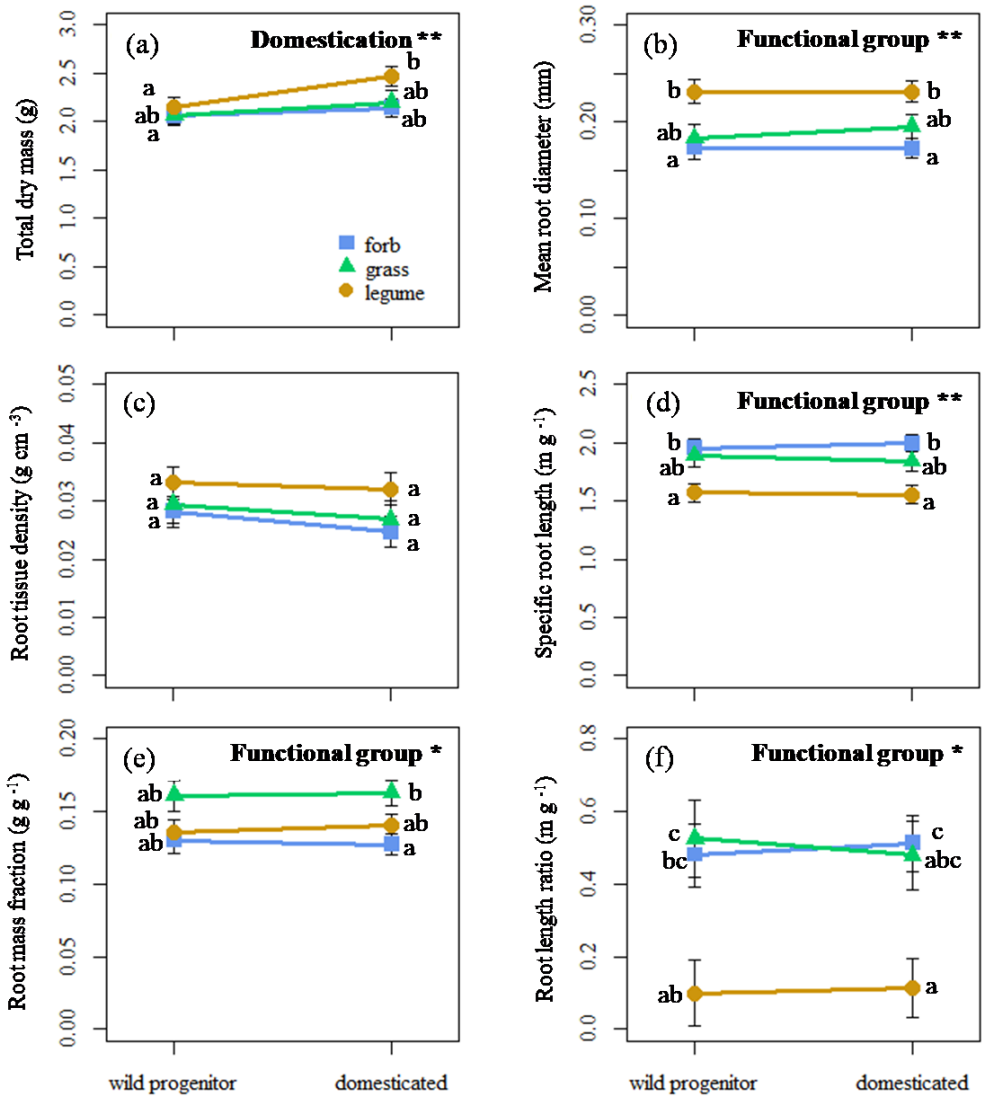


Figure 4. Evolution of total plant dry mass (a) and root traits: mean root diameter (b), root tissue density (c), specific root length (d), root mass fraction and root length ratio (f) under domestication, depending on functional group affiliation: forbs (blue squares), grasses (green triangles) and legumes (yellow dots). The symbols and error bars show the estimated least squares values means and 95% confidence limits, respectively, obtained by mixed effect models. The significances (*, $P<0.05$; **, $P<0.01$) of domestication and functional group, as taken from table 2, are displayed in the right corner of each graph.

Multi-trait response of roots to domestication

Plants increased their total dry mass in response to domestication (path = 0.27, *P*= 0.004, Fig. 1b). However, MRD, RTD and RMF were not directly affected by domestication (*P*> 0.05, Fig. 1b). The overall goodness of fit of the data to the theoretical model was high (C-statistic of 20.6 and associated *P* 0.55, Fig. 1b). The relationships between TDM and root traits fitted the *a priori* model (Poorter & Ryser, 2015; Fig. 1a), with the exception of the relationship between TDM and RTD (see significance and path scores in Fig. 1b). Larger plants had thicker fine roots (MRD, path = 0.33, *P*= 0.003, Fig. 1b). Both MRD and RTD had negative effects on SRL (path = -0.76 and *P*< 0.001 for MRD; path -0.5 and *P*< 0.001 for RTD, Fig. 1b) and RLR was more dependent on changes in SRL than in RMF (path = 0.95, *P*< 0.001, and path = 0.27, *P*< 0.001, respectively, Fig. 1b). In line with univariate analyses, domestication had negligible effects on root traits via indirect effects. The positive effect on plant size driven by domestication was not strong enough to trigger significant net effects on MRD, RTD, SRL and RMF (Fig. 5). from standardized path coefficients, taken from Fig. 1b

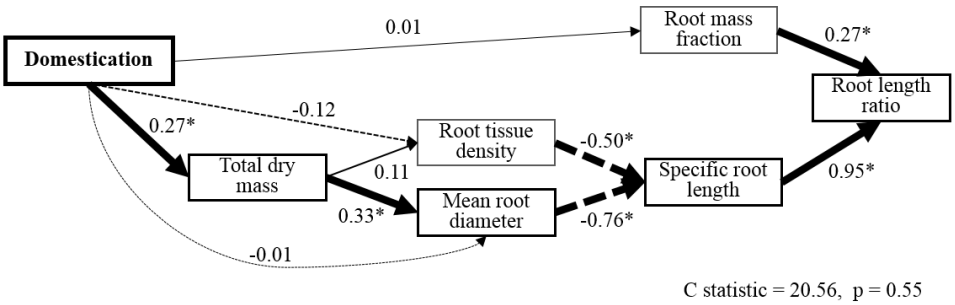


Figure 6. Fit of the domesticated plant and wild progenitor dataset to the conceptual model (a) using phylogenetic path analysis. Here, the nutrient availability effect is replaced by the domestication effect, as argued in the Introduction Section. Standardized path coefficients (obtained from phylogenetic generalized least squares models) are shown in each arrow. Negative paths coefficients are indicated with dashed arrows. Statistically significant paths (*P*<0.05) are marked in bold and an asterisk. The *P* value associated to the C-statistic is obtained using the *P* values of the conditional independencies tested (see Shipley, 2002). *P*> 0.05 indicates that the data fits the model.

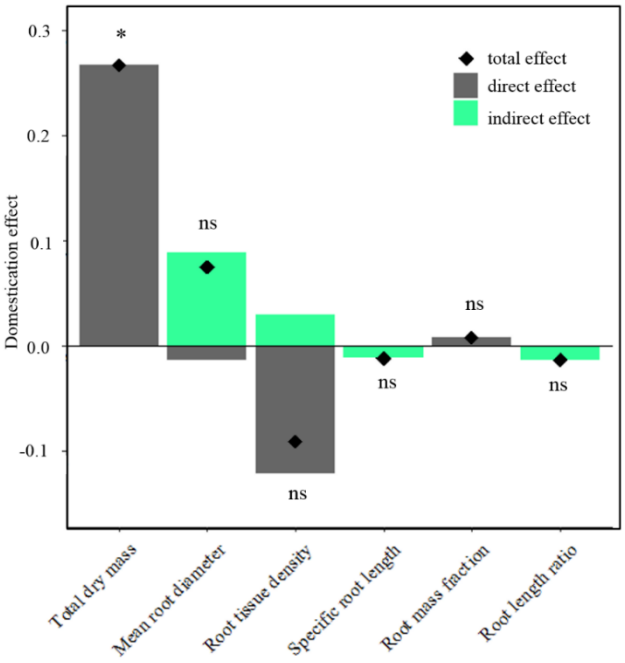


Figure 5. Effect sizes of the direct (grey bars), indirect (green bars), and total effects (sum of direct and indirect effects, black diamond) of domestication on total plant dry mass, mean root diameter, root tissue density, specific root length, root mass fraction, and root length ratio. All the effects were calculated

Discussion

Based on the screening of root traits of a uniquely large set of crop species, our analyses revealed new correlates of plant domestication. Specifically, we found that the evolution towards larger plants during domestication implies correlated evolution of thicker roots. However, since (i) the direct effect of domestication on plant size, and of plant size on root thickness, were modest, and (ii) indirect effects are small, due to their multiplicative nature; the overall effect of domestication on root thickness was of small magnitude. Additionally, root trait responses to domestication were diverse among the several crop species. That variation was however unrelated to phylogenetic or peculiarities of domestication process of the 30 crops. More interestingly, we showed that root traits of domesticated plants and of their wild progenitors are not a random sample of global functional trait

variation of other wild herbaceous species; they are biased towards trait scores indicative of plants adapted to highly fertile conditions. This result leads us to suggest that the crop root phenotypes, and their adaptability to agricultural habitats, were mainly determined by early selection of wild species which were already pre-adapted to highly fertile and frequently disturbed habitats, rather than by further evolution with domestication. These results have important implications for our understanding of resource acquisition strategies of crop roots and portend applied approaches to develop improved cultivars.

The roots of crop wild progenitors were pre-adapted to agricultural conditions

Current crop phenotypes are the outcome of centuries of selection under agriculture, but also reflect the choices of early farmers among available wild plants (Sauer, 1952; Preece et al., 2015; Mercuri, Fornaciari, Gallinaro, Vanin, & di Lernia, 2018). Although crop evolution under domestication exerted a modest impact on root traits in our study, as discussed below, early farmers already showed a bias on root phenotypes of agricultural plants. Specifically, roots of crops' wild progenitors, in comparison with those of other wild herbs, were less dense and thicker (Fig. S2), which is typical of fast-growing species from fertile habitats (Kramer-Walter et al., 2016; Reich, 2014; Ryser, 1996). Furthermore, thicker but less dense roots are suggested to be caused by roots with more cortex area than stele (xylem vessels) area, because cortex area is less dense (Kong et al., 2014). Species with such a root phenotype rely more on mycorrhizal associations for mineral nutrition (Brundrett, 2002; Ma et al., 2018) and indicate acquisitive strategies (Kong et al., 2016). Nevertheless, some evidences, from maize and bean, suggest that domestication led to roots with larger vessel area (York, Galindo-Castañeda, Schussler, & Lynch, 2015; Peña-Valdivia et al.,

2010, Burton & Lynch, 2013). Future studies would be needed to test the proportion of vessel and cortex area of wild progenitors and domesticated plants in the context of botanical variation, and the effect of domestication.

Further, the high diameter and low SRL displayed by the roots of wild progenitors are consistent with a recent worldwide meta-analysis, where these attributes were generally associated with fertile environments (Freschet et al., 2017). Indeed, thicker, lower SRL roots may be generally found where plants are less dependent on soil exploitation by fine roots. Finally, larger biomass allocation to the roots of wild progenitors, as compared to that of other wild herbs, is more surprising in light of the typical species in fertile soils but fits the theory of balanced organ biomass and morphology above- versus below-ground, as postulated by Freschet, Kichenin, & Wardle (2015b). Specifically, since crops and their wild progenitors have relatively higher specific leaf area than average Milla, Osborne, Turcotte, & Violle, 2015; Tribouillois et al., 2015), they rely less on leaf biomass investment to capture light and could therefore invest more biomass into belowground organs. Further studies comparing the biomass investment below and aboveground in domesticated plants with this in wild herbs would be necessary to test this hypothesis.

The fact that wild progenitors exhibit a root phenotype adapted to agricultural habitats is in line with the Dump Heap hypothesis. This hypothesis suggests that early domestication started with the species growing around human settlements, in anthropogenic environments which are characterized by relatively high nutrient availabilities and disturbance frequencies (Sauer, 1952; Zeven, 1973; Hawkes, 1983). Fast growing and short-lived plants would become more abundant around settlements, would thrive better in early agricultural habitats, and thus would respond better to

the early attempts of cultivation and further domestication (Hawkes, 1983; Mercuri, Fornaciari, Gallinaro, Vanin, & di Lernia, 2018). Although rigorous comprehensive tests are still pending, wild progenitors tend to show specific leaf area and nitrogen content of leaves typical of fast-growing species, when compared with other wild herbaceous plants (Cunniff et al., 2014; Milla, Osborne, Turcotte, & Violle, 2015). Our screening of root analysis traits is in line with aboveground evidence that wild plants with nutrient acquiring strategies were more successful candidates for domestication by being pre-adapted to the cultivation conditions.

Root traits changed modestly, and in idiosyncratic ways, after domestication

We hypothesized that root morphology and allocation would change towards resource-acquisitive strategies alongside domestication processes. However, contrary to our hypothesis, we found a wide diversity of root morphology and allocation responses to domestication. For most root traits, trait scores decreased in some species or increased in others, which is consistent with a generalized species-specific response, as observed in previous case studies that compared wild progenitors to domesticated species. For example, SRL decreases with domestication in beans (Perez-Jaramillo et al., 2017) but not in maize (Gaudin, Mc Clymont & Raizada, 2011). Even case studies reporting on the same crop species show opposite responses to domestication, depending on growth conditions or the identities of crop varieties under study. For instance, similar allocation to roots was reported for wild progenitors and domesticated species of wheat and maize (Gaudin, McClymont & Raizada, 2011; Nakhforoosh, Grausgruber, Kaul, & Bodner, 2014), whereas others found lower allocation to roots in domesticated species for the same two species

(Waines & Ehdaie, 2007; Burton, Brown, & Lynch, 2013; Szoboszlay et al., 2015; Roucou, Violle, Fort, Roumet, Ecarnot, & Vile, 2017). Our broader screening, together with previous case studies, supports that the effects of domestication on root morphology and allocation are diverse. Nevertheless, to assess the generality of our results, it will be necessary to conduct similar experiments on root traits under more realistic field conditions (Poorter et al. 2016) and under contrasting growth conditions such as competition or fertilization level.

Acknowledging that the response of root traits to domestication is species-specific, we further investigated other explanatory variables that might account for the diversity in the size and directionality of domestication effects among crops. First, we asked whether crops belonging to different functional groups showed contrasting responses to domestication. In accordance with the literature, grasses tend to allocate more biomass to roots than forbs (Fig. 4c; Roumet, Lafont, Sari, Warembourg, & Garnier, 2008; Poorter et al. 2015; Roumet et al. 2016). Similarly, legumes had lower SRL than forbs, also in line with previous evidence Tjoelker, Craine, Wedin, Reich, & Tilman, 2005). However, the effect of domestication on root traits was generally insignificant among groups (Table 3, Fig.4), ruling out that functional groups could account for the observed diversity in crop responses to domestication. Similarly, taxonomic affinities have previously been used to explain variation in root morphology among taxa (Kong et al., 2014; Valverde-Barrantes, Freschet, Roumet, & Blackwood, 2017). However, phylogenetic relationships did not contribute to explain the diversity in crop reactions to domestication (Table S4, Methods S1). Lastly, we explored whether the variation of reactions was explained by variability in domestication processes (timing of the domestication event and organ under focal selection). Interestingly, plant size has increased more in older

than in younger crops (Table S4; Fig. S3), which is consistent with a longer selective pressure on size. However, the size of the domestication effect for root traits was not explained by those aspects of the domestication process (Table S4; Figs. S3-4). Further characteristics of the domestication processes such as intensity of the selective efforts or geographical location of domestication event may help elucidate the observed diversity of root traits responses to domestication.

Conclusions

Our comparative analysis revealed that none of the root traits reacted to domestication in accordance with evolution towards faster-growth strategies. Root traits changed during most of the 30 domestication processes surveyed here, but this occurred in diverse directions, depending on the crop species, and irrespective of phylogenetic and functional group affiliations, or of variability in the domestication processes. The diversity of responses to domestication encountered here emphasizes the importance of studying multiple crops with a comparative focus. Finally, the less dense and thicker roots with low SRL of crop wild progenitors suggests that the root phenotype of the wild species selected by early farmers were already adapted to fertile and disturbed conditions, thereby supporting the Dump Heap hypothesis. Thus, the adaptation of root phenotypes to fertile soil appears to be largely determined by the choice of wild species by the first farmers rather than by further evolution under domestication.

Acknowledgments

We thank J.M. Alonso, J. Margalet and T. de la Fuente for assistance in data gathering. We also thank all public seed banks that provided seeds for the project (complete list in Supplementary Table S1). This work was supported by MINECO (grants CGL2014-56567-R, BES-2012-054356, PCIN-2014-053).

REFERENCES

Arsenault, J. L., Poulcur, S., Messier, C. & Guay, R. (1995). WinRHlZO™, a root-measuring system with a unique overlap correction method. *HortScience*, *30*, 906-906.

Abbo, S., Pinhasi van-Oss, R., Gopher, A., Saranga, Y., Ofner, I. & Peleg, Z. (2014). Plant domestication versus crop evolution: A conceptual framework for cereals andgrain legumes. *Trends in Plant Science*, *19*, 351–360.

Bartoń, K. (2013). MuMIn: Multi-model inference. R package version 1.9. 13. *The Comprehensive R Archive Network (CRAN), Vienna, Austria*.

Bishopp, A. & Lynch, J.P. (2015). The hidden half of crop yields. *Nature Plants*, *1*, 15117.

Burton, A.L., Brown, K.M. & Lynch, J.P. (2013). Phenotypic diversity of root anatomical and architectural traits in Zea species. *Crop Science*, *53*, 1042–1055.

Buuren, S.& Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of statistical software*, *45*(3).

Craine, J.M. (2009). *Resource strategies of wild plants*. Princeton, NJ: Princeton university press.

Chapin III, F.S. (1980). The mineral nutrition of wild plants. *Annual review of ecology and systematics*, *11*, 233-260.

Cunniff, J., Wilkinson, S., Charles, M., Jones, G., Rees, M. & Osborne, C.P. (2014). Functional traits differ between cereal crop progenitors and other wild grasses gathered in the neolithic fertile crescent. *PLoS ONE*, *9*.

Delgado-Baquerizo, M., Reich, P.B., García-Palacios, P. & Milla, R. (2016). Biogeographic bases for a shift in crop C: N: P stoichiometries during domestication. *Ecology Letters*, *19*, 564–575.

Denison, R.F., Kiers, E.T. & West, S.A. (2003). The Quarterly Review of Biology solutions beyond the reach of natural selection?. *Review Literature And Arts Of The Americas*, *78*, 145–168.

Evans, L.T. (1996) *Crop evolution, adaptation and yield*. Cambridge university press, Cambridge.

Freschet, G.T. & Roumet, C. (2017). Sampling roots to capture plant and soil

functions. *Functional Ecology*, *31*, 1506-1518

Freschet, G.T., Kichenin, E., Wardle, D.A. (2015b). Explaining within-community variation in plant biomass allocation: a balance between organ biomass and morphology above vs below ground? *Journal of Vegetation Science, 26*, 431-440.

Freschet, G.T., Swart, E.M. & Cornelissen, J.H.C. (2015a). Integrated plant phenotypic responses to contrasting above- and below-ground resources: Key roles of specific leaf area and root mass fraction. *New Phytologist*, *206*, 1247–1260.

Freschet, G.T., Valverde-Barrantes, O.J., Tucker, CM, Craine, J.M., McCormack, M.L., Violle, C., … Roumet, C. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *Journal of Ecology*, *105* (5), 1182-1196

Gaudin, A.C.M., McClymont, S.A. & Raizada, M.N. (2011). The nitrogen adaptation strategy of the wild teosinte ancestor of modern maize, *Zea mays* subsp. *parviglumis*. *Crop Science*, *51*, 2780–2795.

Gepts, P. (2004). Crop Domestication as a Long-term Selection Experiment. *Plant Breeding*, *24*, 1–44.

Gonzalez-Voyer, A. & Von Hardenberg, A. (2014). An introduction to phylogenetic path analysis. In *Modern phylogenetic comparative methods and their application in evolutionary biology* (pp. 201-229). Springer, Berlin Heidelberg.

Grace, J. B.& Bollen, K. A. (2005). Interpreting the results from multiple regression and structural equation models.*Bulletin of the Ecological Society of America*, *86*, 283-295.

Hawkes, J. G. (1983). *The diversity of crop plants*. Harvard University Press, Cambridge.

Johnson, P.C. (2014). Extension of Nakagawa & Schielzeth's R^2GLMM to random slopes models. *Methods in Ecology and Evolution, 5*, 944-946.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., ... & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*, 1463-1464.

Kong, D., Wang, J., Zeng, H., Liu, M., Miao, Y., Wu, H., Kardol, P. (2016). The

nutrient absorption-transportation hypothesis: optimizing structural traits in absorptive roots. *New Phytologist*, *213*, 1569–1572.

Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H. & Guo, D. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist*, *203*(3), 863-872.

Kramer-Walter, K.R., Bellingham, P.J., Millar, T.R., Smissen, R.D., Richardson, S.J., Laughlin, D.C. & Mommer, L. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *Journal of Ecology*, *104*, 1299–1310.

Lambers, H. & Poorter, H. (1992), Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in ecological research*, *23*, 187-261.

Lynch, J.P. & Brown, K.M. (2012). New roots for agriculture: exploiting the root phenome. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*, 1598–1604.

Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R. D., Eissenstat, D.M., ... Hedin, L.O. (2018). Evolutionary history resolves global organization of root functional traits. *Nature, 555*, 94.

McKey, D.B., Elias, M., Pujol, B. & Duputié, A. (2012). Ecological Approaches to Crop Domestication. *Biodiversity in agriculture: domestication, evolution, and sustainability*, *377*.

Mercuri, A. M., Fornaciari, R., Gallinaro, M., Vanin, S., & di Lernia, S. (2018). Plant behaviour from human imprints and the cultivation of wild cereals in Holocene Sahara. *Nature plants*, *1*.

Milla, R. & Morente-López, J. (2015). Limited evolutionary divergence of seedlings after the domestication of plant species. *Plant Biology*, *17*, 169–176.

Milla, R., De Diego-vico, N. & Martín-Robles, N. (2013). Shifts in stomatal traits following the domestication of plant species. *Journal of Experimental Botany*, *64*, 3137–3146.

Milla, R., Osborne, C.P., Turcotte, M.M. & Violle, C. (2015). Plant domestication through an ecological lens. *Trends in Ecology and Evolution*, *30*, 463–469.

Nakagawa, S. & Freckleton, R.P. (2008). Missing inaction: the dangers of ignoring missing data. *Trends in Ecology and Evolution*, *23*, 592–596.

Nakhforoosh, A., Grausgruber, H., Kaul, H.P. & Bodner, G. (2014). Wheat root diversity and root functional characterization. *Plant and Soil*, *380*, 211–229.

Penone, C., Davidson, A.D., Shoemaker, K.T., DiMarco, M., Rondinini, C., Brooks, T.M., … & Costa, G.C. (2014). Imputation of missing data in life-history trait datasets: which approach performs the best? *Methods in Ecology and Evolution*, *5*, 961–970.

Peña-Valdivia, C.B., Sánchez-Urdaneta, A.B., Rangel, J.M., Muñoz, J.J., García-Nava, R., Velázquez, R.C. (2010). Anatomical root variations in response to water deficit: Wild and domesticated common bean (*Phaseolus vulgaris* L.). *Biological Research*, *43*, 417–427.

Perez-Jaramillo, J.E., Carrion, V.J., Bosse, M., Ferrao, L.F. V, de Hollander, M., Garcia, A.A.F. *et al.* (2017). Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. *Isme J*, 1–14.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D.& Team, R.C. (2015). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3, 1–120.

Poorter, H., Bühler, J., Van Dusschoten, D., Climent, J.& Postma, J.A. (2012). Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, *39*, 839–850.

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., Putten, W. H., Kleyer, M., ... Postma, J. (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*, *212*, 838-855.

Poorter, H., Jagodzinski, A.M., Ruiz-Peinado, R., Kuyah, S., Luo, Y., Oleksyn, J., … & Sack, L. (2015). How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytologist*, *208*, 736–749.

Poorter, H. & Ryser, P. (2015). The limits to leaf and root plasticity: What is so special about specific root length? *New Phytologist*, *206*, 1188–1190.

Preece, C., Livarda, A., Christin, P.A., Wallace, M., Martin, G., Charles, M., Jones, G., Rees, M. & Osborne, C.P. (2017). How did the domestication of Fertile Crescent grain crops increase their yields? *Functional Ecology*, *31*, 1–11.

Preece, C., Livarda, A., Wallace, M., Martin, G., Charles, M., Christin, P.A., ... & Osborne, C.P. (2015). Were Fertile Crescent crop progenitors higher yielding than other wild species that were never domesticated? *New Phytologist*, *207*, 905–913.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Reich, P.B. (2014). The world-wide "fast-slow" plant economics spectrum: A traits manifesto. *Journal of Ecology*, *102*, 275–301.

Revell, L.J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223.

Roucou, A., Violle, C., Fort, F., Roumet, P., Ecarnot, M., & Vile, D. (2018). Shifts in plant functional strategies over the course of wheat domestication. *Journal of Applied Ecology*, *55*, 25-37.

Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S.*et al.* (2016). Root structure–function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytologist*, *210*, 815-826.

Roumet, C., Lafont, F., Sari, M., Warembourg, F. & Garnier, E. (2008). Root traits and taxonomic affiliation of nine herbaceous species grown in glasshouse conditions. *Plant and Soil*, *312*, 69–83.

Ryser, P. (1996). The Importance of Tissue Density for Growth and Life Span of Leaves and Roots: A Comparison of Five Ecologically Contrasting Grasses. *Source: Functional Ecology British Ecological Society Functional Ecology*, *10*, 717–723.

Ryser, P. & Lambers, H. (1995). Root and leaf attributes accounting for the performance of fast-and slow-growing grasses at different nutrient supply. *Plant and Soil*, *170*, 251-265.

Sauer, C.O. (1952). *Agricultural origins and dispersals*. American Geographical Society, New York.

Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*, *90*, 363-368.

Singmann, H., Bolker, B.& Westfall, J. (2015). Afex: analysis of factorial experiments. R package version 0.13–145.

Smith, B. D. (2007). Niche construction and the behavioral context of plant and animal domestication. *Evolutionary Anthropology: Issues, News, and Reviews*, *16*, 188-199.

Smith, B. D. (2011). General patterns of niche construction and the management of 'wild'plant and animal resources by small-scale pre-industrial societies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*, 836-848.

Symonds, M. R., & Blomberg, S. P. (2014). A primer on phylogenetic generalised least squares. *In Modern phylogenetic comparative methods and their application in evolutionary biology* (pp. 105-130). Springer, Berlin, Heidelberg.

Szoboszlay, M., Lambers, J., Chappell, J., Kupper, J. V., Moe, L.A. & McNear, D.H. (2015). Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. *Soil Biology and Biochemistry*, *80*, 34–44.

Tjoelker, M.G., Craine, J.M., Wedin, D., Reich, P.B.& Tilman, D. (2005). Linking leaf and root trait syndromes among 39 grassland and savannah species. *New Phytologist*, *167*, 493–508.

Tribouillois, H., Fort, F., Cruz, P., Charles, R., Flores, O., Garnier, E., & Justes, E. (2015). A functional characterisation of a wide range of cover crop species: Growth and nitrogen acquisition rates, leaf traits and ecological strategies. *PLoS ONE*, *10*, 1–17.

Turcotte, M.M., Turley, N.E.& Johnson, M.T.J. (2014). The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytologist*, *204*, 671–681.

Valverde-Barrantes, O.J., Freschet, G.T., Roumet, C. & Blackwood, C.B. (2017). A worldview of root traits: the influence of ancestry, growth form, climate

and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. *New Phytologist*, *215*, 1562-1573.

Waines, J.G.& Ehdaie, B. (2007). Domestication and crop physiology: Roots of green-revolution wheat. *Annals of Botany*, *100*, 991–998.

Whitehead, S.R., Turcotte, M.& Poveda, K. (2016). Domestication impacts on plant-herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B*.

York, L.M., Galindo-Castañeda, T., Schussler, J.R., Lynch, J.P. (2015). Evolution of US maize (*Zea mays* L.) root architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress. *Journal of Experimental Botany, 66*, 2347–2358.

Zanne, A.E., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., FitzJohn, R.G., … & Beaulieu, J.M. (2014). Corrigendum: Three keys to the radiation of angiosperms into freezing environments. *Nature*, *514*, 394–394.

Zeven, A.C. (1973). Dr. Th. H. Engelbrecht's views on the origin of cultivated plants. *Euphytica*, *22*, 279-286.

Zohary, D. (2004). Unconcious selection and the evolution of domesticated plants. *Economic Botany*, *58*, 5–10.

Supporting Information

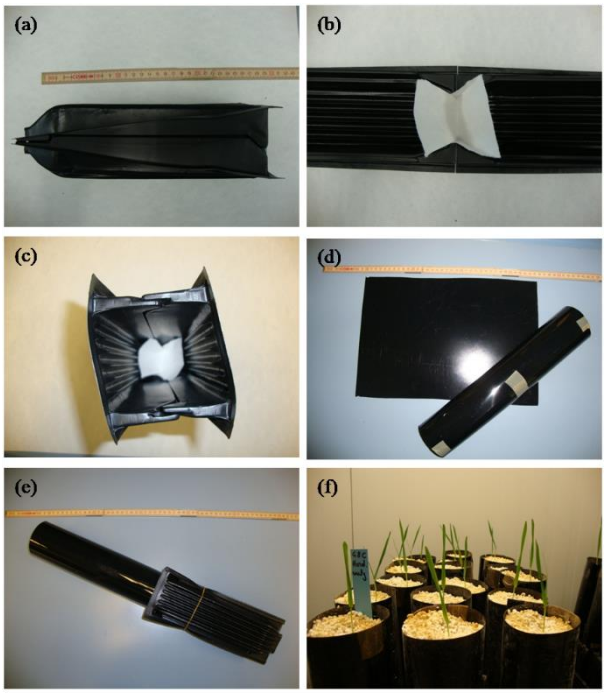


Figure S1. Pictures of the containers. (a) Jumbo Rootrainer (25 cm long, Haxnicks Ltd., Wiltshire). (b and c) A piece of paper was placed at the bottom of the Jumbo Rootrainer. (d) The round plastic cylinder (42 cm deep, 8 cm diameter).(e) The final container of 42 cm depth x 50 cm^2 area. (f) Plants growing in the resulting container.

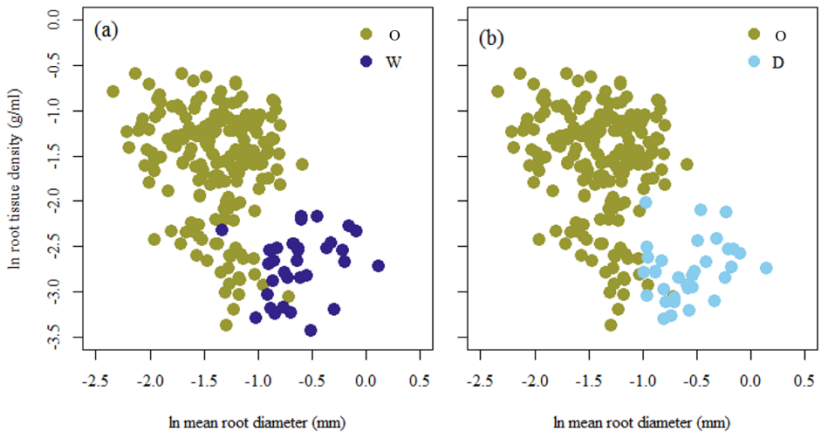


Figure S2. Wild progenitors and domesticated plants of the 30 crops used in the study in the context of wild herbaceous diversity of root traits: mean root diameter and root tissue density. (a) Wild progenitors (W, dark blue dots) versus other wild herbaceous species (O, green dots). (b) Domesticated plants (D, blue dots) versus other wild herbaceous species (O, green dots).

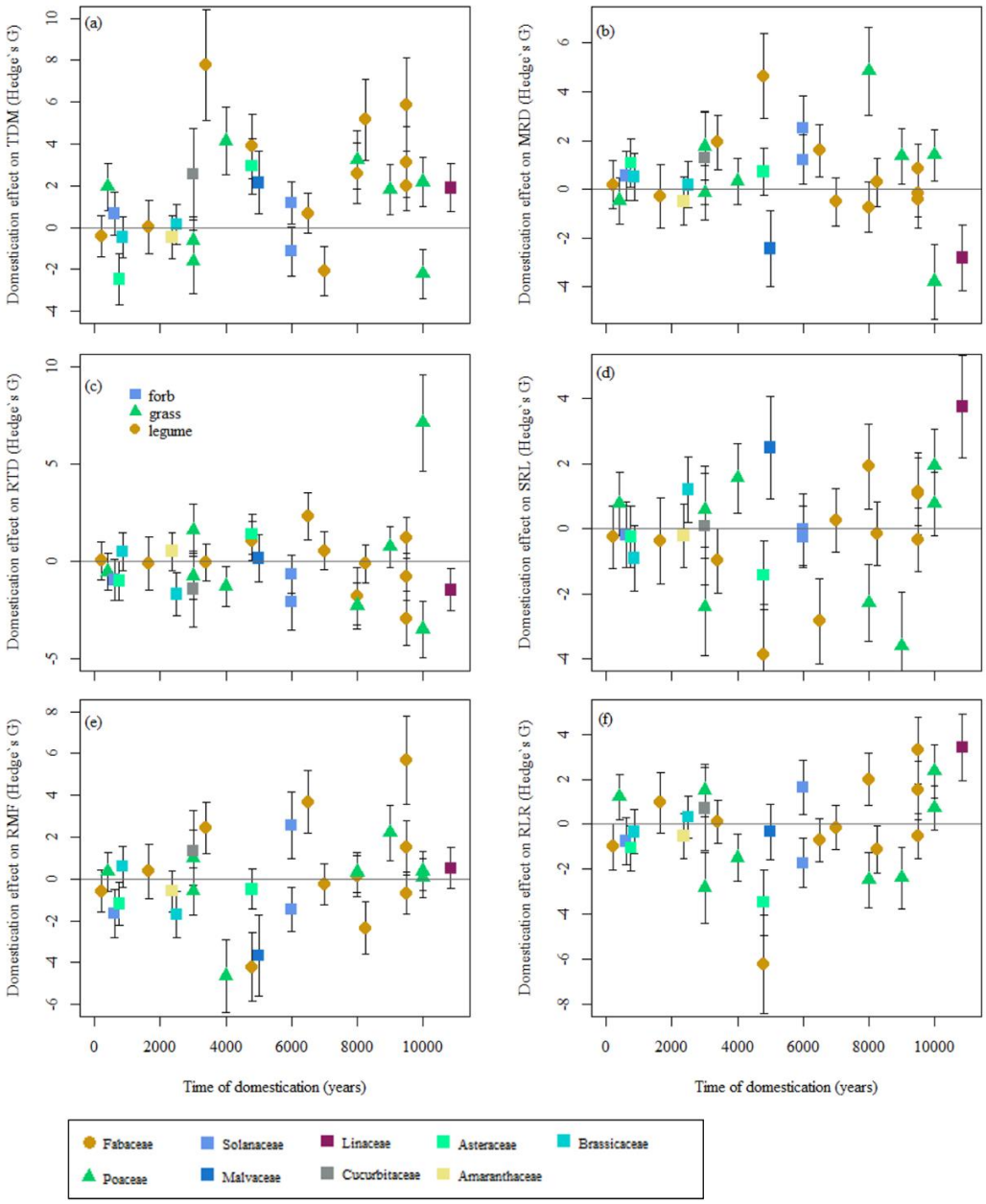


Figure S3. Domestication effect size (Hedges´G) estimates and 95% confidence interval on the total dry mass (TDM) and root traits: mean root diameter (MRD), root tissue density (RTD), specific root length (SRL), root mass fraction (RMF) and root length ratio (RLR), related with the antiquity (years) of each crop.

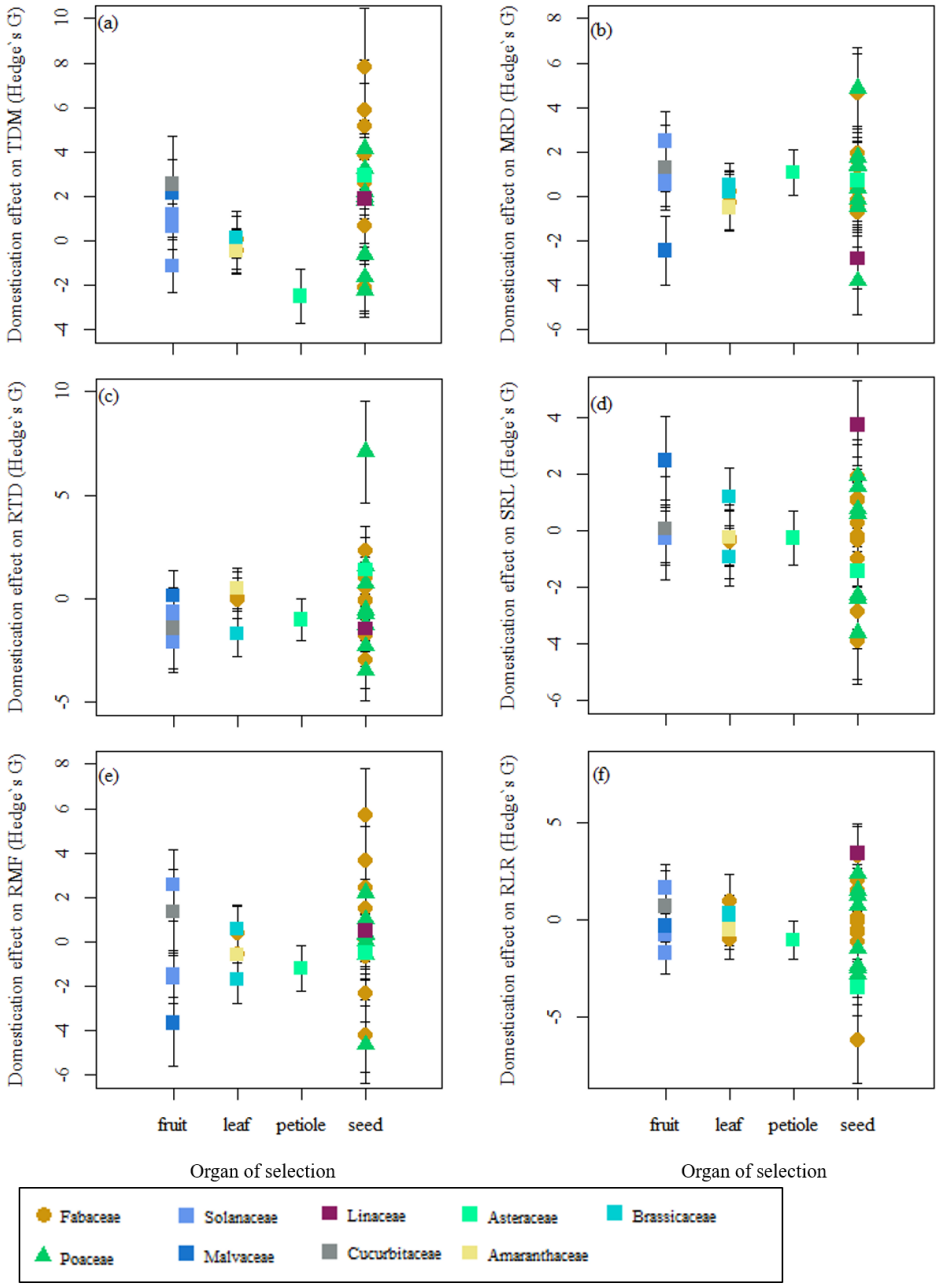


Figure S4. Domestication effect size (Hedges´G) estimates and 95% confidence interval on the total dry mass (TDM) and root traits: mean root diameter (MRD), root tissue density (RTD), specific root length(SRL), root mass fraction (RMF) and root length ratio (RLR), related with the organ of selection under domestication.

Table S1. Common and botanical names of each of the 30 crops used in this experiment, the domesticated and progenitor identities. Domestication status (dom: domesticated; wild: wild progenitor). Time under domestication refers to the years since domestication started. Seed donor (CGN: Center for Genetic Resources, The Netherlands; CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Devélopemment, France; CRF: Centro Nacional de Recursos Fitogenéticos-INIA, Spain; GRU: Germoplasm Resource Unit, United Kingdom; ICARDA: International Center for Agricultural Research in Dry Areas, Syria; * commercial company; IPK: Germplasm bank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; IRRI: International Rice Research Institute, China; NPGS: National Plant Germplasm System-USDA, U.S.A.). Accession identifier refers to the code assigned by each seed donor excepting the commercial companies. Accession country refers to the country where the seeds were collected. Ref. dom: reference source for wild progenitor assignment. Ref. time: reference source for time under domestication. N.A.: data not available.

Family	Common name	*Botanical name*	Dom. status	Time since domestication (years)	Seed donor	Accesion identifier	Accesion country	Ref. dom	Ref. time
Amaranthaceae	Chard	*Beta vulgaris cycla*	dom	2360	Clause	N.A.	commercial	4	1
Amaranthaceae	Chard	*Beta vulgaris maritima*	wild	2360	IPK	1582	Italy	4	1
Asteraceae	Cardoon	*Cynara cardunculus*	dom	750	Rocalba	N.A.	Spain	3	3
Asteraceae	Cardoon	*Cynara cardunculus*	Wild	750	Semillas Silvestres	ES-01-14-0256 lote:113.08	Spain	3	3
Asteraceae	Sunflower	*Helianthus annuus*	dom	4800	IPK	HEL 226	USA	1	1
Asteraceae	Sunflower	*Helianthus annuus*	wild	4800	NPGS	PI413093	USA	1	1
Brassicaceae	Cabbage	*Brassica oleracea acephala*	dom	2500	Rocalba	N.A.	commercial	1	1
Brassicaceae	Cabbage	*Brassica oleracea*	wild	2500	CGN	CGN18947	Germany	1	1
Brassicaceae	Rucola	*Eruca vesicaria*	dom	850	Rocalba	N.A.	commercial	9	9

Family	Common name	*Botanical name*	Dom. status	Time since domestication (years)	Seed donor	Accesion identifier	Accesion country	Ref. dom	Ref. time
Brassicaceae	Rucola	*Eruca vesicaria*	wild	850	IPK	ERU 115	Pakistan	9	9
Cucurbitaceae	Cucumber	*Cucumis sativus*	dom	3000	CGN	CGN19820	India	1	1
Cucurbitaceae	Cucumber	*Cucumis sativus hardwickii*	wild	3000	CGN	CGN24495	India	1	1
Fabaceae	Bean	*Phaseolus lunatus*	dom	4800	NPGS	PI347798	commercial	2	2
Fabaceae	Bean	*Phaseolus lunatus*	wild	4800	NPGS	PI260406	N.A.	2	2
Fabaceae	Chickpea	*Cicer arietinum*	dom	9500	CRF	BGE024684	commercial	1	1
Fabaceae	Chickpea	*Cicer reticulatum*	wild	9500	ICARDA	IG72945 ILWC116	Turkey	1	1
Fabaceae	Cowpea	*Vigna unguiculata*	dom	6500	NPGS	PI599213	USA	6	4
Fabaceae	Cowpea	*Vigna unguiculata*	wild	6500	NPGS	PI447516	Nigeria	6	4
Fabaceae	Lentil	*Lens culinaris*	dom	9500	CRF	BGE024692	commercial	1	4
Fabaceae	Lentil	*Lens culinaris orientalis*	wild	9500	ICARDA	IG 72642 IFWL 119	Syria	1	4
Fabaceae	Lupin	*Lupinus luteus*	dom	200	CRF	LO4500	N.A.	8	8
Fabaceae	Lupin	*Lupinus luteus*	wild	200	CRF	LO4579	Portugal	8	8
Fabaceae	Pea	*Pisum sativum*	dom	9500	GRU	2600	commercial	1	4
Fabaceae	Pea	*Pisum sativum* subsp. *elatius*	wild	9500	GRU	1794	Israel	1	4
Fabaceae	Soya-bean	*Glycine max*	dom	3400	Biográ	N.A.	commercial	10	10
Fabaceae	Soya-bean	*Glycine max* subsp. *soja*	wild	3400	IPK	1039	Russia	10	10

Family	Common name	*Botanical name*	Dom. status	Time since domestication (years)	Seed donor	Accesion identifier	Accesion country	Ref. dom	Ref. time
Fabaceae	White clover	*Trifolium repens*	dom	1650	Intersemillas	N.A.	commercial	11	11
Fabaceae	White clover	*Trifolium repens*	wild	1650	CGN	CGN22513	Kyrgystan	11	11
Fabaceae	Broadbean	*Vicia faba*	dom	8250	CRF	BGE011505	commercial	4	4
Fabaceae	Broadbean	*Vicia narbonensis*	wild	8250	CRF	BGE013234	Spain	4	4
Fabaceae	Lucerne	*Medicago lupulina*	dom	7000	Intersemillas	N.A.	commercial	12	12
Fabaceae	Lucerne	*Medicago lupulina*	wild	7000	ICARDA	IG 58734 IFMA 6092	Turkey	12	12
Fabaceae	Grass pea	*Lathyrus sativus*	dom	8000	CRF	BGE014724	Spain	13	4
Fabaceae	Grass pea	*Lathyru scicera*	wild	8000	CRF	BGE019570	Spain	13	4
Linaceae	Flax	*Linum usitatissimum*	dom	10850	CRF	BGE030455	commercial	7	14
Linaceae	Flax	*Linum usitatissimum*	wild	10850	CRF	BGE033614	Spain	7	14
Malvaceae	Cotton	*Gossypium hirsutum*	dom	5000	CRF	BGE006434	USA	1	1
Malvaceae	Cotton	*Gossypium hirsutum*	wild	5000	CIRAD	BG 6050	Isl. Guadalupe	1	1
Poaceae	Barley	*Hordeum vulgare*	dom	10000	CRF	BGE000214	commercial	1	1
Poaceae	Barley	*Hordeum spontaneum*	wild	10000	CRF	BGE025385	Morocco	1	1
Poaceae	Corn	*Zea mays mays*	dom	8000	NPGS	Ames26252	Brazil	5	1
Poaceae	Corn	*Zea mexicana*	wild	8000	NPGS	PI566674	Mexico	5	1

Family	Common name	*Botanical name*	Dom. status	Time since domestication (years)	Seed donor	Accesion identifier	Accesion country	Ref. dom	Ref. time
Poaceae	Milllet	*Pennisetum glaucum*	dom	3000	NPGS	PI586660	Burkina Faso	4	4
Poaceae	Milllet	*Pennisetum glaucum*	wild	3000	NPGS	PI537068	Nigeria	4	4
Poaceae	Oat	*Avena sativa*	dom	4000	CRF	BGE024681	Spain	4	4
Poaceae	Oat	*Avena sterilis*	wild	4000	ICARDA	IG 100379 IFMI 3096	Turkey	4	4
Poaceae	Rice	*Oryza sativa*	dom	8000	Calasparra	N.A.	commercial	1	1
Poaceae	Rice	*Oryza rufipogon*	wild	8000	IRRI	IRGC 104969	China	1	1
Poaceae	Rye	*Secale cereale*	dom	3000	CRF	BGE010915	commercial	1	1
Poaceae	Rye	*Secale cereale*	wild	3000	NPGS	PI618666	Turkey	1	1
Poaceae	Sorghum	*Sorghum sudanense*	dom	4000	Rocalba	N.A.	commercial	1	1
Poaceae	Sorghum	*Sorghum bicolor*	wild	4000	NPGS	PI524718	Sudan	1	1
Poaceae	Wheat	*Triticum durum*	dom	10000	CRF	BGE020911	Italia	1	1
Poaceae	Wheat	*Triticum dicoccoides*	wild	10000	NPGS	352322	Lebanon	1	1
Solanaceae	Chillipepper	*Capsicum bacattum pendulum*	dom	6000	CGN	CGN22181	N.A.	1	1
Solanaceae	Chillipepper	*Capsicum bacattum baccatum*	wild	6000	CGN	CGN23278	Argentina	1	1
Solanaceae	Pepper	*Capsicum anuum*	dom	6000	Mascarell	N.A.	Spain	1	1
Solanaceae	Pepper	*Capsicum anuum glabriusculum*	wild	6000		PI631137	Guatemala	1	1
Solanaceae	Tomato	*Solanum esculentum*	dom	600	Clause	N.A.	commercial	1	1
Solanaceae	Tomato	*Solanum pimpinellifolium*	wild	600	NPGS	LA1383	Peru	1	1

References of Table S1

1 Sauer, JD. 1993. *Historical geography of crop plants. A select roster*. CRC Press. Boca Raton, USA.

2 Maquet, A., X. Vekemans y J-P. Baudoin. 1999. Phylogenetic study on wild allies of Lima bean, *Phaseolus lunatus* (Fabaceae), and implications on its origin. *Plant Systematics and Evolution* 218: 43-54.

3 Sonnante, G., Pignone, D., & Hammer, K. (2007). The domestication of artichoke and cardoon: from Roman times to the genomic age. *Annals of Botany*, 100(5), 1095-1100.

4 Hancock, JF. 2004. *Plant Evolution and the origin of crop species*. CABI Publishing, NY, USA.

5 Wilkes G. 2007. Urgent notice to all maize researchers: disappearance and Extinction of the last wild teosinte population is more than half completed. A modest proposal for teosinte evolution and conservation in situ: the Balsas, Guerrero, Mexico. *Maydica* 52:49-60

6 Tomooka N, Kaga A, Isemura T, Vaughan D (2011) *Vigna. In Wild Crop Relatives: Genomic and Breeding Resources, Legume Crops and Forages* (Kole C, ed).Pp. 291-311. Springer-Verlag, Berlin.

7 Jhala AJ, Hall LM, Hall JC. 2008. Potential hybridization of flax with wild and weedy relatives: An avenue for movement of engineered genes. *Crop Science* 48:825–840.

8 Wolko B et al. 2011. *Lupins. In Wild Crop Relatives: Genomic and Breeding Resources, Legume Crops and Forages* (Kole C, ed). Pp. 153-206. Springer-Verlag, Berlin.

9 Pignone D, and Gómez-Campo C. 2011. *Eruca. In Wild Crop Relatives: Genomic and Breeding Resources, Oilseeds (Kole C, ed). Pp. 149-160*. Springer-Verlag, Berlin.

10 Hymowitz, T., & Newell, C. A. (1981). Taxonomy of the genus *Glycine*, domestication and uses of soybeans. *Economic botany*, *35*(3), 272-288.

11 Frame J, Newbould P. 1986. Agronomy of white clover. *Advances in Agronomy* 40: 1-88.

12 Chandra A, Verma S, Pandey KC (2011) Genetic similarity based on isoenzyme banding pattern among fifty species of *Medicago* representing eight sections (Fabaceae).*Biochemical Systematics and Ecology* 39:711-717.

13 Sarker et al. *Grasspea and chicklinks. In Plant Genetic Resources of Legumes in the Mediterranean*. Maxted and Bennett eds, pp. 159-180. Kluwer Acad. Publishers, Dordrecht, Netherlands.

14 Allaby RG, Peterson G, Merriwether DA, Fu Y- B. 2005. Evidence of the domestication history of flax (*Linum usitatissimum* L.) from genetic diversity of the sad2 locus. *TheorApplGenet* 112: 58–65.

	Mean root diameter	**Root tissue density**	**Specific root length**	**Root mass fraction**
Functional group	2.04	0.03	0.79	0.73
Plant type	169.68***	82.42***	9.36***	20.59***
Plant type X Func group	3.09	5.00***	1.45	0.27
Pagel´s λ	0.44	0.81	0.88	0.47

*, $P< 0.05$; **, $P< 0.01$; ***, $P< 0.001$.

Table S2. Effects of functional group, plant type (wild progenitor, domesticated plant and other wild species) and the interaction between plant type and functional group on root traits: mean root diameter, root tissue density, specific root length and root mass fraction. The table shows *F* and significance values of dependent variables and phylogenetic signal (Pagel´s λ), obtained by phylogenetic generalized least squares.

Table S3. Mean traits scores of the 30 domesticated plants (D) and their wild ancestors (W) used in the experiment. Arithmetic means and standard deviation of total dry mass (TDM), mean root diameter (MRD), root tissue density (RTD), specific root length (SRL), root mass fraction (RMF) and root length ratio (RLR).

Family	Botanical name	Dom. status	TDM (mg)	MRD (mm)	RTD (g/ cm^3)	SRL (m/g)	RMF (g/g)	RLR (m/g)
Amaranthaceae	*Beta vulgaris* var.*cycla*	D	68.327 (31.258)	0.442 (0.013)	0.070 (0.006)	98.092 (5.257)	0.245 (0.021)	2.388 (0.725)
	Beta vulgaris var. *Maritima*	W	104.719 (15.092)	0.470 (0.023)	0.061 (0.005)	104.356 (13.049)	0.294 (0.033)	3.137 (1.923)
Asteraceae	*Cynara cardunculus*	D	106.041 (7.054)	0.584 (0.011)	0.052 (0.002)	72.804 (3.062)	0.346 (0.010)	2.527 (0.461)
	Cynara cardunculus var. *sylvestris*	W	187.466 (12.943)	0.544 (0.012)	0.058 (0.002)	75.583 (3.913)	0.398 (0.017)	2.967 (0.359)
	Helianthus annuus	D	98.351 (5.260)	0.382 (0.008)	0.047 (0.003)	194.803 (15.837)	0.360 (0.025)	6.683 (0.642)
	Helianthus annuus	W	47.520 (5.670)	0.363 (0.009)	0.037 (0.001)	269.037 (17.116)	0.394 (0.018)	10.368 (1.344)
Brassicaceae	*Brassica oleracea*	D	140.694 (13.006)	0.417 (0.010)	0.061 (0.003)	123.741 (7.060)	0.262 (0.008)	3.201 (0.441)
	Brassica oleracea var. *acephala*	W	130.829 (26.014)	0.410 (0.012)	0.078 (0.004)	99.752 (5.415)	0.308 (0.009)	3.050 (0.499)
	Eruca vesicaria	D	147.797 (15.301)	0.494 (0.009)	0.044 (0.002)	119.786 (6.971)	0.282 (0.013)	3.357 (0.617)
	Eruca vesicaria	W	175.505 (22.214)	0.469 (0.020)	0.042 (0.002)	145.774 (10.776)	0.253 (0.018)	3.581 (0.709)
Cucurbitaceae	*Cucumis sativus*	D	547.128 (38.787)	0.594 (0.017)	0.062 (0.004)	60.579 (4.243)	0.442 (0.017)	2.633 (0.349)
	Cucumis sativus var. *hardwickii*	W	263.607 (85.550)	0.535 (0.016)	0.078 (0.013)	59.376 (13.503)	0.378 (0.031)	2.284 (0.984)
Fabaceae	*Phaseolus lunatus*	D	679.015 (36.972)	0.731 (0.015)	0.089 (0.003)	27.162 (1.057)	0.337 (0.014)	0.911 (0.131)
	Phaseolus lunatus	W	328.438 (16.280)	0.533 (0.011)	0.080 (0.002)	56.955 (3.264)	0.495 (0.009)	2.802 (0.406)

	Botanical name	Dom. status	TDM (mg)	MRD (mm)	RTD (g/ cm^3)	SRL (m/g)	RMF (g/g)	RLR (m/g)
	Cicer arietinum	D	489.705 (18.096)	0.904 (0.018)	0.075 (0.004)	21.401 (1.428)	0.512 (0.010)	1.087 (0.186)
	Cicer reticulatum	W	229.059 (7.312)	0.854 (0.021)	0.103 (0.002)	17.236 (0.783)	0.336 (0.010)	0.580 (0.103)
	Vigna unguiculata	D	173.784 (20.464)	0.798 (0.021)	0.120 (0.007)	17.773 (1.611)	0.283 (0.009)	0.502 (0.155)
Fabaceae	*Vigna unguiculata*	W	139.787 (8.822)	0.700 (0.017)	0.081 (0.002)	32.947 (1.763)	0.184 (0.008)	0.613 (0.159)
	Lens culinaris	D	313.143 (35.807)	0.631 (0.016)	0.122 (0.002)	26.713 (1.241)	0.433 (0.017)	1.166 (0.259)
	Lens culinaris orientalis	W	149.414 (16.004)	0.637 (0.012)	0.113 (0.002)	28.046 (1.316)	0.463 (0.012)	1.305 (0.268)
	Lupinus luteus	D	263.424 (26.019)	0.816 (0.026)	0.079 (0.004)	24.893 (1.505)	0.324 (0.016)	0.793 (0.117)
	Lupinus luteus	W	289.683 (12.545)	0.804 (0.012)	0.079 (0.006)	26.087 (1.485)	0.348 (0.011)	0.895 (0.084)
	Pisum sativum	D	395.896 (32.764)	0.792 (0.025)	0.058 (0.006)	38.722 (4.423)	0.409 (0.009)	1.575 (0.476)
	Pisum sativum subsp. *elatius*	W	154.295 (19.527)	0.824 (0.034)	0.069 (0.002)	27.904 (1.918)	0.326 (0.033)	0.927 (0.338)
	Glycine max	D	209.867 (10.258)	0.667 (0.027)	0.069 (0.005)	47.289 (8.207)	0.342 (0.016)	1.524 (0.515)
	Glycine max subsp. *soja*	W	27.834 (1.957)	0.530 (0.018)	0.070 (0.005)	68.293 (5.047)	0.220 (0.016)	1.476 (0.337)
	Trifolium repens	D	126.121 (26.541)	0.482 (0.025)	0.038 (0.004)	160.764 (30.757)	0.492 (0.037)	7.346 (1.394)
	Trifolium repens	W	123.226 (31.904)	0.499 (0.025)	0.039 (0.007)	242.373 (122.157)	0.444 (0.066)	5.898 (1.535)
	Vicia faba	D	1477.818 (105.624)	1.156 (0.028)	0.064 (0.005)	15.430 (0.923)	0.352 (0.022)	0.545 (0.154)
	Vicia narbonensis	W	274.457 (36.488)	1.126 (0.040)	0.066 (0.001)	15.928 (1.206)	0.471 (0.010)	0.756 (0.211)
	Medicago lupulina	D	50.904 (8.455)	0.388 (0.014)	0.072 (0.004)	124.292 (11.706)	0.405 (0.038)	4.824 (1.202)
	Medicago lupulina	W	97.315 (6.036)	0.406 (0.009)	0.067 (0.002)	117.161 (4.861)	0.426 (0.010)	4.964 (0.508)

	Botanical name	Dom. status	TDM (mg)	MRD (mm)	RTD (g/ cm^3)	SRL (m/g)	RMF (g/g)	RLR (m/g)
	Lathyrus cicera	D	614.466 (91.851)	0.838 (0.035)	0.065 (0.007)	31.488 (3.359)	0.335 (0.020)	1.196 (0.327)
	Lathyrus sativus	W	118.710 (11.139)	0.919 (0.037)	0.096 (0.004)	16.524 (1.579)	0.321 (0.041)	0.527 (0.337)
Linaceae	*Linum usitatissimum*	D	32.344 (3.153)	0.384 (0.011)	0.081 (0.006)	111.599 (7.309)	0.390 (0.028)	4.322 (1.114)
	Linum usitatissimum	W	19.106 (1.105)	0.553 (0.024)	0.110 (0.007)	41.801 (4.714)	0.350 (0.023)	1.429 (0.482)
Malvaceae	*Gossypium hirsutum*	D	569.408 (23.912)	0.611 (0.020)	0.087 (0.004)	40.046 (2.070)	0.307 (0.012)	1.216 (0.106)
	Gossypium hirsutum	W	442.551 (22.088)	0.726 (0.015)	0.086 (0.003)	28.545 (1.343)	0.444 (0.018)	1.279 (0.254)
Poaceae	*Hordeum spontaneum*	D	100.269 (8.313)	0.585 (0.018)	0.059 (0.001)	64.675 (4.250)	0.386 (0.010)	2.498 (0.566)
	Hordeum vulgare	W	150.875 (5.844)	0.747 (0.007)	0.041 (0.001)	56.512 (2.107)	0.385 (0.008)	2.174 (0.283)
Poaceae	*Zea mays mays*	D	260.052 (13.439)	0.858 (0.025)	0.079 (0.004)	22.667 (1.432)	0.673 (0.011)	1.515 (0.253)
	Zea mexicana	W	140.798 (9.499)	0.550 (0.012)	0.114 (0.006)	38.213 (2.686)	0.657 (0.020)	2.489 (0.491)
	Pennisetum glaucum	D	127.947 (23.673)	0.567 (0.033)	0.040 (0.007)	204.692 (79.337)	0.474 (0.046)	6.512 (3.279)
	Pennisetum glaucum	W	181.840 (38.113)	0.580 (0.027)	0.059 (0.010)	87.187 (30.753)	0.557 (0.050)	3.171 (2.657)
	Avena sativa	D	207.346 (10.212)	0.496 (0.014)	0.046 (0.003)	116.019 (5.269)	0.308 (0.007)	3.540 (0.309)
	Avena sterilis	W	87.964 (7.873)	0.483 (0.008)	0.058 (0.003)	95.222 (2.820)	0.418 (0.008)	3.962 (0.250)
	Oryza sativa	D	262.791 (55.440)	0.381 (0,045)	0.132 (0.024)	54.793 (11.174)	0.479 (0.017)	2.548 (1.429)
	Oryza rufipogon	W	71.335 (9.772)	0.265 (0,004)	0.098 (0.004)	190.926 (13.270)	0.341 (0.023)	6.496 (1.894)
	Secale cereale	D	124.991 (7.431)	0.718 (0.047)	0.045 (0.007)	62.470 (13.390)	0.410 (0.031)	2.442 (0.725)
	Secale cereale	W	161.423 (7.728)	0.604 (0.016)	0.032 (0.001)	110.881 (5.493)	0.366 (0.011)	4.023 (0.476)
	Sorghum bicolor	D	166.275 (13.079)	0.414 (0.012)	0.062 (0.004)	128.054 (12.542)	0.519 (0.019)	6.455 (1.289)
	Sorghum sudanense	W	100.090 (7.594)	0.429 (0.008)	0.069 (0.005)	103.672 (6.412)	0.498 (0.019)	5.107 (0.893)

	Botanical name	Dom. status	TDM (mg)	MRD (mm)	RTD (g/ cm^3)	SRL (m/g)	RMF (g/g)	RLR (m/g)
	Triticum dicoccoides	D	161.943 (8.159)	0.562 (0.007)	0.051 (0.001)	79.209 (3.048)	0.434 (0.007)	3.426 (0.335)
	Triticum durum	W	101.164 (9.482)	0.512 (0.014)	0.084 (0.004)	59.664 (3.296)	0.425 (0.008)	2.531 (0.419)
Solanaceae	*Capsicum bacattum*	D	202.426 (25.011)	0.448 (0.008)	0.051 (0.002)	126.498 (5.778)	0.330 (0.014)	4.126 (0.46)
	Capsicum bacattum var. *pendulum*	W	128.421 (12.870)	0.423 (0.005)	0.056 (0.003)	130.483 (4.116)	0.414 (0.021)	5.379 (0.909)
	Capsicum anuum	D	156.116 (23.889)	0.516 (0.019)	0.058 (0.002)	84.736 (6.545)	0.325 (0.004)	2.746 (0.472)
	Capsicum anuum var. *glabriusculum*	W	212.878 (14.140)	0.439 (0.006)	0.080 (0.004)	85.011 (4.847)	0.261 (0.009)	2.157 (0.311)
	Solanum esculentum	D	105.889 (10.910)	0.448 (0.017)	0.036 (0.002)	184.021 (15.313)	0.356 (0.012)	6.476 (1.589)
	Solanum pimpinellifolium	W	82.324 (12.995)	0.415 (0.024)	0.041 (0.001)	194.177 (23.953)	0.419 (0.014)	8.055 (2.560)

Independent variable	Blomberg´s K	Phylogenetic generalized least squares models		
		Dependent variable		
		Model A	Model B	Model C
		Time of domestication p-value	Organ of selection p-value	Functional group p-value
Total dry mass	0.059	**0.052**	**0.011**	**0.000**
Mean root diameter	0.060	0.295	0.755	0.823
Root tissue density	0.171	0.502	0.994	0.986
Specific Root Length	0.049	0.1718	0.844	0.836
Root Mass Fraction	0.047	0.295	0.472	0.339
Root Length Ratio	0.026	0.173	0.980	0.983

Table S4. Phylogenetic signal and the influence of three characteristic of the domestication events in the domestication effect rate on total dry mass and root traits: mean root diameter, root tissue density, specific root length, root mass fraction and root length ratio. The table shows the phylogenetic signal Blomberg's K, and the the effect of time of domestication (model A), organ of selection (model B) and functional group (model C) in the domestication effect size obtained by phylogenetic generalized least squares.

Methods S1

Analysis of phylogenetic signal on root traits across the 30 crops used in the experiment.

To assess whether the effect of domestication on root traits is explained by phylogeny, we calculated the domestication effect and quantified the phylogenetic signal. We calculated the domestication effect size on total dry mass, root mean diameter, root tissue density, specific root length, root mass fraction and root length ratio of each crop with the Hedge's G statistic (Hedges et al., 2008). The phylogenetic signal of each trait was calculated with Blomberg´s K (Blomberg et al., 2003). Blomberg´s K = 0 indicates that the trait has evolved independently of phylogeny, and Blomberg´s K =1 indicates that the evolution of the trait is strongly related with the phylogeny, thus the close relatives had similar domestication effect sizes than distant relatives. To facilitate the phylogenetic signal analysis, a phylogenetic tree with all 27 crops used in our extensive experiment was derived from the largest reference tree of the angiosperms (Zanne et al., 2014), with the drop.tip function of 'phytools' R package (Revell, 2012). There were no polytomies in the tree. Phylogenetic signal was implemented using the phylosig function of the 'picante' R package (Kembel et al., 2010). The phylogenetic signals of the domestication effect size on root traits are shown in Table S4.

References of Methods S1

Blomberg, S.P., Garland, T., Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717–745.

Freschet, G.T., Valverde-Barrantes, O.J., Tucker, CM, Craine, J.M., McCormack, M.L., Violle, C… Roumet, C. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *Journal of Ecology*, **105**(5), 1182-1196.

Hedges, L.V., Gurevitch, J., Curtis, P.S. (2008). The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150-1156.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D…Webb, C.O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.

Revell, L.J. (2012). Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217-223.

Zanne, A.M., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., FitzJohn, R.G., McGlinn, DJ, … Beaulieu, J.M. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**, 89-92.

Capítulo 2 / Chapter 2

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

Nieves Martín-Robles, Anika Lehmann, Erica Seco, Ricardo Aroca, Matthias C. Rillig, Rubén Milla

(2018) Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species.
New Phytologist 218(1), 322-334

Summary

The arbuscular mycorrhizal (AM) symbiosis is key to plant nutrition, 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 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.

Key words

Arbuscular mycorrhiza, domestication, crop evolution, mycorrhizal growth response, phosphorus fertilization, sustainable agriculture, symbiosis, wild progenitors.

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, 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 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 phosphorus response (MPR) are the effects of AM on plant growth and phosphorus concentration, when compared to 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 (Comas & Eissenstat, 2009; Tawaraya 2003; 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, phosphorus 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. Particularly, 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 behavior 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 tends to be low compared with more natural ecosystems (Helgason *et al.*, 1998; Oehl *et*

al., 2010; Verbruggen *et al.*, 2010). Agricultural practices like 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 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 to the amount of carbohydrates moved belowground 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 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 barley (*Hordeum vulgare*), wheat (*Triticum*) and sunflower (*Helianthus annuus*) (Turrini *et al.*, 2016). Additionally, in a few crops, mycorrhizal response has been reported to decrease alongside domestication (Manske, 1989; Hetrick *et al.*, 1993, wheat), (An *et al.*, 2010, maize (*Zea mays*)), (Bryla & Koide, 1998, tomato (*Solanum lycopersicum*)). However, lower MGR among domesticated plants does not hold in other

crops, such as barley (Baon *et al.*, 1993). Although case studies have provided useful insight, 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 when compared with their wild progenitors across 27 independent domestication events. Specifically, we asked the following questions: (i) Have mycorrhizal colonization and mycorrhizal responsiveness decreased during crop domestication? (ii) Do crops react differently to the presence of AM fungi in P rich and P poor environments? (iii) 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, aboveground biomass and leaf phosphorus 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 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 'non mycorrhizal' 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 non-inoculated control). To address our second question on the interaction between 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 phosphorus treatment (high and low).

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. o*rientalis* (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.

Table 1. Common and botanical names of domesticated and wild progenitor taxa of each of the 27 crops used in the *extensive experiment*. The 14 crops used in the *fertilization experiment* are in bold letters. See Table S1 for more detailed information, particularly on bibliographic references used for assigning wild progenitors for each crop.

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 (40º18´48´´N, 3º52´57´´W). All species, but 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 and 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 is frequently used in this sort of experiments (i.e. Koide *et al.*, 1998; 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 (*ca.*60 AM fungal propagules per gram, according with 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 pre-germinated in dark and cold (4ºC) growth chambers. Once the radicle emerged, seedlings were individually transplanted to pots (1.8l volume, 22x10.5x10.5 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 100ºC for one hour during three consecutive days. To introduce the AM fungus to the plants, pots were inoculated with 25g of mycorrhizal inoculum, placed at 5cm depth in the pot one week before seedling transplanting. Control (non-inoculated) plants received a 3ml aliquot of a microbial wash, to supply non-AM microbes (Koide & Li, 1989). The microbial wash was made by filtering (<20 μm pore size) 2l of suspension prepared from 25g 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 100ml of fertilizer solution. The fertilization solution was a modified Hoagland´s solution (Hoagland & Arnon, 1950). KH_2PO_4 concentration was 1mM for the whole *extensive experiment* and for the low P treatment of the *fertilization experiment*, and 4mM for the high P treatment in the *fertilization experiment*. The base Hoagland´s solution consisted of 5mM Ca $(NO_3)_2$, 5mM KNO_3, 2mM $MgSO_4$, 180μM FeEDTA, 46.2μM H_3BO_3, 9.1μM $MnCl_2$, 0.76μM $ZnSO_4$ and 0.32μM $CuSO_4$. Finally, KCl was used to maintain constant potassium concentrations across the different fertilization solutions.

Before flowering, approximately six to nine weeks after sowing depending on the crop species, we randomly harvested 5-10 plants per accession and per treatment. We oven-dried the aboveground biomass of each plant at 60ºC for 72 hours. 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 (approximately 80 mg). Root samples were cleared with 10% KOH, were stained with ink and vinegar solution 5% at 100ºC and were rinsed in acidified water for 30 minutes (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 35x (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 co-ordinately, we took morphological fine root trait data of the same species from parallel experiment (methodological details in Methods S1). We used mean root diameter (mm), root tissue density (gml^{-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 mycorrhizal growth response (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 aboveground dry mass of inoculated plants and M_{ni} is arithmetic mean of the dry masses of the non-inoculated plants (Hetrick *et al.*, 1992). Using

the same equation with the P tissue concentration of *i* and *ni* plants, we quantified the mycorrhizal phosphorus response (MPR).

Statistical analyses

Prior to data analysis six individuals with extreme trait values, which were randomly distributed across accessions, were excluded from the data set. All subsequent analyses were conducted with 1014 plants for the *extensive experiment* and 1015 plants for the *fertilization experiment*. All analyses were performed with R software 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 (GLMM) with a binomial error distribution; and the differences in plant biomass, P tissue concentration, MGR and MPR were quantified using linear mixed-effect models (LME). 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 phosphorus 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 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^2s 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 the 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 run phylogenetic generalized least squares models (PGLSs). We used 24 of the 27 crops used 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 Hedge's 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 variables 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 non-independence 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 '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 non-inoculated plants, 2% showed AM colonization and hence were removed from the analysis. Brassicaceae and Amaranthaceae representatives showed negligible AM colonization and mycorrhizal responsiveness (Table 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 percentage of variation explained by fixed-effect, and random-effect predictors, respectively; Table 2, Fig. 1a and 1b). AM fungal colonization increased in some crops (for example *Lens*), and decreased in others (such as *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).

	Plant biomass (g)			**P in green leaves (%)**			**AM colonization (%)**			**MGR (%)**			**MPR (%)**		
	Estim values (SE)	$F_{1,1015}$	*P*	Estim values (SE)	$F_{1,148}$	*P*	Estim values (SE)	$F_{1,1015}$	*P*	Estim values (SE)	$F_{1,492}$	*P*	Estim 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 x Myc	-0.19 (0.11)	2.77	0.1	0.02 (0.01)	3.46	0.06	-	-	-	-	-	-	-	-	-
Dom x 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 dependent variables: domestication and AM root colonization were not transformed. Domestication was a factor (domesticated and wild progenitor). Interactions are indicated by x.

Table 2. Results of mixed-effect models of data from the *extensive experiment*, where 27 crops were grown under low P availability. The models tested (i) if plant biomass and phosphorus (P) tissue concentration in green leaves were affected by mycorrhizal treatment (Myc) and domestication status (Dom); (ii) if arbuscular mycorrhizal (AM) colonization was affected by domestication status; and (iii) 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.

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*) than their wild progenitors (Table S3, Fig. S1). The overall effect 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 and 1e).

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 (Table 3, Table S4, Fig. S1). However, in the *fertilization experiment*, the growth response to mycorrhizal inoculation differed between domestication status and P treatment (dom x myc interaction term P=0.03 in plant biomass model; and dom x 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).

AM fungal colonization decreased with P treatment regardless of domestication status (phosphorus effect -0.475%, P<0.001, Table 3, Fig 2c). However, as indicated by a significant domestication and P treatment interaction (Table 3), domesticated plants reduced AM fungal colonization more strongly than wild progenitors in response to increased P availability (Fig. 2c). The contribution of AM fungal colonization to MGR was similar in domesticated and wild progenitor species, and was independent of P

treatment (Dom x 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 wild progenitors (dom x 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 to shifts in root morphology. Shifts in MGR, AM fungal colonization and MPR were generally unrelated with 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).

	Plant biomass (g)			**P in green leaves (%)**			**AM colonization (%)**			**MGR(%)**			**MPR(%)**		
	Estim values (SE)	$F_{1, 1015}$	*P*	Estim values (SE)	$F_{1, 148}$	*P*	Estim values (SE)	$F_{1,498}$	*P*	Estim values (SE)	$F_{1,492}$	*P*	Estim 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	.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	**<.00**	0.00 (0.00)	0.75	0.4
P treat	0.73 (0.13)	111.5	**<.00**	0.06 (0.01)	113. 91	**<.00**	-0.47 (0.03)	307.46	**<.00**	-0.25 (0.08)	3.44	0.06	-0.02 (0.07)	0.09	0.77
Dom x myc	-0.51 (0.19)	4.81	**0.03**	0.00 (0.02)	0.25	0.62	-	-	-	-	-	-	-	-	-
Dom x AM col	-	-	-	-	-	-	-	-	-	0.00 (0.00)	2.48	0.13	0.00 (0.00)	7.23	**0.02**
Dom x P treat	0.12(0.18)	0.62	0.43	-0.01 (0.02)	1.14	0.29	-0.31 (0.04)	77.47	**<.00**	0.28 (0.11)	6.24	**0.01**	-0.08 (0.09)	0.66	0.42
P treat x Myc	-0.19 (0.18)	0.06	0.8	0.00 (0.02)	0.44	0.51	-	-	**-**	-	-	-	-	-	-
P treat x AM col	-	-	-	-	-	-	-	-	-	0.00 (0.00)	0.1	0.75	0.00 (0.00)	0.29	0.59
Dom x P treat x Myc	-0.44 (0.26)	2.85	0.09	-0.01 (0.03)	0.24	0.63	-	-	-	-	-	-	-	-	-
Dom x P treat x 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 dependent variables were not transformed. Domestication and P fertilization were factors. Interactions are indicated by x.

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. The models tested (i) if plant biomass and P tissue concentration in green leaves were affected by mycorrhizal treatment (Myc), domestication status (Dom) and P treatment (P treat). (ii) If arbuscular mycorrhizal (AM) colonization was affected by domestication status and P treatment (P treat). (iii) If mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) is 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 table also reports the R^2marginal (R^2m), the variance of the model explained by the fixed effects; and the R^2conditional (R^2c) the variance explained by both: fixed and random effects.

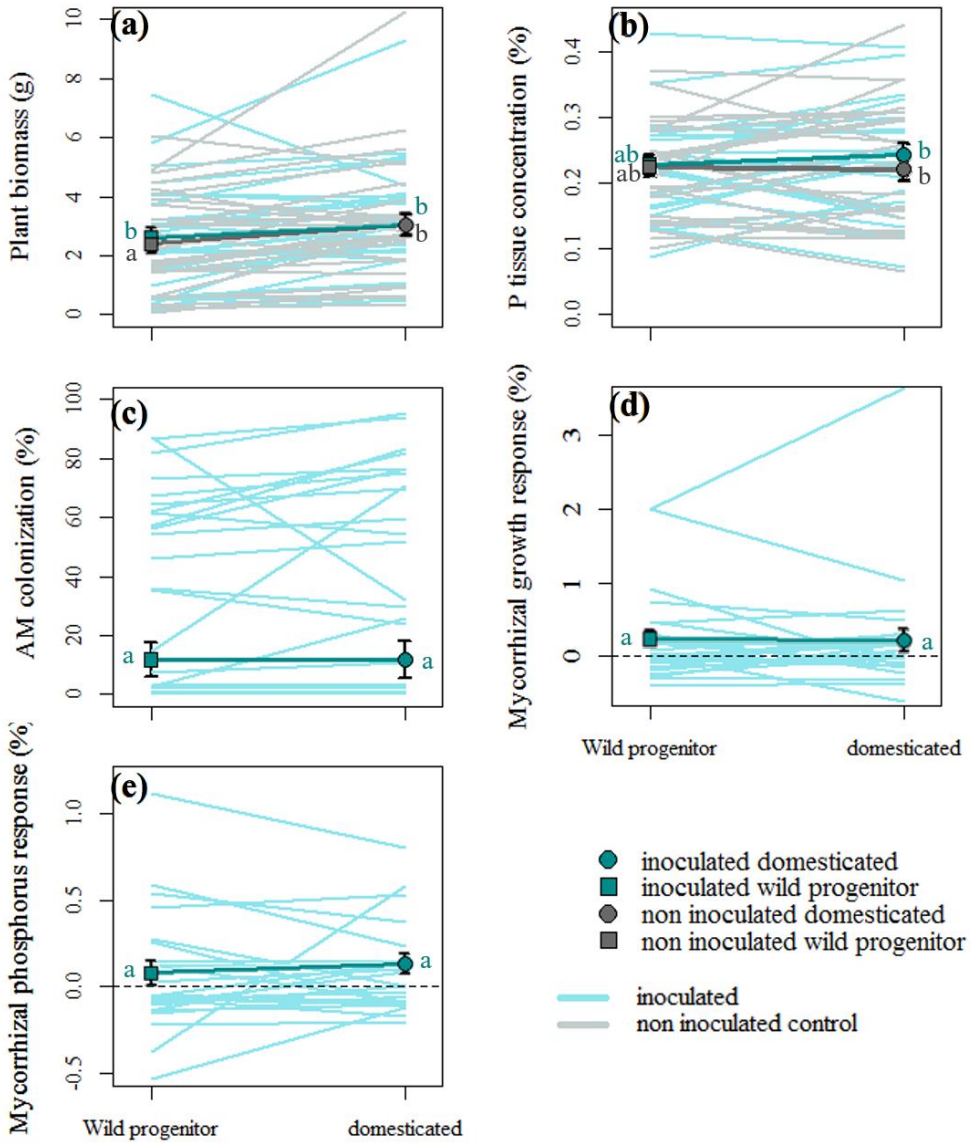


Figure 1. The reaction of inoculated plants (blue lines) and non-inoculated controls (grey lines) to domestication on 27 crops. Arbuscular mycorrhizal performance as measured as arbuscular mycorrhizal (AM) fungi was measured as plant biomass (a), leaf P tissue concentration (b), AM colonization (c), mycorrhizal growth response (d) and mycorrhizal phosphorus response (e). The letters and error bars (standard error) 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.

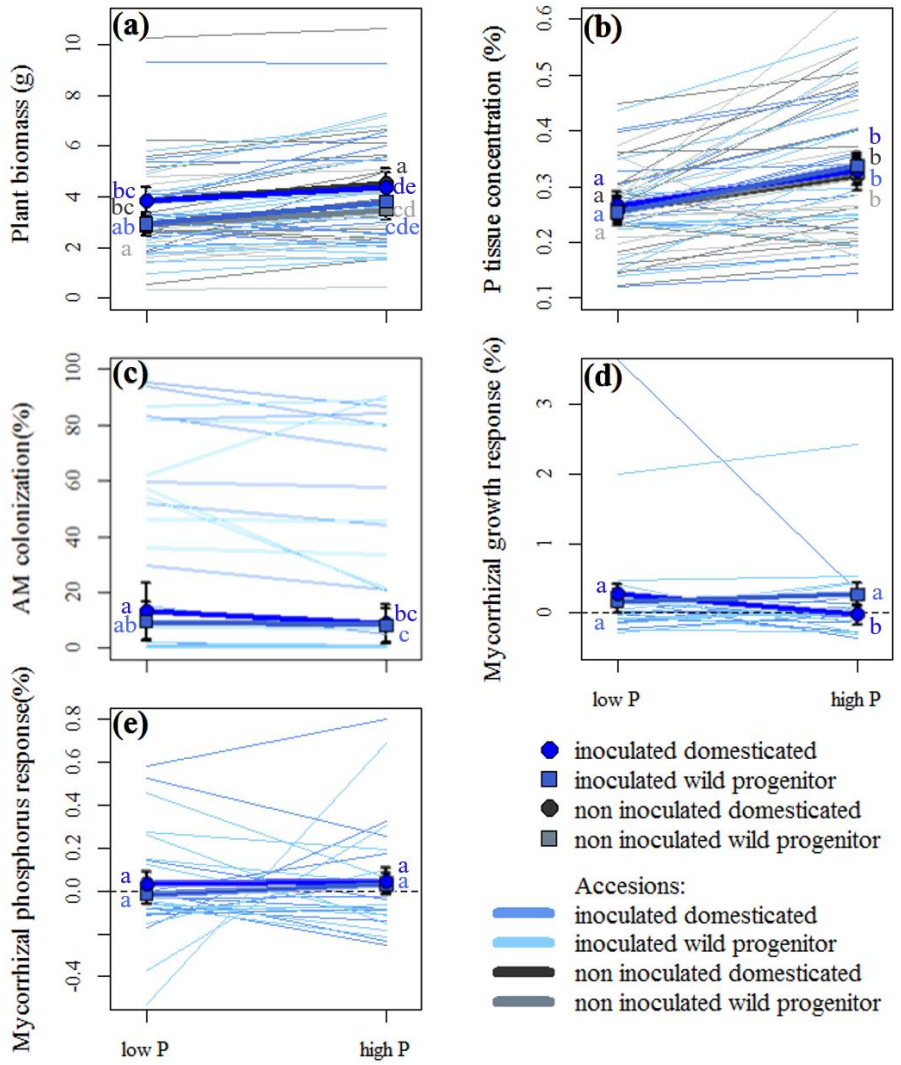


Figure 2. The reaction of inoculated plants (blue lines) and non-inoculated controls (grey lines) to phosphorus (P) treatment on 14 domesticates species (dark lines) and their wild progenitors (light lines). Arbuscular mycorrhizal (AM) symbiosis performance was measured as plant biomass (a), leaf P tissue concentration (b), AM fungal colonization (c), mycorrhizal growth response (d) and mycorrhizal phosphorus response (e). The letters and error bars (standard error) 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.

	Domestication effect on AM colonization		**Domestication effect on MGR**		**Domestication effect on MPR**	
	Estim values (SE)	*P*	Estim values (SE)	*P*	Estim 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

Table 4. Phylogenetic generalised least squared models (PGLSs) testing whether the domestication effect (Hedge´s G) on arbuscular mycorrhizal (AM) colonization, mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) correlates 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). The table shows the estimated values with standard error (SE) and significance. The significant p-values are marked in bold.

Discussion

Here 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 domesticated plants and to their wild progenitors alike, even though the strength and direction of the response to domestication varied depending on the crop species. However, wild progenitors benefitted from the AM symbiosis irrespective of P availability, while domesticated species only profited from the AM symbiosis under P limited conditions (Fig. 3). We therefore have 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 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 to 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 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 with 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 due to 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 with increased plant size under domestication, and be independent of the mycorrhizal symbiosis. Thus, different selective pressures on mycorrhizal and architectural root traits under domestication might explain such discrepancy.

P fertilization reduced mycorrhizal benefits to domesticated plants

Phosphorous fertilization reduced mycorrhizal response of domesticated plants. In contrast, wild progenitors kept positive MGRs even at high P supply (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 mycorrhizal growth response, even to negative rates (Johnson, 2010), and decreases mycorrhizal colonization (Kaeppler *et al.*, 2000; Treseder, 2004; Nijjer *et al.*, 2001; Kovalinková *et al.*, 2017). Several experiments reporting such 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 mycorrhizal growth response 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 non-beneficial 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 and 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 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). Therefore, our results are compatible with the hypothesis that the abilities to regulate AM fungi might have evolved under domestication similarly to those regulating rhizobia. However, further evidence is needed to test this hypothesis.

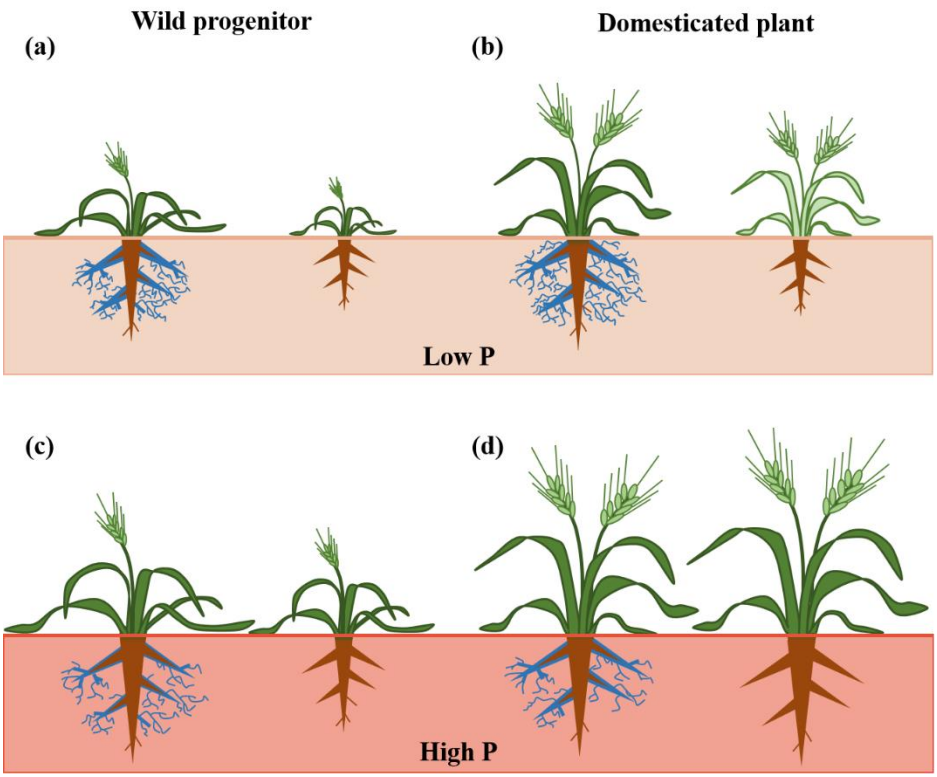


Figure 3. Conceptual diagram summarizing the main results of this study. Evolution of the plant-mycorrhizal fungi symbiosis under domestication in two scenarios of phosphorus (P) availability. Wild progenitors are represented in (a) and (c), and domesticated plants in (b) and (d). Upper and lower plots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

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 turned negligible or costly to domesticated plants when P availability increased. Since crop plants are raised under high fertilization in agricultural lands, this result has far-reaching implications. We hypothesize that our finding could be due to 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 abilities to regulate host selection and reward the cooperative AM fungi underlay this effect. Our results provide useful information for future plant breeding programs aimed to develop crops that benefit from mycorrhizal fungi effectively. However, to generalize our work, it will be important to analyze the mycorrhizal responsiveness with more AM fungal species, under more diverse experimental conditions, and in the field.

Acknowledgments

We thank Melchor Maestro, Marta Rodríguez, José M. Alonso, Leila Rico and José Margalet for assistance in data gathering; Dulce-Nombre Rodríguez for microbiological support with nitrogen fixing bacteria; Gloria Barzana and Susana Rodríguez-Echevarría for assistance with mycorrhizal procedures, and Ian Dickie and anonymous reviewers for their useful comments. We also thank all public seed banks that provided seeds for the project (complete list in Supplementary Table S1). This work was supported by MINECO (grants CGL2014-56567-R, BES-2012-054356, PCIN-2014-053), and the European Union (Eco-serve project, 2013-2014 BiodivERsA/FACCE-JPI, with the national funders ANR, NWO, FCT, MINECO, FORMAS, and SNSF).

References

Allen SE, Grimsban HM, Parkinson JA, Quarmby C, Roberts JD. 1976. Chemical analysis. In: Chapman SB, ed. *Methods in plant ecology*. Oxford, UK: Blackwell, 411– 466.

Aghili F, Jansa J, Khoshgoftarmanesh AH, Afyuni M, Schulin R, Frossard E, Gamper HA. **2014**. Wheat plants invest more in mycorrhizae and receive more benefits from them under adverse than favorable soil conditions. *Applied Soil Ecology* **84**: 93–111.

An GH, Kobayashi S, Enoki H, Sonobe K, Muraki M, Karasawa T, Ezawa T. **2010**. How does arbuscular mycorrhizal colonization vary with host plant genotype? An example based on maize (*Zea mays*) germplasms. *Plant and Soil* **327**: 441–453.

Argüello A, O'Brien MJ, van der Heijden MGA, Wiemken A, Schmid B, Niklaus PA. **2016**. Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. *Ecology Letters* **19**: 648–656.

Arsenault JL, Poulcur S, Messier C, Guay R. 1995. WinRHlZO™, a root-measuring system with a unique overlap correction method. *HortScience* **30**: 906-906.

Baon JB, Smith SE, Alston AM. **1993**. Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant and Soil* **157**: 97–105.

Bates D, Sarkar D, Bates MD, Matrix L. 2007. The lme4 package. *R package version*, *2*, 74.

Barton K. 2014. MuMIn: multi-model inference.–R package ver. 1.10. 0.

Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. New York, NY, USA: Academic Press, 373–389.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. **2009**. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12**: 13–21.

Blomberg SP, Garland T, Ives AR. **2003**. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717.

Brundrett MC. **2002**. Coevolution of roots and mycorrhiza of land plants. *New*

Phytologist **154**: 275–304.

Brundrett MC. **2009**. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* **320**: 37–77.

Bryla DR, Koide RT. **1998**. Mycorrhizal response of two tomato genotypes relates to their ability to acquire and utilize phosphorus. *Annals of Botany* **82**: 849–857.

Chagnon PL, Bradley RL. **2013**. Evidence that soil nutrient stoichiometry controls the competitive abilities of arbuscular mycorrhizal vs. root-borne non-mycorrhizal fungi. *Fungal Ecology* **6**: 557-560.

Chagnon PL, Bradley RL, Maherali H, Klironomos JN. **2013**. A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science* **18**: 484–491.

Comas LH, Callahan HS, Midford PE. **2014**. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: Implications for the evolution of belowground strategies. *Ecology and Evolution* **4**: 2979–2990.

Comas LH, Eissenstat DM. **2009**. Patterns in root trait variation among 25 co-existing North American forest species. *New Phytologist* **182**: 919–928.

Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT. **2015**. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytologist* **208**: 114–124.

Gamper H, Hartwig UA, Leuchtmann A. 2005. Mycorrhizas improve nitrogen nutrition of Trifolium repens after 8 yr of selection under elevated atmospheric CO2 partial pressure. *New Phytologist* **167**: 531-542.

Giovanetti M, Mosse B. **1980**. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New phytologist* **84**: 489–500.

Grman E. 2012. Plant species differ in their ability to reduce allocation to non-beneficial arbuscular mycorrhizal fungi. *Ecology* **93**: 711-718.

Hamel C. 1996. Prospects and problems pertaining to the management of arbuscular mycorrhizae in agriculture. *Agriculture, ecosystems & environment* **60**: 197-210.

Hart MM, Reader RJ. **2002**. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**: 335–344.

Hedges LV, Gurevitch J, Curtis PS. 2008. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**: 1150-1156.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. **1998**. Ploughing up the wood-wide web?. *Nature* **394**: 431.

Hetrick BAD, Wilson WT, Cox TS. **1993**. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Canadian Journal of Botany* **71**: 512–518.

Hetrick BAD, Wilson GWT, Leslie JF. **1991**. Root architecture of warm- and cool-season grasses: relationship to mycorrhizal dependence. *Canadian Journal of Botany* **69**: 112–118.

Hetrick BAD, Wilson GWT, Todd TC. **1992**. Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. *Canadian Journal of Botany* **70**: 1521–1528.

Hoagland D, Arnon DI. **1950**. The water-culture method for growing plants without soil. *California agricultural experiment station* **347**: 1–32.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, *et al.* 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394–407.

Ivanov S, Fedorova EE, Limpens E, Mita SD, Genre A, Bonfante P. **2012**. *Rhizobium* – legume symbiosis shares an exocytotic pathway required for arbuscule formation. *PNAS* **109**: 8316–8321.

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae?. *Ecological applications* **3**: 749-757.

Johnson NC. **2010**. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New phytologist* **185**: 631–647.

Johnson NC. Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New phytologist* **135**: 575-585.

Johnson NC, Pfleger FL. **1992**. Vesicular-arbuscular mycorrhizae and cultural stresses. *Mycorrhizae in sustainable agriculture* **54**: 71-99.

Johnson PC. 2014. Extension of Nakagawa & Schielzeth's R²GLMM to random slopes models. *Methods in Ecology and Evolution* **5**: 944-946.

Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF. **2000**. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Science* **40**: 358–364.

Kapulnik Y, Kushnir U. 1991. Growth dependency of wild, primitive and modern cultivated wheat lines on vesicular-arbuscular mycorrhiza fungi. *Euphytica* **56**: 27-36.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**: 1463-1464.

Kiers ET, Denison RF. 2014. Inclusive fitness in agriculture. *Phil. Trans. R. Soc. B* **369**: 20130367.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Kiers ET, Hutton MG, Denison RF. **2007**. Human selection and the relaxation of legume defences against ineffective rhizobia. *Proceedings of the Royal Society of London B: Biological Sciences* **274**: 3119–3126.

Kirk A, Entz M, Fox S, Tenuta M. 2011. Mycorrhizal colonization, P uptake and yield of older and modern wheats under organic management. *Canadian Journal of Plant Science* **91**: 663-667.

Klironomos JN, Hart MM. **2002**. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza* **12**: 181–4.

Koide R, Li M, Lewis J, Irby C. 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. *Oecologia* **77(4)**: 537-543.

Koide RT, Li M. **1989**. Appropriate controls for vesicular-arbuscular mycorrhiza research. *New Phytologist* **111**: 35–44.

Koltai H, Kapulnik Y. 2010. *Arbuscular mycorrhizas: physiology and function*. Dordrecht, Netherlands: Springer.

Kong D, Ma C, Zhang Q, Li L, Chen X, Zeng H, Guo D. 2014. Leadingdimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist* **203**: 863–872.

Konvalinková T, Püschel D, Řezáčová V, Gryndlerová H, Jansa J. 2017. Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant and Soil* **419**: 319-333.

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson S J, Laughlin DC. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *Journal of Ecology* **104(5)**: 1299-1310.

Lehmann A, Barto EK, Powell JR, Rillig MC. **2012**. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. *Plant and Soil* **355**: 231–250.

Lekberg Y, Koide RT. **2005**. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *The New phytologist* **168**: 189–204.

Lenth RV. 2016. Least-squares means: the R package lsmeans. *J Stat Softw* **69**: 1-33.

Mäder P, Edenhofer S, Boller T, Wiemken A, Niggli U. **2000**. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and fertility of Soils* **31**: 150–156.

Mäder P, Edenhofer S, Boller T, Wiemken A, Niggli U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and fertility of Soils* **31(2)**: 150-156.

Maherali H. **2014**. Is there an association between root architecture and mycorrhizal growth response? *New Phytologist* **204**: 192–200.

Manske GGB. **1989**. Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. *Agriculture, Ecosystems and environment* **29**: 273–280.

Milla R, Matesanz S. **2017**. Growing larger with domestication: a matter of physiology, morphology or allocation? *Plant Biology* **19**: 475–483.

Newbold T, Hudson LN, Arnell AP, Contu S, De Palma A, Ferrier S, Hill S, Hoskins A, Lysenko I, Phillips H *et al* 2016. Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment. *Science* **353 (6296)**: 288-291.

Newsham KK, Fitter AH, Watkinson AR. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution* **10(10)**: 407-411.

Nijjer S, Rogers WE, Siemann E. 2001. The Impacts of Fertilization on Mycorrhizal Production and Investment in Western Gulf Coast Grasslands. *Am. Midl. Nat.* **163**: 124–133.

Oehl F, Laczko E, Bogenriede A, Stahr K, Bösch R, van der Heijden M, Sieverding E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry* **42(5):** 724-738.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A, Ma P. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Applied and Environmental Microbiology* **69**: 2816–2824.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell Wk, Craine JM, Gurvich DE *et al.* 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of botany* **61**: 167-234

Pinheiro J, Bates D, DebRoy S, Sarkar D. 2015. nlme: Linear and Nonlinear Mixed Effects Models R package version 3.1–117.

Poorter H, Ryser P. 2015. The limits to leaf and root plasticity: What is so special about specific root length? *New Phytologist* **206**: 1188–1190.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Redecker D, Kodner R, Graham LE. 2000. Glomalean fungi from the Ordovician. *Science* **289**: 1920–1921.

Reinhart KO, Wilson GWT, Rinella MJ. 2012. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. *Ecology letters* **15**:

689–95.

Revell LJ. **2012**. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Rillig MC, Sosa-hernández MA, Roy J. **2016**. Towards an integrated mycorrhizal technology : harnessing mycorrhiza for sustainable intensification in agriculture. *Frontiers in plant science* **7**: 1–5.

Singmann H, Bolker B, Westfall J. (2015). Afex: analysis of factorial experiments. R package version 0.13–145.

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. Cambridge, UK: Academic Press.

Steinkellner S, Hage-Ahmed K, García-Garrido JM, Illana A, Ocampo JA, Vierheilig H. **2012**. A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*. *Mycorrhiza* **22**: 189–194.

Symonds MRE, Blomberg SP. 2014. A Primer on Phylogenetic Generalised Least Squares. In: Garamszegi L. eds. *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*. Berlin, Heidelberg: Springer, 105-130.

Tawaraya K. **2003**. Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition* **49**: 655–668.

Thirkell TJ, Charters M, Elliott A, Sait SM, Field KJ. 2017. Are mycorrhizal fungi our sustainable saviours? Considerations for achieving food security. *Journal of Ecology* **105**: 921-929.

Treseder KK. **2004**. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist* **164**: 347-355.

Tromas A, Parizot B, Diagne N, Champion A, Hocher V, Cissoko M, Crabos A, Prodjinoto H, Lahouze B, Bogusz D, *et al.v*2012. Heart of endosymbioses: transcriptomics reveals a conserved genetic program among arbuscular mycorrhizal, actinorhizal and legume-Rhizobial symbioses. *PLoS ONE* **7**: 1–7.

Turrini A, Giordani T, Avio L, Natali L, Giovannetti M, Cavallini A. **2016**. Large variation in mycorrhizal colonization among wild accessions, cultivars, and inbreds of sunflower (*Helianthus annuus* L.). *Euphytica* **207**: 331–342.

Veiga RSL, Faccio A, Genre A, Pieterse CMJ, Bonfante P, Van der Heijden MGA. 2013. Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant Arabidopsis thaliana. *Plant, Cell and Environment* **36**: 1926–1937.

Verbruggen E, Kiers ET. 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evolutionary Applications* **3**: 547–560.

Verbruggen E, Röling WF, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MG. 2010. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist* **186(4):** 968-979.

Vierheilig H, Coughlan AP, Wyss URS, Piché Y. 1998. Ink and Vinegar , a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. *Applied And Environmental Microbiology* **64**: 5004–5007.

Xing X, Koch AM, Jones AMP, Ragone D, Murch S, Hart MM. 2012. Mutualism breakdown in breadfruit domestication. *Proceedings of the Royal Society of London B: Biological Sciences* **279(1731):** 1122-1130.

Wang B, Qiu YL. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299–363.

Werner GD a, Kiers ET. 2015. Partner selection in the mycorrhizal mutualism. *New Phytologist* **205**: 1437–1442.

West SA, Kiers ET, Pen I, Denison RF. 2002. Sanctions and mutualism stability: When should less beneficial mutualists be tolerated? *Journal of Evolutionary Biology* **15**: 830–837.

Wissuwa M, Mazzola M, Picard C. 2009. Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil* **321**: 409–430.

Wright DP, Scholes JD, Read DJ, Rolfe SA. 2005. European and African maize cultivars differ in their physiological and molecular responses to mycorrhizal infection. *New phytologist* **167**: 881–96.

Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlinn DJ, O'Meara BC, Moles AT, Reich PB, *et al.*2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**: 89-92

Zhu L, Zhang DY. **2013**. Donald's Ideotype and Growth Redundancy: A Pot Experimental Test Using an Old and a Modern Spring Wheat Cultivar. *PLoS ONE* **8**: 1–7.

Supporting Information

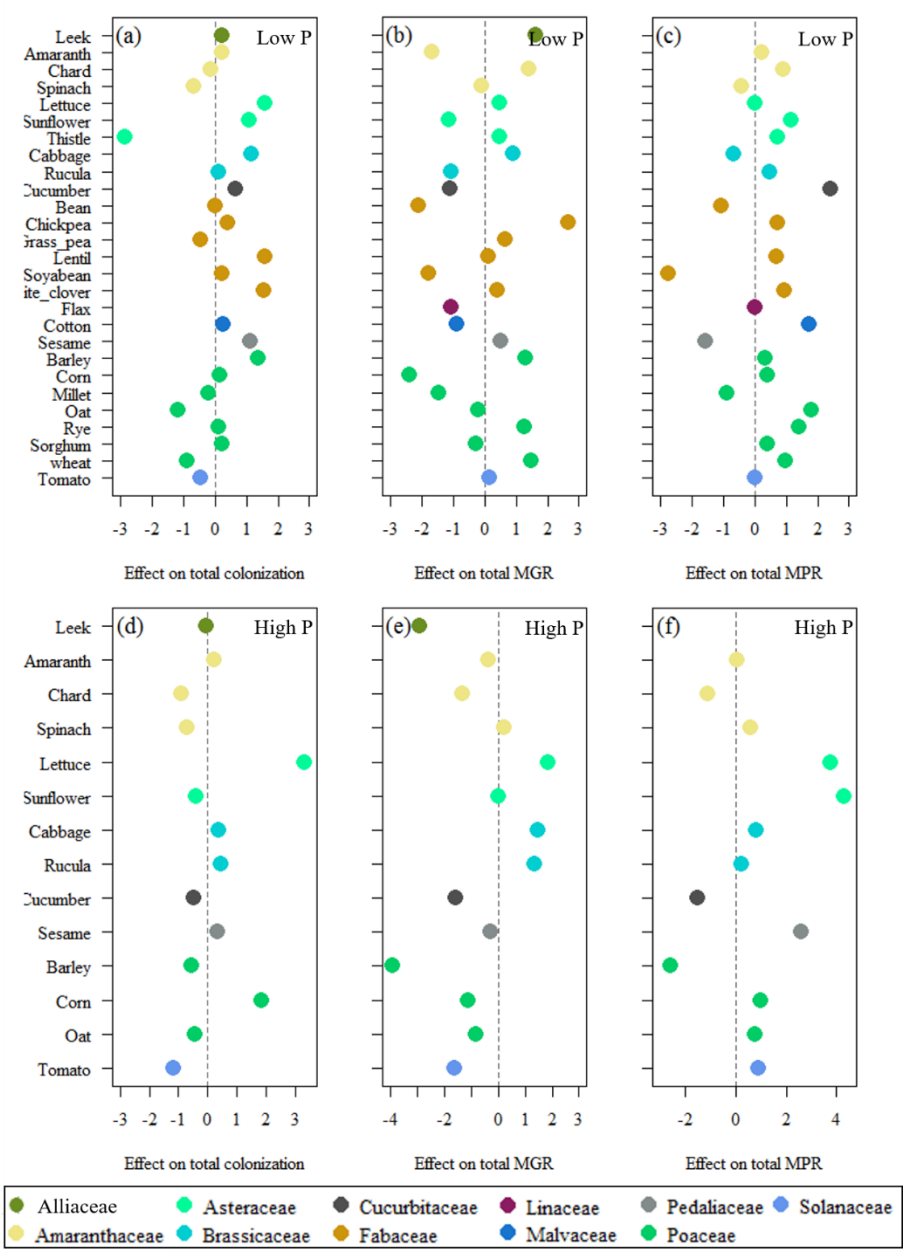


Figure S1. Domestication effect size on mycorrhizal traits at low and high phosphorus treatment of all crops used in the study. Effect size (Hedges´G) estimates for each crop showing the effect of domestication on total AM colonization (A, D), mycorrhizal growth response (MGR, B, E) and mycorrhizal phosphorus response (MPR, C, F) in the low phosphorus (A,B,C) and high phosphorus (D,E,F). Colors of points correspond to botanical families.

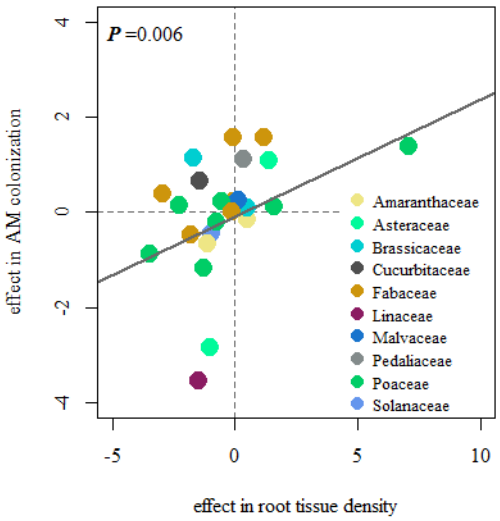


Figure S2. Correlation plot between domestication effect in arbuscular mycorrhizal (AM) colonization and root tissue density. Points represent the domestication effect size on root tissue density and AM colonization of each of the 24 crops. Colors of points correspond to botanical families. The *P*-value from the phylogenetic generalised least squared model is given in the left corner.

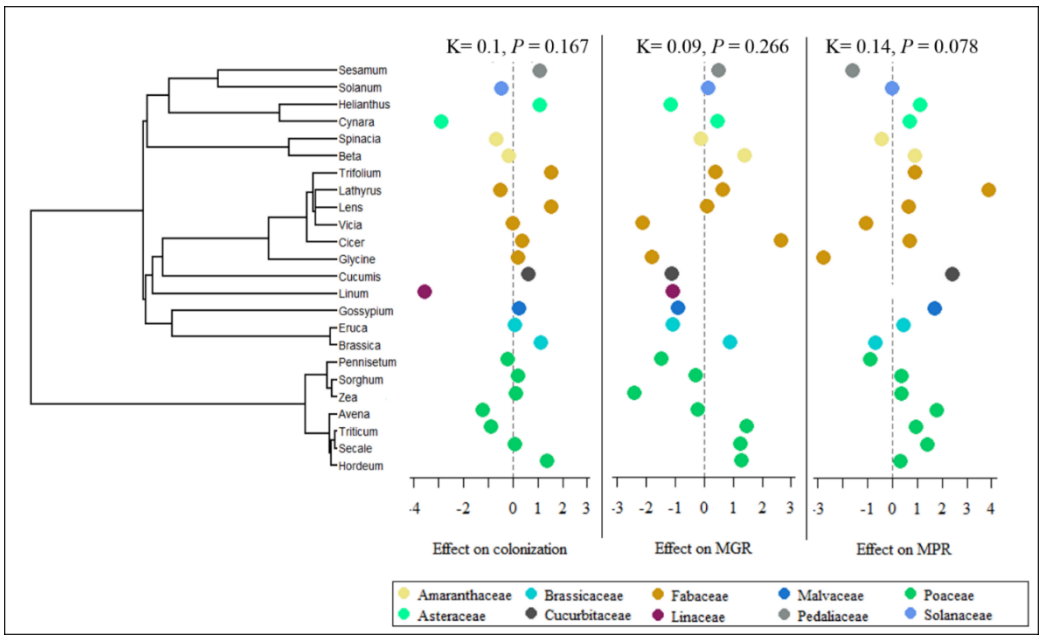


Figure S3. Visualization and phylogenetic signal of the domestication effect on arbuscular mycorrhizal colonization, mycorrhizal growth response (MGR) and mycorrhizal phosphorus response (MPR) mapped along the phylogeny of the 27 crops used in the study. The Blomberg´s K value, indicating the phylogenetic signal, and P value are displayed on the top of each "trait" panel. The colors of the crops represent the different botanical families.

Table S1. Detailed information of each of the 27 domesticated-wild progenitor used in this experiment, and reference sources for wild progenitor assignment. Common and botanical names of each crop, the domesticated and progenitor identities, and botanical family. Domestication status (dom: domesticated; wild: wild ancestor). Seed donor (IPK: Germplasm bank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; NPGS: National Plant Germplasm System-USDA, U.S.A.; CRF: Centro Nacional de Recursos Fitogenéticos-INIA, Spain; ICARDA: International Center for Agricultural Research in Dry Areas, Syria; * commercial company; CGN: Center for Genetic Resources, The Netherlands; CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Devélopemment, France). Accession identifier refers to the code assigned by each seed donor excepting the commercial companies. Accession country refers to the country where the seeds were collected. Time under domestication refers to the years since domestication started. Ref. dom: reference source for wild ancestor assignment. Ref. time: reference source for time under domestication. N.A.: data not available.

Family	Common name	Botanical name	Dom. status	Seed donor	Accession identifier	Accession country	Time under domestication (y)	Ref. dom	Ref. time
Alliaceae	**leek**	*Allium porrum* L.	dom	Clause	N.A.	commercial	4500	1	1
Alliaceae	**leek**	*Allium ampeloprasum* L.	wild	CGN	CGN20776	Turkey	4500	1	1
Amaranthaceae	**amaranth**	*Amaranthus cruentus* L.	dom	IPK	AMA 169	Nepal	4000	2	2
Amaranthaceae	**amaranth**	*Amaranthus hybridus* L.	wild	grin	PI652417	Brazil	4000	2	2
Amaranthaceae	**Chard**	*Beta vulgaris* L.	dom	Clause	N.A.	commercial	2360	3	2
Amaranthaceae	**Chard**	*Beta vulgaris* L.	wild	IPK	1582	Italy	2360	3	2
Amaranthaceae	**Spinach**	*Spinacia oleracea* L.	dom	Rocalba	N.A.	commercial	1250	2	2

Family	Common name	Botanical name	Dom. status	Seed donor	Accession identifier	Accession country	Time under domestication (y)	Ref. dom	Ref. time
Amaranthaceae	**Spinach**	*Spinacia turkestanica* Iljin.	wild	CGN	CGN9546	Uzbequistan	1250	2	2
Asteraceae	**Lettuce**	*Lactuca sativa* L.	dom	Verdecora	N.A.	commercial	4500	4	4
Asteraceae	**Lettuce**	*Lactuca serriola* L.	Wild	CRF	BGE034705	Madrid	4500	4	4
Asteraceae	**Cardoon**	*Cynara cardunculus* L.	dom	Rocalba	N.A.	Spain	750	5	5
Asteraceae	**Cardoon**	*Cynara cardunculus* L.	Wild	S. Silvestres	ES-01-14-0256 lote:113.08	Spain	750	5	5
Asteraceae	**Sunflower**	*Helianthus annuus* L.	dom	IPK	HEL 226	USA	4800	2	2
Asteraceae	**Sunflower**	*Helianthus annuus* L.	wild	NPGS	PI413093	USA	4800	2	2
Brassicaceae	**Cabbage**	*Brassica oleracea* L.	dom	Rocalba	N.A.	commercial	2500	2	2
Brassicaceae	**Cabbage**	*Brassica oleracea* L.	wild	CGN	CGN18947	Germany	2500	2	2
Brassicaceae	**Rucola**	*Eruca vesicaria* L.	dom	Rocalba	N.A.	commercial	850	6	6
Brassicaceae	**Rucola**	*Eruca vesicaria* L.	wild	IPK	ERU 115	Pakistan	850	6	6
Cucurbitaceae	**Cucumber**	*Cucumis sativus* L.	dom	CGN	CGN19820	India	3000	2	2
Cucurbitaceae	**Cucumber**	*Cucumis sativus* L.	wild	CGN	CGN24495	India	3000	2	2
Fabaceae	**Chickpea**	*Cicer arietinum* L.	dom	CRF	BGE024684	commercial	9500	2	2
Fabaceae	**Chickpea**	*Cicer reticulatum* Ladiz.	wild	ICARDA	IG72945 ILWC116	Turkey	9500	2	2

Family	Common name	Botanical name	Dom. status	Seed donor	Accession identifier	Accession country	Time under domestication (y)	Ref. dom	Ref. time
Fabaceae	**Lentil**	*Lens culinaris* L.	dom	CRF	BGE024692	commercial	9500	2	3
Fabaceae	**Lentil**	*Lens culinaris* subsp. o*rientalis* (Boiss.) Ponert	wild	ICARDA	IG 72642 IFWL 119	Syria	9500	2	3
Fabaceae	**Soybean**	*Glycine max* (L.) Merr	dom	Biográ	N.A.	commercial	3400	7	7
Fabaceae	**Soybean**	*Glycine max* subsp. *soja* (Siebold & Zucc.) H.Ohashi	wild	IPK	1039	Russia	3400	7	7
Fabaceae	**White clover**	*Trifolium repens* L.	dom	Intersemillas	N.A.	commercial	1650	8	8
Fabaceae	**White clover**	*Trifolium repens* L.	wild	CGN	CGN22513	Kyrgystan	1650	8	8
Fabaceae	**Faba bean**	*Vicia faba* L.	dom	CRF	BGE011505	commercial	8250	3	3
Fabaceae	**Faba bean**	*Vicia narbonensis* L.	wild	CRF	BGE013234	Spain	8250	3	3
Fabaceae	**Vetch**	*Lathyrus sativus* L.	dom	CRF	BGE014724	Spain	8000	9	3
Fabaceae	**Vetch**	*Lathyrus cicera* L.	wild	CRF	BGE019570	Spain	8000	9	3
Linaceae	**Flax**	*Linum usitatissimum* L.	dom	CRF	BGE030455	commercial	10850	10	11
Linaceae	**Flax**	*Linum usitatissimum* L.	wild	CRF	BGE033614	Spain	10850	10	11
Malvaceae	**Cotton**	*Gossypium hirsutum* L.	dom	CRF	BGE006434	USA	5000	2	2
Malvaceae	**Cotton**	*Gossypium hirsutum* L.	wild	CIRAD	BG 6050	Isl. Guadalupe	5000	2	2

Family	Common name	Botanical name	Dom. status	Seed donor	Accession identifier	Accession country	Time under domestication (y)	Ref. dom	Ref. time
Pedaliaceae	**Sesame**	*Sesamum indicum* L.	dom	Rocalba	N.A.	commercial	5300	12	13
Pedaliaceae	**Sesame**	*Sesamum indicum* L.	wild	IPK	17	Yemen	5300	12	13
Poaceae	**Barley**	*Hordeum vulgare* L.	dom	CRF	BGE000214	commercial	10000	2	2
Poaceae	**Barley**	*Hordeum spontaneum* K.Koch	wild	CRF	BGE025385	Morocco	10000	2	2
Poaceae	**Corn**	*Zea mays*	dom	NPGS	Ames26252	Brazil	8000	14	2
Poaceae	**Corn**	*Zea mexicana* (Schrad.) Kuntze	wild	NPGS	PI566674	Mexico	8000	14	2
Poaceae	**Milllet**	*Pennisetum glaucum* (L.) R.Br.	dom	NPGS	PI586660	Burkina Faso	3000	3	3
Poaceae	**Milllet**	*Pennisetum glaucum* (L.) R.Br.	wild	NPGS	PI537068	Nigeria	3000	3	3
Poaceae	**Oat**	*Avena sativa* L.	dom	CRF	BGE024681	Spain	4000	3	3
Poaceae	**Oat**	*Avena sterilis* L.	wild	ICARDA	IG 100379 IFMI 3096	Turkey	4000	3	3
Poaceae	**Rye**	*Secale cereale* L.	dom	CRF	BGE010915	commercial	3000	2	2
Poaceae	**Rye**	*Secale cereale* L.	wild	NPGS	PI618666	Turkey	3000	2	2
Poaceae	**Sorghum**	*Sorghum drummondii* (Nees ex Steud.) Millsp. & Chase	dom	Rocalba	N.A.	commercial	4000	2	2
Poaceae	**Sorghum**	*Sorghum arundinaceum* (Desv.) Stapf	wild	NPGS	PI524718	Sudan	4000	2	2

Family	Common name	Botanical name	Dom. status	Seed donor	Accession identifier	Accession country	Time under domestication (y)	Ref. dom	Ref. time
Poaceae	**Wheat**	*Triticum durum* Desf.	dom	CRF	BGE020911	Italia	10000	2	2
Poaceae	**Wheat**	*Triticum dicocoides* (Körn. ex Asch. & Graebn.) Schweinf.	wild	NPGS	352322	Lebanon	10000	2	2
Solanaceae	**Tomato**	*Solanum lycopersicum* L.	dom	Clause	N.A.	commercial	600	2	2
Solanaceae	**Tomato**	*Solanum pimpinellifolium* (L.) Mill.	wild	NPGS	LA1383	Peru	600	2	2

References of the table

1. De Clercq H, Van Bockstaele E. 2002. Leek: Advances in Agronomy and Breeding. In: Rabinowitch HD, Currah L, eds. *Allium crop science: recent advances*. Wallingford, UK: CABI, 431.

2. Sauer JD. 1993. *Historical geography of crop plants. A select roster*. Boca Raton, USA: CRC Press.

3. Hancock, JF. 2004. *Plant Evolution and the origin of crop species*. CABI Publishing, NY, USA.

4. De Vries IM 1997. Origin and domestication of Lactuca sativa L. *Genetic Resources and Crop Evolution,* 44(2): 165-174.

5. Sonnante G, Pignone D, Hammer K. 2007. The domestication of artichoke and cardoon: from Roman times to the genomic age. *Annals of Botany,* 100(5): 1095-1100.

6. Pignone D. Gómez-Campo C. 2011. Eruca. In (Kole C, ed) *Wild Crop Relatives: Genomic and Breeding Resources, Oilseeds*. Berlin, Germany: Springer, 149-160.

7. Hymowitz T, Newell CA. 1981. Taxonomy of the genus Glycine, domestication and uses of soybeans. *Economic botany,* 35(3): 272-288.

8. Frame J, Newbould P. 1986. Agronomy of white clover. Advances in *Agronomy,* 40: 1-88.

9. Sarker A, El Moneim AA, Maxted N. 2001. Grasspea and chicklinks. In: Maxted N, Bennett SJ, eds. *Plant Genetic Resources of Legumes in the Mediterranean*. Dordrecht, Netherlands: Kluwer Acad. Publishers, 159-180.

10. Jhala AJ, Hall LM, Hall JC. 2008. Potential hybridization of flax with wild and weedy relatives: An avenue for movement of engineered genes. *Crop Science,* 48:825–840.

11. Allaby RG, Peterson G, Merriwether DA, Fu YB. 2005. Evidence of the domestication history of flax (Linum usitatissimum L.) from genetic diversity of the sad2 locus. *Theor Appl Genet*, 112: 58–65.

12. Fuller DQ. 2003. Further evidence on the prehistory of sesame. *Asian Agri-History*,7(2): 127-137.

13. Bedigian D. 2003. Evolution of sesame revisited: domestication, diversity and prospects. Genetic resources and crop evolution 50(7): 779-787.

14. Wilkes G. 2007. Urgent notice to all maize researchers: disappearance and Extinction of the last wild teosinte population is more than half completed. A modest proposal for teosinte evolution and conservation in situ: the Balsas, Guerrero, Mexico. Maydica 52:49-60.

Crop name	Crop genus	Root nodule bacteria	Strain
Chikpea	*Cicer*	*Mesorhizobium ciceri*	UPM Ca7+
Faba bean	*Vicia*	*Rhizobium legominosarum*	ISL-39
Lentil	*Lens*	*Rhizobium legominosarum*	ISL-56
Soya bean	*Glycine*	*Bradirhizobium diazoeficiens*	USDA 110
Grass pea	*Lathyrus*	*Rhizobium legominosarum*	ISL-56
White Clover	*Trifolium*	*Rhizobium legominosarum* bv. *Trifolii*	IST 80

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

Table S3. Mean traits scores of the 27 domesticated plants (D) and their wild ancestors (W) used in the low phosphorus treatment. Arithmetic means and standard error of mycorrhizal growth response (MGR), mycorrhizal phosphorus response (MPR), plant biomass, arbuscular mycorrhizal (AM) colonization and phosphorus (P) content, on inoculated (myc+) and non-inoculated (myc -) plants. N.A.: data not available.

Family	Genus	Dom. status	MGR (%)	MPR (%)	Plant biomass myc- (g)	Plant biomass myc+ (g)	AM colonization myc+ (%)	AM colonization myc- (%)	P content myc+ (%)	P contentmyc- (%)
Alliaceae	*Allium*	D	3,64 (0,97)	NA	0,57 (0,03)	2,65 (0,17)	51,88 (15,27)	0 (0)	0,19 (0,02)	0,12 (0.01)
	Allium	W	2 (0,96)	NA	0,32 (0,05)	0,98 (0,09)	46,2 (25,97)	1 (0)	0,32 (0,04)	NA
Amaranthaceae	*Amaranthus*	D	-0,09 (0,08)	-0,11 (0,03)	10,28 (0,52)	9,31 (0,27)	0,32 (0,41)	2 (0)	0,23 (0,01)	0,26 (0.02)
	Amaranthus	W	0,18 (0,2)	-0,52 (0,04)	4,88 (0,28)	5,8 (0,32)	0,2 (0,38)	3 (0)	0,16 (0,01)	0,35 (0)
	Beta	D	0,22 (0,23)	0,14 (0,22)	3,38 (0,23)	4,13 (0,25)	2,08 (1,02)	0,04 (0,12)	0,4 (0,08)	0,35 (0.02)
	Beta	W	-0,08 (0,16)	0,15 (0,21)	3,02 (0,13)	2,77 (0,16)	2,24 (1,57)	0,16 (0,27)	0,43 (0,07)	0,37 (0.03)
	Spinacea	D	0,14 (0,15)	-0,1 (0,02)	2,59 (0,18)	2,97 (0,12)	0,52 (0,37)	0 (0)	0,39 (0,01)	0,44 (0.02)
	Spinacea	W	0,18 (0,5)	0,26 (0,12)	1,71 (0,23)	2,02 (0,91)	1,02 (0,79)	0 (0)	0,35 (0,03)	0,28 (0.12)
Asteraceae	*Cynara*	D	0,62 (0,4)	0,8 (0,14)	1,34 (0,1)	2,18 (0,17)	76,48 (19,18)	0 (0)	0,21 (0,01)	0,11 (0)
	Cynara	W	0,45 (0,32)	1,11 (0,17)	1,49 (0,07)	2,15 (0,15)	56,2 (17,63)	0,04 (0,12)	0,24 (0,02)	0,11 (0)

	Genus	Dom. status	MGR (%)	MPR (%)	Plant biomass myc- (g)	Plant biomass myc+ (g)	AM colonization myc+ (%)	AM colonization myc- (%)	P content myc+ (%)	P contentmyc- (%)
	Helianthus	D	0 (0,18)	0,52 (0,18)	5,6 (0,34)	3,71 (0,28)	81,86 (16,11)	0 (0)	0,28 (0,03)	0,18 (0.18)
	Helianthus	W	-0,07 (0,25)	0,45 (0,02)	4,45 (0,53)	4,14 (0,36)	62,12 (16,81)	0,24 (0,42)	0,28 (0)	0,19 (0.2)
	Lactuca	D	0,21 (0,22)	0 (0,07)	2,49 (0,23)	3,02 (0,2)	83,25 (16,56)	0 (0)	0,14 (0,01)	0,14 (0.07)
	Lactuca	W	0,01 (0,54)	-0,1 (0,08)	1,42 (0,14)	1,44 (0,34)	57,28 (11,19)	0,1 (0,28)	0,22 (0,02)	0,25 (0.08)
Brassicaceae	*Brassica*	D	0 (0,26)	-0,08 (0,08)	3,3 (0,3)	3,33 (0,28)	0,72 (0,83)	0 (0)	0,33 (0,03)	0,36 (0.08)
	Brassica	W	-0,24 (0,27)	0,12 (0,07)	3,95 (0,98)	2,97 (0,35)	0 (0)	0 (0)	0,23 (0,01)	0,21 (0.07)
	Eruca	D	-0,21 (0,13)	-0,03 (0,19)	2,66 (0,21)	2,08 (0,11)	0,68 (0,7)	0 (0)	0,3 (0,06)	0,31 (0.19)
	Eruca	W	0,29 (0,71)	-0,06 (0,03)	1,62 (0,26)	2,1 (0,4)	0,68 (0,71)	0 (0)	0,28 (0)	0,3 (0.3)
Cucurbitaceae	*Cucumis*	D	-0,12 (0,18)	-0,16 (0,08)	6,25 (0,67)	5,48 (0,38)	94,04 (8,95)	0 (0)	0,24 (0,02)	0,29 (0.08)
	Cucumis	W	0,05 (0,11)	-0,06 (0,02)	4,79 (0,24)	5,03 (0,17)	86,6 (11,21)	0,12 (0,26)	0,22 (0)	0,24 (0.03)
Fabaceae	*Cicer*	D	0,32 (0,18)	-0,1 (0,05)	0,36 (0,05)	0,48 (0,06)	0,72 (0,59)	0 (0)	0,23 (0,01)	0,26 (0.01)
	Cicer	W	-0,16 (0,16)	-0,13 (0,05)	0,21 (0,05)	0,18 (0,03)	0,4 (0,49)	0 (0)	0,16 (0,01)	0,18 (0.01)
	Glycine	D	-0,14 (0,57)	0,14 (0,19)	3,04 (1,16)	2,59 (1,74)	69,72 (32,41)	0 (0)	0,13 (0,02)	0,11 (0)

	Genus	Dom. status	MGR (%)	MPR (%)	Plant biomass myc- (g)	Plant biomass myc+ (g)	AM colonization myc+ (%)	AM colonization myc- (%)	P content myc+ (%)	P contentmyc- (%)
Fabaceae	*Glycine*	W	0,91 (0,57)	-0,05 (0,05)	1,58 (0,59)	3,03 (0,9)	64,64 (34,55)	0,12 (0,37)	0,18 (0,01)	0,19 (0)
	Lathyrus	D	0,18 (0,29)	0,1 (0,69)	1,87 (1,23)	2,22 (0,54)	23,73 (22,05)	1,24 (1,09)	0,07 (0,04)	0,06 (0)
	Lathyrus	W	-0,02 (0,34)	0,02 (0,04)	1,44 (0,44)	1,4 (0,49)	35,64 (24,89)	1,32 (1,01)	0,13 (0)	0,13 (0)
	Lens	D	-0,35 (0,45)	0,37 (0,3)	0,96 (0,35)	0,62 (0,43)	25,6 (38,16)	2,76 (1,35)	0,16 (0,03)	0,11 (0)
	Lens	W	-0,39 (0,37)	0,53 (0,13)	0,54 (0,13)	0,32 (0,2)	2,68 (3,11)	1,08 (1,42)	0,22 (0,01)	0,14 (0)
	Trifolium	D	0,08 (0,52)	-0,2 (0,07)	0,59 (0,12)	0,65 (0,31)	70,57 (33,36)	0 (0)	0,25 (0,02)	0,31 (0)
	Trifolium	W	-0,17 (0,75)	-0,21 (0,08)	0,6 (0,41)	0,5 (0,45)	14,66 (20,71)	0 (0)	0,18 (0,02)	0,23 (0)
	Vicia	D	-0,6 (0,28)	0,14 (0,15)	2,74 (1,14)	1,07 (0,78)	3,14 (5,05)	0 (0)	0,19 (0,02)	0,16 (0)
	Vicia	W	0,02 (0,26)	-0,13 (0,15)	0,57 (0,16)	0,58 (0,14)	3,28 (4,52)	0 (0)	0,08 (0,01)	0,1 (0)
Linaceae	*Linum*	D	1,03 (0,72)	NA	0,91 (0,06)	1,87 (0,2)	32,04 (14,91)	0 (0)	0,41 (0,07)	0,17 (0)
	Linum	W	2 (0,97)	NA	0,06 (0)	0,19 (0,01)	87,24 (14,52)	0 (0)	NA	NA
Malvaceae	*Gossypium*	D	0,48 (0,38)	0,92 (0,32)	2,99 (0,18)	5,26 (0,19)	76,64 (25,47)	0 (0)	0,26 (0,04)	0,13 (0)
	Gossypium	W	0,74 (0,2)	NA	3,01 (0,56)	4,44 (0,52)	73,2 (18,66)	0 (0)	NA	NA

Family	Genus	Dom. status	MGR (%)	MPR (%)	Plant biomass myc- (g)	Plant biomass myc+ (g)	AM colonization myc+ (%)	AM colonization myc- (%)	P content myc+ (%)	P contentmyc- (%)
Pedaliaceae	*Sesamum*	D	0,03 (0,18)	0,58 (0,29)	5,19 (0,23)	5,36 (0,3)	95,32 (5,22)	0 (0)	0,25 (0,04)	0,16 (0.29)
	Sesamum	W	-0,08 (0,23)	-0,37 (0,04)	4,09 (0,39)	3,75 (0,3)	81,76 (14,93)	0,8 (0,62)	0,15 (0)	0,24 (0.4)
Poaceae	*Avena*	D	-0,11 (0,37)	-0,08 (0,22)	2,7 (0,27)	2,38 (0,41)	0 (0)	0(0)	0,28 (0,07)	0,31 (0.22)
	Avena	W	-0,02 (0,44)	-0,04 (0,07)	1,79 (0,18)	1,75 (0,29)	0,28 (0,38)	0,08 (0,17)	0,26 (0,02)	0,28 (0.04)
	Hordeum	D	0,18 (0,39)	-0,05 (0,07)	2,82 (0,22)	3,3 (0,33)	10,75 (6,24)	0,2 (0,28)	0,25 (0,02)	0,26 (0.07)
	Hordeum	W	-0,27 (0,22)	-0,14 (0,1)	4,27 (0,22)	0,34 (2,92)	7,48 (4,44)	0 (0)	0,24 (0,03)	0,29 (0.01)
	Pennisetum	D	-0,13 (0,25)	0,08 (0,09)	5,07 (0,48)	4,37 (0,49)	54,45 (21,17)	0,16 (0,38)	0,17 (0,01)	0,16 (0)
	Pennisetum	W	0,23 (0,23)	-0,09 (0,1)	6,05 (0,3)	7,47 (0,45)	61,6 (32,08)	0 (0)	0,12 (0,01)	0,14 (0)
	Secale	D	0,3 (0,19)	-0,09 (0,11)	3,06 (0,12)	3,99 (0,19)	0,12 (0,19)	0 (0)	0,28 (0,03)	0,31 (0)
	Secale	W	0,09 (0,12)	-0,06 (0,1)	3,71 (0,13)	4,05 (0,14)	0,12 (0,19)	0 (0)	0,27 (0,03)	0,29 (0)
	Sorghum	D	-0,31 (0,1)	0,23 (0,12)	4,43 (0,24)	3,03 (0,14)	75,28 (29,6)	0 (0)	0,15 (0,01)	0,12 (0)
	Sorghum	W	-0,28 (0,1)	0,58 (0,22)	2,96 (0,11)	2,11 (0,09)	67,28 (20,77)	0 (0)	0,21 (0,03)	0,13 (0)
	Triticum	D	0,17 (0,08)	0,12 (0,01)	3,23 (0,22)	3,81 (0,08)	0,36 (0,51)	0,12 (0,26)	0,33 (0)	0,3 (0)

Family	Genus	Dom. status	MGR (%)	MPR (%)	Plant biomass myc- (g)	Plant biomass myc+ (g)	AM colonization myc+ (%)	AM colonization myc- (%)	P content myc+ (%)	P contentmyc- (%)
Poaceae	*Triticum*	W	-0,04 (0,18)	0,12 (0,02)	3,19 (0,1)	3,05 (0,19)	0,92 (0,75)	0,28 (0,42)	0,27 (0)	0,24 (0)
	Zea	D	0 (0,2)	-0,02 (0)	3,89 (0,29)	3,9 (0,25)	59,56 (24,46)	0 (0)	0,12 (0)	0,12 (0.2)
	Zea	W	0,46 (0,16)	-0,06 (0)	2,18 (0,09)	3,2 (0,11)	54,24 (14,54)	0 (0)	0,14 (0)	0,15 (0.23)
Solanaceae	*Solanum*	D	-0,01 (0,4)	0 (0,1)	1,85 (0,07)	1,83 (0,24)	29,51 (12,63)	0 (0)	0,23 (0,02)	0,23 (0)
	Solanum	W	-0,15 (0,2)	0,27 (0,19)	2,72 (0,45)	2,3 (0,17)	35,73 (16,06)	0 (0)	0,23 (0,03)	0,18 (0)

Methods S1.

Root trait data gathering. Fine root morphological data were collected as part of other experiments (unpublished). In short, to obtain the morphological and allocational root traits of our set of 24 herbaceous crops, we grew 20 individuals of the domesticated and wild accession of each crop in long containers to avoid plants from becoming severely pot-bound. Plants were harvested before the roots reached to the bottom of the container. Root systems were scanned, and their dry weight measured. Root traits were determined using WinRHIZO software (WinRHIZOTM 2009; Regents Instruments, Quebec City, Canada; Arsenault et al. 1995).

Growth conditions: The two accessions (domesticated plant and wild progenitor) belonging to each crop were simultaneously grown from January to June 2012 in the glasshouse (located in Móstoles, central Spain, 40º18´48´´N, 3º52´57´´W). To determine the root system phenotype, we built special long containers to allow roots growing for several weeks before reaching the bottom of the container. For this purpose, a round plastic cylinder (42 cm deep, 8 cm diameter) was embedded inside, and down to the bottom end, of a 25 cm long Jumbo Rootrainer (Haxnicks Ltd., Wiltshire, UK), resulting a final container of 42 cm depth x 50 cm^2 area (2.1 L). The bottom of this final container was removable without root or substrate disturbance, to analyze the depth of the deepest root. Containers were filled with pure sand to facilitate recovery of the complete root system. Plants were fertirrigated twice a week with 50 mL of a complete nutrient solution and watered through regular water sprinkling to maintain plants under optimal conditions.

Plant root harvest and trait measurements: Root lengths were checked every second day to harvest all the individuals belonging to a crop (domesticated plant and wild progenitor) before the fastest root reached the bottom of the container. We harvested 5-10 (median 9) well developed plants per accession (domesticated plant and wild progenitor), which were 30 - 40 days old after germination, depending on the crop pair. The whole root system of each individual was carefully cleaned and transferred to a transparent tray filled with water, where the root branches were carefully extended to avoid overlapping. Then, root systems were scanned as grey scale images at a resolution of 400 dpi (Epson scan GT 15000). We determined total root length (cm) and root volume (cm3) for the whole root system using a scanner-based, digital image analysis system

(WinRHIZOTM 2009). Following root scanning, the root system of each plant was oven dried (60ºC) and weighed to estimate: root tissue density (RTD, g root cm-3root), specific root length (SRL, m root g-1 root), root mass fraction (RMF, g root g-1 plant) and root length ratio (RLR, m root g-1 plant).

References of Methods S1

Arsenault JL, Poulcur S, Messier C, Guay R. 1995. WinRHlZO™, a root-measuring system with a unique overlap correction method. *HortScience* 30: 906-906.

Capítulo 3 / Chapter 3

Crops and their wild progenitors recruit beneficial and detrimental root-associated biota in opposing ways

Nieves Martín-Robles, Pablo García-Palacios,
Marta Rodríguez, Daniel Rico, Rocío Vigo,
Sara Sánchez-Moreno, Gerlinde B. De Deyn,
Rubén Milla

Manuscript in preparation

Summary

It is widely known that agricultural practices, as tillage, monocropping or fertilization, promote negative plant soil feedbacks (PSF). However, the effect of crop domestication on PSF are unknown. Artificial and natural selection pressures under cultivation impact crop genotypes, which might alter the interactions with soil organisms, reducing the mutualisms and resistance to pathogens, leading to shifts in soil legacies and promoting negative feedbacks.

We conducted a classical feedback experiment of two consecutive phases, with ten crop species and their wild progenitors. First, we grew the ten crop-wild progenitors pairs to condition the soils. Second, we inoculated the conditioned soils in a new generation of plants to test the microbial feedback effects. We measured the arbuscular mycorrhizal colonization, root feeding nematode infection and plant biomass and calculated the PSF index.

Plants grown in soils conditioned by domesticated plants showed less mycorrhizal colonization and more nematode infection. Moreover, domesticated plants were less colonized by mycorrhiza but more infected by nematodes than theirs wild progenitors. However, magnitudes and directions of plant biomass and PSF were diverse among the ten crops, and unrelated with mycorrhizal and nematodes colonization.

Soil legacies differed between domestication statuses, suggesting an impact of domestication on the recruitment of rhizosphere organisms through an overall negative effect on plant resistance to herbivores and mycorrhizal mutualism. Thus, crop domestication would alter soil legacy promoting negative feedbacks. This knowledge highlights the importance to undertake plant breeding strategies to optimize the profitable functions from the plant-soil interactions towards a sustainable agriculture.

Key words

Domestication, crop evolution, mycorrhizal colonization, nematodes infection, plant soil feedback, wild progenitors.

Introduction

Interactions between plants and soil organisms are key determinants to plant performance (Bever, 2003; Wardle *et al.*, 2004; Van der Heijden *et al.*, 2008; Bever *et al.*, 2010). Plants influence soil organisms in a species-specific manner, through changes in abiotic or biotic conditions, determining the composition and diversity of soil communities (Klironomos, 2002; Kulmatiski *et al.*, 2008; Van der Heijden *et al.*, 2008; Van der Putten *et al.*, 2013). These plant-mediated changes in belowground organisms can potentially improve or decline the plant performance and productivity of plants that subsequently occupy that soil. These reciprocal effects between plants and soil microorganisms are named plant soil feedbacks (PSFs). Mycorrhizal fungi, growth-promoting bacteria or nitrogen fixing bacteria promote positive feedbacks; while soil pathogens, such as root feeding nematodes or pathogenic fungi or bacteria promote negative feedbacks (Van der Heijden *et al.*, 2008). The net effects of PSF play a determinant role in plant coexistence, functioning and dynamics in natural and agricultural ecosystems (van der Putten *et al.*, 2007). Indeed, the proliferation of negative feedbacks by cultivated plants is the reason behind the success of cropping rotations, a common agricultural practice that consists on alternating sequences of crops with fallows (Van der Putten *et al.*, 2013; Mariotte *et al.*, 2017). Domestication has modified the morphology of crops as compared with their wild progenitors (Milla *et al.*, 2015). These shifts might have modified the interactions of crops with soil organisms, reducing the ability to recruit beneficial root microbiota and resistance to herbivores, which would lead to the accumulation of negative feedbacks. Similarly, high nutrient availability and low plant diversity, which are typical conditions of agricultural fields, also promote the accumulation of negative feedbacks (Maron *et al.*, 2011; Van der Putten *et al.*, 2013; Zuppinger-

Dingley *et al.*, 2014). Thus, the proliferation of negative feedbacks under crop cultivation might be due to changes in the plant influence on soil rhizosphere arisen with plant domestication and agricultural practices.

Agriculture alters physical and chemical properties of soil, with consequences in soil biota with key role in PSF effects (Thiele-Bruhn *et al.*, 2012; Pieterse *et al.*, 2016). Agriculture intensification eliminates the disturbance sensitive soil microorganisms and promotes the domination of few species (Stevens *et al.*, 2004; Moora *et al.*, 2014; Bell & Tylianakis, 2016). Specifically, agricultural practices as monocropping increase fungal pathogens (Maron *et al.*, 2011), while others like tillage or fertilization reduce mycorrhizal diversity (Helgason *et al.*, 1998; Mäder *et al.*, 2000; Oehl *et al.*, 2003). In addition, the majority of mycorrhizal fungi thriving in agricultural soils tends to be poor mutualistic partners, showing traits less beneficial to plants (Verbruggen & Kiers, 2010; Chagnon *et al.*, 2013). Likewise, agricultural practices also alter the bacterial community composition (Thiele-Bruhn *et al.*, 2012; Fierer *et al.*, 2013; Leff *et al.*, 2015). For instance, nitrogen addition reduces rhizobia diversity and abundance (Yan *et al.*, 2014; but see Treseder, 2008), leading to the appearance of less mutualistic rhizobia, which provides fewer benefits to their hosts (Vargas *et al.*, 2000; Kiers *et al.*, 2002; Weese *et al.*, 2015). Conversely, communities of root feeding nematodes are increased by tillage and monocropping (Abawi & Widmer, 2000; van der Putten *et al.*, 2006; Postma-Blaauw *et al.*, 2010). Thus, agricultural practices promote soil legacies which might decline plant growth of subsequent generation of plants, by decreasing the mutualistic partners and increasing pathogenetic communities (Mariotte *et al.*, 2017). While our knowledge about the effect of agricultural practices on feedback is quite advanced, little is known about domestication effect on recruitment

ability of plants on soil microorganisms and the consequences in the soil legacy and feedbacks.

Crop domestication has modified plant genotypes, through artificial and natural selection (Milla *et al.*, 2015). These shifts might affect the plant ability to interact with soil organisms, leading to changes in the rhizosphere microbiome composition and functions (Wissuwa *et al.*, 2009; Hale *et al.*, 2014; Pérez-Jaramillo *et al.*, 2016). In this line, previous studies reported differences in rhizosphere composition between domesticated plants and their wild progenitors (Mao *et al.*, 2013; Zachow *et al.*, 2014; Cardinale *et al.*, 2015; Pérez-Jaramillo *et al.*, 2017). Shifts on rhizosphere community suggest that crop evolution would alter the outcome of plant soil biota interactions. However, whether the changes in the rhizosphere composition driven by crop domestication are reflected in PSF effects is not well known to date.

Crop domestication is hypothesized to impact the soil rhizosphere composition promoting negative feedbacks, through complementary evolutionary mechanisms. Firstly, trade-off between growth and defense against enemies exists (Coley *et al.*, 1985; Herms & Mattson, 1992; Kulmatiski *et al.*, 2008; Craine, 2009). This trade off would be reinforced in cultivated plants by selective pressures exerted to increase the yield. Thus, crop evolution would promote more productive plants with negative consequences in the defense ability (Rosenthal & Dirzo, 1997). Likewise, a reduction on plant resistance to aboveground herbivores has been reported for several crops (Chen & Welter, 2007; Turcotte *et al.*, 2015; Whitehead, Turcotte & Poveda, 2016; Simpson *et al.*, 2017). Belowground, the reduction in the defense ability might trigger the accumulation of root feeding herbivores in the rhizosphere of cultivated plants, which would promote negative feedbacks. Secondly, crops have evolved under high

nutritional conditions and with pest control, which might have altered the selective regime of plant defense and root mutualisms. Fertile conditions deter plants from investing in rhizobia (Kiers *et al.*, 2002) and mycorrhizal fungi (Johnson, 1993; Mäder *et al.*, 2000; Nijjer *et al.*, 2010), which reduces the proliferation of these organisms in the soil. In addition, mycorrhizal and rhizobia symbiosis become less mutualistic under fertile conditions (*i.e.* Johnson & Pfleger, 1992; Tawaraya, 2003; Johnson, 2010; Remigi *et al.*, 2016), reducing mycorrhizal benefits in domesticated plants under such circustances (Kiers *et al.*, 2002; Martín-Robles *et al.*, 2018). Finally, plant traits and strategies for nutrient acquisition can predict PSF effects (Baxendale *et al.*, 2014; Kardol *et al.*, 2015; Cortois *et al.*, 2016; Laliberté, 2017; Faucon *et al.*, 2017). Although both domesticates and wild progenitors usually fall within the resource-acquisitive part of the economic spectrum (Milla *et al.*, 2015; Roucou *et al.*, 2018; Martín-Robles *et al.*, chapter1), crop genotypes may show trait values that are even more biased towards this part of the spectrum (García-Palacios *et al.*, 2013; Milla *et al.* submitted). Acquisitive strategists, which occupy rich resource environments, accumulate negative feedbacks (Lemmermeyer *et al.*, 2015; Laliberté, 2017). Therefore, domesticates might promote more negative feedbacks than their wild progenitors (Mariotte *et al.*, 2017). Thus, there are several reasons to expect that crop domestication might trigger plant genotypes which have altered the interactions with soil organisms, leading to accumulate negative feedbacks. Knowledge about the consequences of domestication on PSF would aid to develop plant breeding strategies to optimize associations with soil organisms and reduce the use of pesticides and fertilizers (Pieterse *et al.*, 2016; Schmidt *et al.*, 2016; Mariotte *et al.*, 2017; Faucon *et al.*, 2017).

In this work, we investigated whether crop domestication has modified the strength and direction of plant soil feedbacks through modifications in the

interactions with root-associated organisms with key role in PSF across ten independent domestication events. In order to compare differences in PSFs, we stablished a classical PSF experiment, using a taxonomically diverse set of ten crops. In a first phase, we grew ten domesticated species and each of their wild progenitors in separate containers to condition the soils. In the second phase, we examined the effects of the soil conditioning on mycorrhizal and nematodes root colonization and aboveground biomass by growing the same plant genotypes on soils previously conditioned by themselves or by the domesticated or progenitor partner (Fig. 1). With this experiment we tested two hypotheses: (i) provided that mycorrhizal dependence and herbivore defense might have decreased with domestication, we hypothesized that domesticated crops have lower mycorrhizal root colonization but higher infestation of root feeding nematodes than wild progenitors; and (ii) we hypothesized that aboveground plant growth would be reduced in soils previously conditioned by domesticated plants and this reduction in plant biomass would be greater in domesticated plants than wild progenitors.

Material and Methods

Study system and experimental design

We selected ten phylogenetically diverse herbaceous crop species (Table 1) comprising the most relevant families of herbaceous crops for global agriculture (www.fao.org/statistics). The choice of study species was made to include a wide range of variability in the domestication process, such as different target organs of selection (leaves, seeds and fruits), diverse origins and antiquity of domestication ranging from 10.000 to 600 years (Table S1, Sauer, 1993; Hancock, 2004). For each crop, we obtained seed lots from

two accessions: one belonging to a common domesticated cultivar and another of its recognized wild progenitor. See Table S1 for further information about the criteria for assigning wild progenitors, seed accessions identifiers and seed donors.

Crop name	**Wild progenitor**	**Domesticated plant**
Amaranth	*Amaranthus hybridus L.*	*Amaranthus cruentus* L.
Barley	*Hordeum spontaneum* K.Koch	*Hordeum vulgare* L.
Cardoon	*Cynara cardunculus* L.	*Cynara cardunculus* L.
Chard	*Beta vulgaris L.*	*Beta vulgaris L.*
Leek	*Allium ampeloprasum* L.	*Allium porrum* L.
Maize	*Zea mexicana* (Schrad.) Kuntze	*Zea mays* L.
Pepper	*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill	*Capsicum annuum L.*
Sorghum	*Sorghum bicolor* (L.) Moench	*Sorghum drummondii* (Nees ex Steud.) Millsp. & Chase
Tomato	*Solanum pimpinellifolium* L.	*Solanum esculentum* Dunal
White clover	*Trifolium repens* L.	*Trifolium repens* L.

Table 1. Pairs of domesticates and wild progenitors used in the experiment.

We conducted a classical short-term feedback experiment in the greenhouse consisting of two consecutive phases (*i.e.* Bever 2002; Kulmatiski *et al.*, 2008). In the "training phase", we grew plant individuals belonging to 20 accessions: ten domesticated plants and their wild progenitors (Table 1) in separate pots filled with the same initial soil (Fig. 1). After this phase, we got samples of two soil types (crop soil and wild progenitor soil) for each crop-wild progenitor pair, which are expected to accumulate a genotype-specific suite of soil organisms. The microbial feedback effects of these trainings were tested in the "feedback phase" by using the soil samples trained by wild progenitors and domesticated crops

to inoculate the microbial community in a new generation of plants (Fig. 1). In the feedback phase, plants were also grown under sterile soil conditions to assess the independent effects of changes in soil physicochemistry between soil types. In all the soils of the training phase, we measured the microbial biomass and soil organic matter content; and in all the plants of the feedback phase, we measured the aboveground biomass, mycorrhizal and nematode colonization.

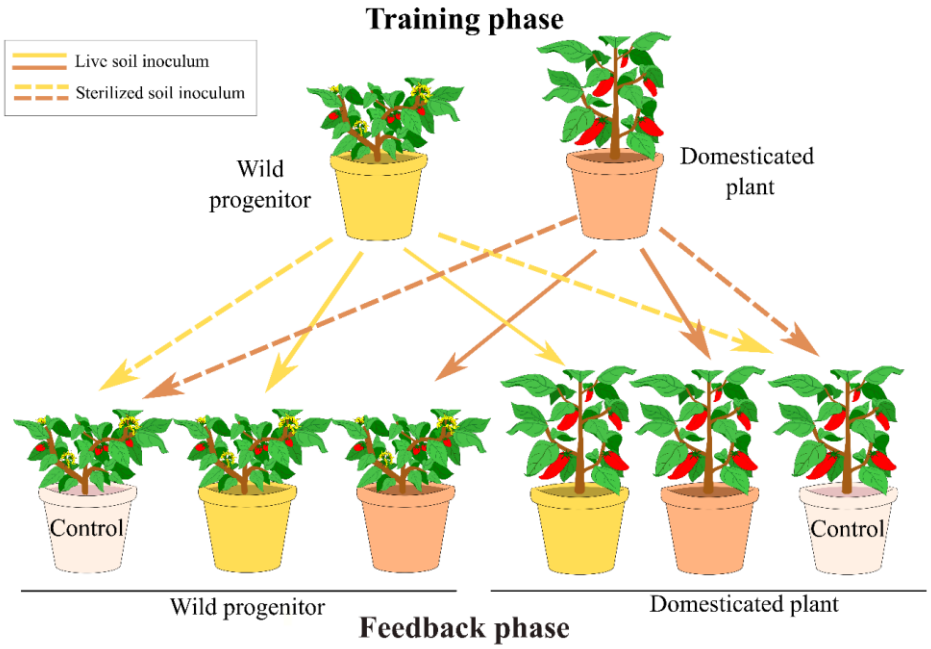


Figure 1. Experimental design to test the effects of plant domestication on plant-soil feedbacks. In the training phase, wild progenitor (up left) and domesticated plant (up right) belonging to ten crops were grown in separate containers with the same initial soil. The conditioned soils were used for growing a new generation of plants in the subsequent feedback phase. In this phase, domesticated plants and wild progenitors of each crop were individually grown in soils conditioned by wild progenitors (yellow), wild progenitors (brown) and sterilized controls (beige)

Training phase

The set of wild progenitors and domesticated crops (Table 1) was grown from March to June 2014 in the greenhouse of the Rey Juan Carlos University, located in Móstoles, central Spain (40º18´48´´N, 3º52´57´´W). Each accession was replicated three times, resulting in 60 pots (10 crops x 2 domestication status x 3 replicates) set up in a randomized design. We used fresh field soil from a roadside grassland, characterized by high organic matter content, microbial functional diversity and high fungal: bacterial ratio (Table S2). The soil was collected from a depth of 15-30 cm, sieved (2mm) to remove coarse root fragments and homogenized before filling the pots for sowing. All seeds were surface-disinfected in 70% ethanol solution for 3 min and pre-germinated on petri dishes on filter paper soaked with sterilized water in dark and cold (4ºC) growth chambers. Pre-germinated seeds were transplanted into pots (6L volume, 20x20x23 cm) to train the soil. The number of seeds per pot varied between 5 to 10 depending on the plant size of the accession, to reduce the differences in biomass production across pots. So, to minimize differences, less seeds were sown per pot in the case of large accessions, as maize, and more seeds were sown for small accessions, as white clover. Pots were watered through automatic water sprinkling to maintain soils near field capacity and rotated every two weeks to control variation in the greenhouse.

We harvested the soils twelve weeks after transplanting, once soils were extensively colonized by roots, which suggests that soil organisms may have responded to the different accessions. The soil of each pot was individually cleaned of visible root fragments, sieved to 2 mm and thoroughly homogenized. Ten soil samples (200 mL of homogenized soil) were taken per pot to inoculate the microbial community trained in this phase in the pots of the feedback phase. The soil inoculums were stored for eight

months at 4ºC until the start of the feedback phase. In addition, two subsamples of soil were collected per pot and frozen (-20ºC), to characterize the microbial and soil organic matter enrichment after plant growth.

To analyze the active soil microbial biomass, we used a substrate-induced respiration method and the MicroResp™ system (Campbell *et al.*, 2003) as in (García-palacios *et al.*, 2011). Before these measurements, soil was incubated in 96-DeepWell Microplates for 5 days at 25ºC and at 50% of their water-holding capacity in order to allow microbial communities to reestablish in defrosting soil. To quantify the soil organic matter content, we used the "loss on ignition" method (Hoogsteen *et al.*, 2015). Briefly, soil samples were weighted before and after the removal of the organic matter by ignition at 550ºC for 4 hours. The difference in the weight indicated the organic matter content of the sample.

Feedback phase

During the feedback phase, each accession was grown in sterilized background soil mixed with one of three different soil inoculums: (1) soil inoculum trained by the domesticated accession of the crop pair (hereafter "domesticated soil"), (2) soil inoculum trained by the wild progenitor accession of the crop pair (hereafter "progenitor soil") and (3) a sterilized mixture of domesticated and progenitor soil (hereafter "control") (Figure 1). This set up was replicated ten times. In total, there were 600 pots (10 crops x 2 domestication status x 3 soil types x 10 replicates) in the feedback phase.

To prepare the potting soil for this phase, we mixed the soil inoculum obtained in the training phase with sterilized background soil in a 10 vs 90%

proportion, respectively. This approach is commonly used in PSF experiments where the conditioned soil typically ranges from 1 to 50% of total volume with the remainder as sterilized soil (Brinkman *et al.*, 2010). The sterilized background soil was prepared by autoclaving a mixture of 75% sand and 25% topsoil (topsoil: 93% sand, 5% silt and 1% clay, 0.38% organic matter; pH=8.3). On March 2015, the set of accessions (Table 1) were pre-germinated in dark and cold (4ºC) growth chambers. Pre-germinated seeds were individually planted in 2L pots (22x10.5x10.5 cm), placed randomly in the greenhouse. At the beginning of the experiment, all plants were fertilized with 16g of slow release fertilizer (8 g/L; 16%N, 8%P and 12%K; Basacote Plus 6M, Compo) to keep plants under high fertile regime and prevent nutrient limitation in a sandy substrate. During the growing season, all pots were watered through automatic water sprinkling to maintain soil near field capacity and were randomly rearranged in the greenhouse every 2 weeks to minimize greenhouse micro-site effects on plant performance. Before flowering, 6-7 weeks after sowing, depending on the crop species, we randomly harvested 5-10 plants per accession and per treatment. We took the aboveground biomass of each plant and oven-dried it at 60ºC for 72 hours to measure plant dry biomass (g).

Measurement of beneficial and detrimental root-associated microorganisms

To calculate arbuscular mycorrhizal colonization and nematode infection, we washed the fresh roots of harvested plants and randomly selected fine root fragments (approximately 80 mg per sample). Root samples were cleared with 10% KOH at 90ºC and transferred to 1% HCl solution to eliminate root pigmentation. After rinsing the root samples in clear water, roots were stained in a staining solution (50% acid lactic, 25% glycerol) with fuchsine 1 g/l at 90ºC and rinsed in distaining solution, identical to staining

solution without fuchsine (Baker & Gowen, 1996). Clearing, staining and distaining times varied among accessions. Once stained, mycorrhizal colonization and nematode infection were measured using the gridline intersect method, with a magnification of 35x (Giovanetti & Mosse, 1980). We quantified arbuscular mycorrhizal colonization (%) as the percentage of intercepts of root colonized by hyphae, vesicles and arbuscules from 250 intercepts per sample. Similarly, nematodes infection (%) was quantified as the percentage of intercepts of roots colonized by adults and eggs from 250 intercepts per sample.

Plant soil feedback calculation

We calculated a plant soil feedback (PSF) index to evaluate the effects of soil legacies in the training phase on aboveground biomass in the feedback phase. PSF index was calculated as $PSF= (M_i-M_{ni})/M_{ni}$, where M_i is the biomass (log transform) of species grown in soil that received a biologically active soil inoculum and M_{ni} is the arithmetic mean of biomass (log transform) of plants that received a sterilized soil inoculum, i.e. control plants (Kardol, 2007; Brinkman *et al.*, 2010). PSF index is positive if biologically active inoculum increases plant biomass, and negative if biomass decreases. This approach provides an estimate of PSFs that it is independent of plant size and that allows comparisons between species and environments (Brinkman *et al.*, 2010).

Statistical analyses

To assess whether soil conditioning has modified the soil parameters in the training phase, we tested microbial biomass and soil organic matter enrichment using linear models. For the microbial biomass model, we used four of the ten crops surveyed in this study: *Amaranthus*, *Cynara, Sorghum* and *Zea*. For the organic matter content model, we used *Amaranthus*, *Beta,*

Solanum and *Zea*. These models contained microbial biomass and soil organic matter enrichment as dependent variables and domestication status (wild progenitor or domesticated plant), crop identity (four crop pairs) and the interaction between them as predictor variables. Linear models were fitted with lm function and performed with R software v.3.3.0 (R Core Team, 2016).

Prior to data analysis of feedback phase, we excluded one pot with an extreme plant biomass value, and 18 control plants that were accidentally colonized by mycorrhizas or nematodes. These contaminated control plants represented the 16% of 110 total controls and were randomly distributed across accessions. All subsequent analyses were done with 470 plants and performed with R software v.3.3.0.

To assess whether crop domestication and soil conditioning have affected the plant ability to interact with beneficial and detrimental root-associated organisms, we used generalized linear mixed-effect model. The differences in mycorrhizal colonization and nematode infection among plants were quantified with binomial error distribution; using the clog-log link function in the case of nematode infection model to deal with the numerous zeros of the variable (Zuur *et al.* 2009). These models contained mycorrhizal or nematodes colonization as dependent variables and domestication status (wild progenitor or domesticated plant), soil conditioning (the domestication status that conditioned the soil in the training phase: "progenitor soil" and "domesticated soil") and the interaction between them as fixed effects predictors. The model with mycorrhizal colonization as dependent variable included nematode infection as fixed effects and *vice versa*. Both models included crop identity (Table 1) as a random effect over the intercept (random intercept term) and as a random effect over

domestication status parameter (random slope term, analogous to an interaction term in the fixed effects). In addition, pot identity (60 pots of the training phase), to which each soil inoculum belonged, was included in the random structure of the models. Specifically, pot identity was nested in crop identity, to model the variation between pot identity within the crop identity as random intercepts. Generalized linear mixed-effects models were fitted with glmer function of the "lme4" package (Bates *et al.*, 2007).

To assess whether crop domestication and soil conditioning have modified the plant biomass and PSF index, we used mixed effect models. Plant biomass data were log-transformed to meet normality assumptions and homogeneity of variance of the model´s residuals. Both models included domestication status, soil conditioning, the interaction between them, mycorrhizal colonization and nematodes colonization as fixed effects. The random structure was as described previously for mycorrhizal and nematodes models. Linear mixed-effect models were fitted with lme function of the "nlme" package (Pinheiro *et al.*, 2015).

In all the models, the significance of the fixed factors was calculated with type III analysis of variance, obtained with the mixed function of the "afex" package (Singman *et al.* 2015). We estimated the pseudo-R^2 of mixed effects models using the marginal R^2 (R^2m, variance explained by fixed factors) and conditional R^2 (R^2c, variance explained by fixed and random factors) according to Johnson (2014), with the r.squaredGLMM function of the "MuMIn" package (Barton, 2014). The r.squaredGLMM function is not applicable to models with clog-log link function. Therefore, to estimate the marginal R^2m of the nematodes model we used an equation with the deviance of the model and the null model, but R^2C was not estimated. Finally, to calculate the significance and least square means of the

interaction of domestication and soil conditioning, we conducted post-hoc Tukey-test pairwise comparisons with the lsmeans function of the "lsmeans" package (Lenth, 2016).

Results

Training effect on microbial biomass and soil organic matter enrichment

The differences on microbial biomass and organic matter enrichment of soils differed among accessions, indicating that plants modified the soil parameters during the training phase. Domesticated plants tended to accumulate more microbial biomass in the soil than wild progenitors (*P* 0.08, Fig. 2a). This positive effect of domestication on microbial biomass (Table S3) was common for the four crop pairs analyzed (Fig. S1). The amount of organic matter removed from soils varied across crop pairs (*P* 0.003, Table S3). All plants reduced the organic matter from the soils in comparison with controls. However, crop domestication did not affect soil organic matter content (Fig. 2b).

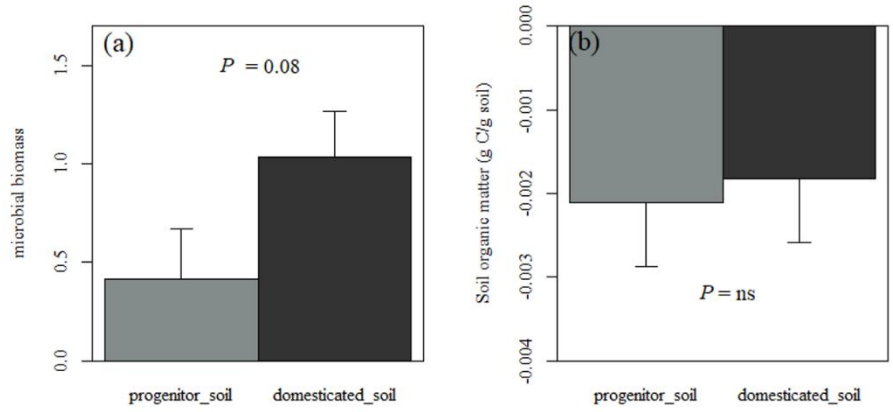


Figure 2. Effect of soil conditioning (conditioned by wild progenitor: "progenitor soil", or domesticated plant: "Dom. Soil") on microbial biomass (a) and soil organic enrichment (b).

Effects of plant domestication and soil conditioning on mycorrhizal colonization and nematode infection

In the feedback phase, domesticated plants were less colonized by mycorrhiza and showed more nematodes than wild progenitors irrespective of the soil conditioning ($P<0.0001$, Table 2). In addition, soil conditioning significantly altered mycorrhizal colonization and nematode infection rates (P 0.01 and $P<0.0001$ respectively, Table 2). Wild progenitors were more colonized by mycorrhizal fungi in progenitor soils than domesticated soils, whereas domesticated plants did not show significant differences on mycorrhizal colonization between soils (domestication status x soil conditioning P 0.001, Table 2, Fig. 3a). The number of nematodes increased in wild progenitors and domesticated plants grown on domesticated soils and decreased in progenitor soils (Fig. 3a,b). The response pattern to domestication and soil conditioning was common to most species in the experiment, although we found substantial variation in mycorrhizal and nematodes colonization rates among the set of accessions (Fig. 4a-d). Root colonization by mycorrhiza ranged between 20-80% depending on crop identity, domestication status and soil conditioning (Fig. 4a,b). For instance, maize showed higher mycorrhizal colonization rates (50-80%), than other crops, as white clover, that showed lower mycorrhizal colonization (10-20%; Fig. 4a,b). Similarly, root colonization by nematodes ranged between 0-5% depending on crop identity, domestication status and soil conditioning (Fig. 4c,d). For instance, crops as chard were more colonized by nematodes than other crops as leek (Fig. 4c,d). Finally, we did not find a significant interaction between the mycorrhizal colonization and nematode infection in the roots (interaction term $P>0.05$, Table 2).

	Mycorrhizal colonization (%)			**Nematodes colonization (%)**			**Plant biomass (g)**			**PSF index**		
	estim value (SE)	*F*	*P*	estim value (SE)	*F*	*P*	estim value (SE)	*F*	*P*	estim value (SE)	*F*	*P*
Intercept	-1.57 (0.11)	-	-	-5.24 (0.17)	-	-	0.09 (0.195)	-	-	0.01 (0.06)	-	-
Soil conditioning	-0.29 (0.11)	6.56	**0.01**	0.93 (0.11)	51.54	**<.0001**	-0.01 (0.021)	0.07	0.8	-0.04 (0.06)	0.01	0.94
Domestication status	-0.35 (0.04)	43.22	**<.0001**	0.48 (0.09)	31.21	**<.0001**	0.08 (0.06)	2.21	0.17	-0.01 (0.06)	0.02	0.89
Mycorrhizal colonization	-	-	-	-0.5 (0.66)	0.58	0.45	-0.31 (0.22)	1.72	0.19	0.01 (0.29)	0.10	0.76
Nematodes colonization	0.08 (2.09)	0.00	0.97	-	-	-	1.19 (1.61)	0.5	0.48	-2.72 (10.00)	0.00	>.99
Dom x soil conditioning	0.15 (0.04)	10.73	**0.001**	0.11 (0.07)	2.46	0.12	0.01 (0.01)	0.3	0.58	-0.01 (0.06)	0.01	0.93
R^2m	0.055			0.08			0.032			0.008		
R^2c	0.232			-			0.903			0.008		

The dependent variable Plant biomass was log transformed. Domestication and soil conditioning were factors. Interactions are indicated by x.

Table 2. Results of mixed-effects models testing the effects of soil conditioning (conditionate in the training phase), on wild progenitors and domesticated plants growth during the feedback phase. The models tested if mycorrhizal and nematodes colonization were affected by soil conditioning, domestication status (Dom) and the interaction; and if plant biomass and plant soil feedback (PSF) index were affected by soil conditioning, domestication status, the interaction, AMF and nematodes colonization. The table shows the estimated values and standard errors (SE), *F* and *P* scores of each variable. The percentage of the variance explained by the fixed effects of the models is indicated by R^2marginal (R^2m), and the variance explained by both the fixed and random effects is indicated by R^2conditional (R^2c).

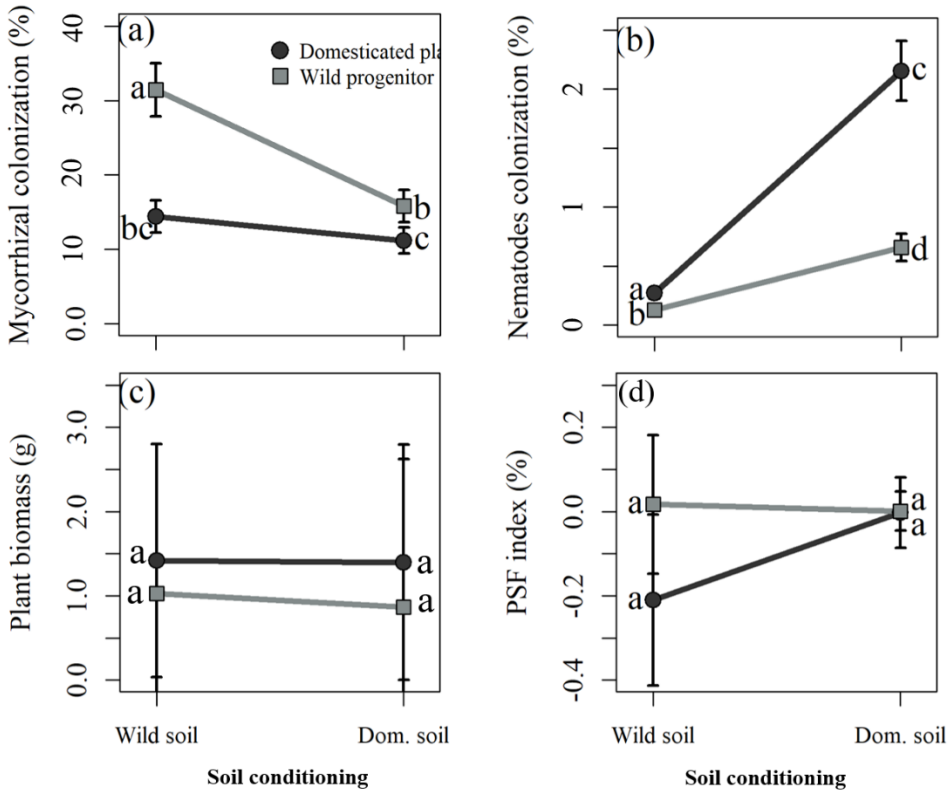


Figure 3. The reaction of domesticated plants (black lines) and wild progenitors (grey lines) to soil type. The reaction to soil type was measured as mycorrhizal colonization (a), nematodes colonization (b), plant biomass (c) and plant soil feedback (PSF) index (d). The symbols, error bars (standard error) and letters show the least squares means and 95% confidence interval of domesticated plants (circles) and wild progenitors (squares), obtained by mixed models.

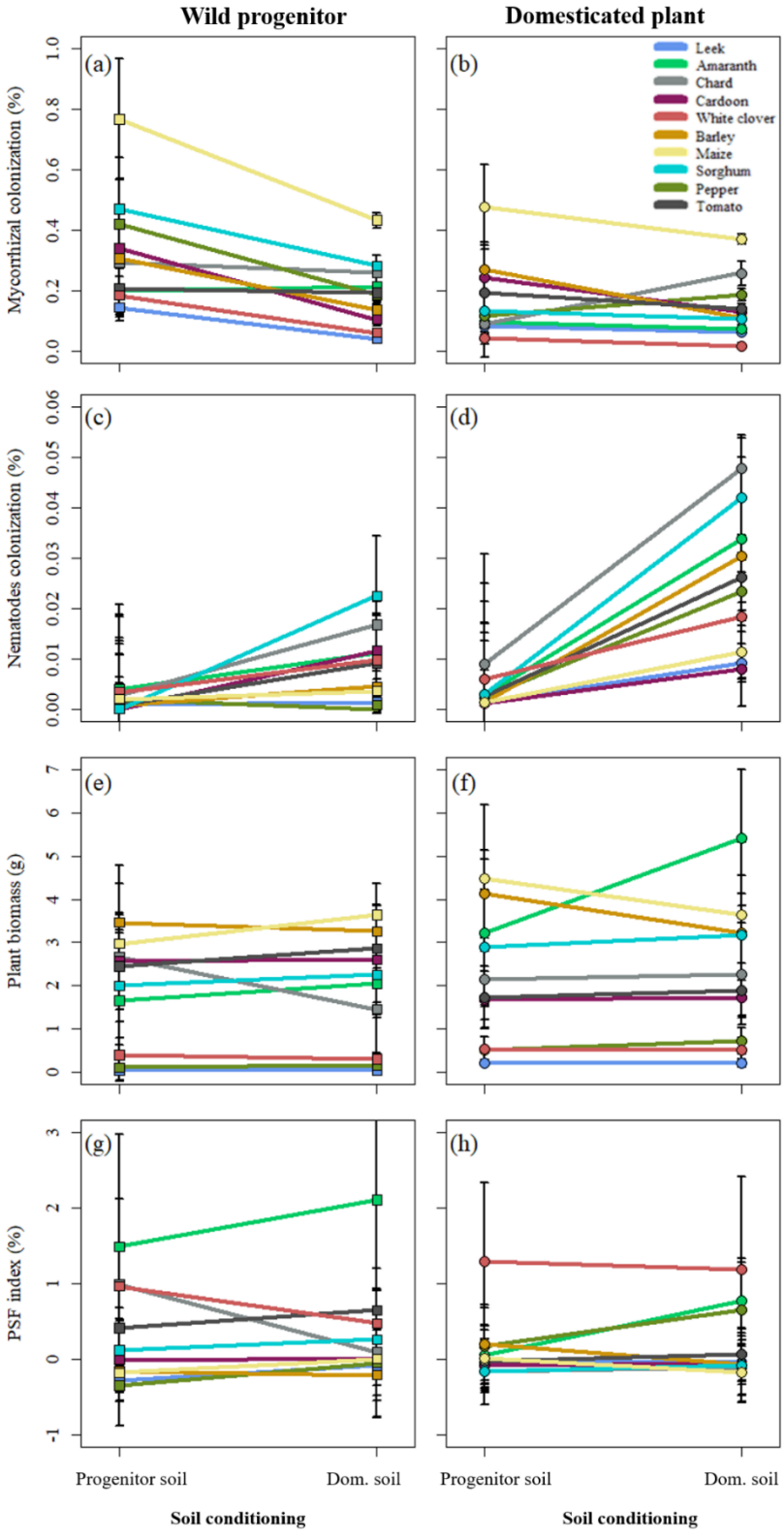
Response of plant biomass and plant soil feedback to domestication and soil conditioning

Aboveground plant biomass was not affected by domestication status and neither soil conditioning in the feedback phase (*P* 0.17 and *P* 0.8 respectively, Table 2). Wild progenitors and domesticated plants did not show significant differences in plant biomass (Fig. 3c). Similarly, the plant biomass was not significantly different in domesticated and progenitor soils

(Fig. 3c). However, the effect of domestication and soil conditioning on plant biomass varied among crops, as informed the high variance associated with the random term of the plant biomass model (indicated by R^2m and R^2c, Table 2). In addition, plant biomass also varied among crops, ranging from 0.5 g, in crops such as leek or white clover, to 5g, in crops such as barley and maize (Fig. 4e,f). Finally, aboveground plant biomass was not affected by either mycorrhizal or nematodes colonization rates (Table 2, Fig. S2).

Plant soil feedback index was not affected by domestication status and soil conditioning (*P* 0.89 and *P* 0.94 respectively, Table 2, Fig. 3d). PSF index showed generally low scores, ranging from -0.5% to 2% depending on the domestication status, soil conditioning and crop identity (Fig. 4g,h). Similarly, the response to soil conditioning differed between crops and domestication statuses (Fig. 4g,h). For instance, PSF index increased in response to domesticated soils for crops as amaranth or tomato and decreased in domesticated soils for crops as white clover. The biomass of wild progenitors in soils conditioned by themselves and domesticated plants was not significantly different to sterilized controls (Fig S3). In addition, mycorrhizal and nematode colonization neither influenced the direction or strength of the feedbacks (Table 2, Fig. S2).

Figure 4. Effect of soil type (trained by wild progenitor: "progenitor soil", or domesticated plant: "Dom. Soil") on mycorrhyzal colonization (a and b), nematodes colonization (c and d), plant biomass (e and f) and plant soil feedback index (PSF, g and h) of wild progenitors (a,c,e and g) and domesticated plants (b,d,f and h). The symbols show the mean score of each wild progenitor (squares) and domesticated plants (circles). Colors of the points correspond to crop pairs.→


Wild progenitor
Domesticated plant
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Leek
Amaranth
Chard
Cardoon
White clover
Barley
Maize
Sorghum
Pepper
Tomato
Mycorrhizal colonization (%)
Nematodes colonization (%)
Plant biomass (g)
PSF index (%)
Progenitor soil
Dom. soil
Soil conditioning

Discussion

Here, we investigated the effect of crop domestication on the interactions with rhizosphere biota with key role in PSF and its consequences on soil legacy and the strength and directions of PSF across ten independent domestication events. In support to our first hypothesis, we found that domesticated plants were less colonized by arbuscular mycorrhiza but more infected by root feeding nematodes than theirs wild progenitors, suggesting shifts in the interactions with these soil organisms. Moreover, wild progenitors and domesticates showed less mycorrhizal colonization and more nematodes when plants grew at soils conditioned by domesticated plants (Fig. 4), indicating that soil legacies differed between domestication statuses. These results suggest an impact of domestication on the recruitment of rhizosphere organisms through an overall negative effect on plant resistance to herbivores and mycorrhizal mutualism. We expected that such shifts in soil legacy should trigger negative feedbacks on plant performance. However, in contrast to our second hypothesis, plants did not reveal growth reduction in domesticated soils. In addition, plant growth response to feedbacks from both conditioned soils and sterilized soils was diverse among accessions. This result could not be predicted based on the variation in mycorrhizal colonization and nematode infection across plant genera and domestication status. In the discussion that follows, we examine the domestication effect on mycorrhizal colonization and nematodes infection and speculate about the mechanism underlying these patterns. We also examine and speculate about the reasons of the lack of a general reaction of plant biomass to the soil trainings.

Domesticated plants were less colonized by arbuscular mycorrhizal fungi than wild progenitors, suggesting that crop domestication has negatively

impacted the mycorrhizal symbiosis. A reduction in the mycorrhizal colonization with domestication has been reported for crops as wheat, breadfruit and sunflower (Hetrick *et al.*, 1993; Xing *et al.*, 2011; Turrini *et al.*, 2016). Plant species tend to limit mycorrhizal colonization when they have less dependence on mycorrhizal symbiosis (Graham *et al.*, 1991). Thereby, the reduction of mycorrhizal colonization intensity might be a consequence of unintended selection for domesticated species less dependent of mycorrhizal symbiosis (Kiers & Denison, 2014; Pérez-Jaramillo *et al.*, 2016). Likewise, the reduction in mycorrhizal dependence with domestication has been reported for several crops (Baon *et al.*, 1993; Hetrick *et al.*, 1993; Zhu *et al.*, 2001; Tawaraya, 2003; Xing *et al.*, 2011; Turrini *et al.*, 2016), with few exceptions (Lehmann *et al.*, 2012). Less dependent plants may arise with domestication through: first the well-known pattern that fertilization decreases mycorrhizal diversity and abundance in natural (Treseder, 2004; Hoeksema *et al.*, 2010) and agricultural ecosystems (Mäder *et al.*, 2000; Oehl *et al.*, 2003; Verbruggen & Kiers, 2010). Moreover, high fertile conditions promote mycorrhizal fungi with less cooperative traits (Kiers & Heijden, 2006; Nijjer *et al.*, 2010; Chagnon *et al.*, 2013), that decreases the mycorrhizal benefits allocated to the host (Verbruggen & Toby Kiers, 2010; Verbruggen *et al.*, 2015). Second, the resource available to the plant is less dependent upon the symbiont´s contribution under fertile conditions (Johnson, 2010). Mycorrhizal colonization represents a carbon cost to the plant, that is directly related to the benefits that the plant received from the partner for the carbon investment (Graham *et al.*, 1991; Johnson, 2010). High nutrient availability lead plants to expend less resource in mycorrhiza even further when the mycorrhiza fails providing benefits (Graham & Eissenstat, 1994). Thus, low mycorrhizal diversity with less mutualistic behavior, in combination with less dependence of mycorrhizal resource contribution might reduce the mycorrhizal dependence of domesticated plants reducing

as well, the mycorrhizal colonization on the root and the abundance on the soil.

Domesticated plants were more infected by root feeding nematodes in both soils, suggesting that crop domestication has reduced the resistance ability against nematodes infection. Evolution under cultivation may decrease the resistance of plants to herbivores (review in Macfadyen & Bohan, 2010; Whitehead, Turcotte & Poveda, 2016). For instance, domesticated plants decrease herbivory resistance to caterpillar in sunflower (Chen & Welter, 2007) and to leafhopper in maize (Dávila-Flores *et al.*, 2013). Consequences of domestication on belowground herbivory are less studied although, a few evidences also suggest that modern cultivars are more infected by root nematodes than wild progenitors (Rivera *et al.*, 2016), landraces cultivars (Sheedy & Thompson, 2009) or weeds (Roberts *et al.*, 1981; Trudgill & Blok, 2001). The reduction of herbivory resistance with crop domestication might be explained by the resource-availability hypothesis, that argues that species in resource rich environments invest resources in growth rather than defense (Coley *et al.*, 1985; Herms & Mattson, 1992; Lemmermeyer *et al.*, 2015). Specifically, domestication may have compromised the defense ability of modern crops in order to increase plant growth and yield (Rosenthal & Dirzo, 1997). In this line, previous studies found trade-offs between resource allocation to mechanical or chemical defense and growth rate in several crop species (Massei & Hartley, 2000; van der Putten *et al.*, 2006; Kempel *et al.*, 2011; Rodriguez-Saona *et al.*, 2011; Turcotte *et al.*, 2014; Simpson *et al.*, 2017). Thus, breeding for high yield and palatability may have unintended consequences reducing the resistance ability against nematodes on crops, leading to the proliferation of nematodes in the rhizosphere.

Plants grown in soils conditioned by domesticated plants were less colonized by mycorrhiza but more infected by nematodes (Fig. 5), indicating that soil microorganism recruitment of plants might have differed between domesticates and wild progenitors. In addition, analysis of soil microbial biomass revealed that domesticated plants tended to promote the accumulation of more microbial biomass in the soil during the conditioning phase. Our result, linked with the fact that mycorrhizal and nematodes colonization in the feedback phase differed among domestication statuses, suggests that rhizosphere of domesticated plants in the training phase might be characterized by less mycorrhizal fungi and more nematodes. Previous studies found differences in the rhizosphere between domesticated plants and wild progenitors for few crops (Zachow *et al.*, 2014; Bulgarelli *et al.*, 2015; Cardinale *et al.*, 2015; Szoboszlay *et al.*, 2015; Leff *et al.*, 2016; Iannucci *et al.*, 2017; Pérez-Jaramillo *et al.*, 2017). A reduction of mycorrhizal fungi and increase of nematodes in the rhizosphere of domesticated plants would promote negative feedbacks. In this line, Miller & Menalled (2015) found negative feedbacks in plants growing in soils previously trained by crops, in comparison with plants growing in soils trained by wild species. The proliferation of negative feedbacks under domesticated plants might be a consequence of their resource strategy. Negative feedbacks are accumulated by plants with acquisitive strategies, which are more susceptible to herbivores and pathogens (Baxendale *et al.*, 2014; Cortois *et al.*, 2016; Bardgett, 2017; Laliberté, 2017). Previous evidence suggest an evolution of plant´s strategies towards acquisitive strategies with crop domestication (Roucou *et al.*, 2018; Milla *et al.*, submitted). Based on acquisitive strategies, domesticated plants would accumulate more negative feedbacks than its progenitors (Mariotte *et al.*, 2017). Thus, crop domestication would have

modified soil interactions altering the soil legacy probably promoting negative feedbacks.

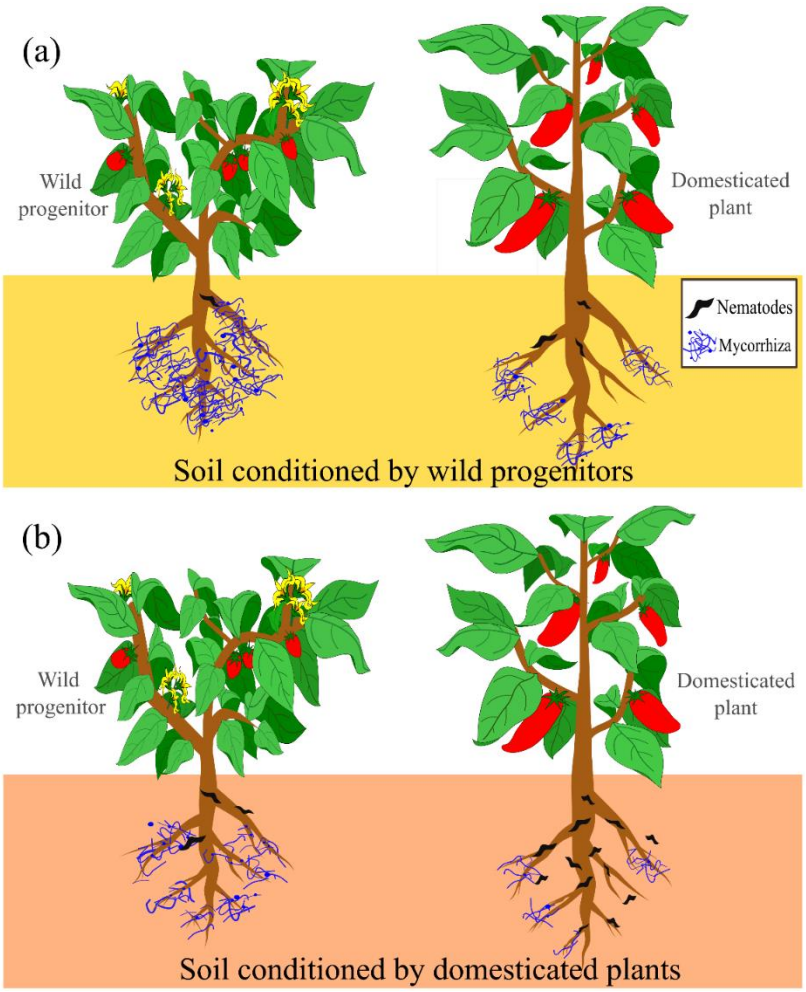


Figure 5. Conceptual diagram summarizing the main results of this study. During the feedback phase, wild progenitors and domesticated plants were growth in soils conditioned by the corresponding wild progenitor (a) and its domesticated plant (b) to test the effect of soil legacies in plant performance. Domesticated plants (right plants) accumulated more root feeding nematodes (black dashes) than wild progenitors (left plants), and this pattern was more pronounced in soils conditioned by domesticated plants (b). In addition, wild progenitors were more colonized by arbuscular mycorrhizal fungi (tangle of blue thick lines) than domesticated plants, and the pattern was stronger in soils conditioned by wild progenitors (a). Nevertheless, the effect on plant microorganism interactions did not impact on plant performance, which were similar irrespective of the soil conditioning identity.

Soil conditioning did not influence plant growth, even though mycorrhizal colonization and nematode infection varied between soils and domestication statuses. The fact that differential colonization of root mutualists and antagonists did not impact on plant performance could be due to diverse causes. Environmental conditions, such as nutrient availability, play a relevant role in determining the strength and directions of the feedbacks (Kardol *et al.*, 2013). For instance, mycorrhizal symbiosis can decrease plant growth when nutritional conditions increase, suggesting that positive feedback of mycorrhizal symbiosis would decrease when nutrient availability increases (Bennett *et al.*, 2017). In this line, Luo *et al.* (2017) found that fertilization decreases the negative and positive feedbacks effects of soil biota on eight woody species. Thus, the high nutritional conditions of our experiment might have suppressed the mycorrhizal positive effects on plant growth. Another potentially explanatory factor might be the initial density of organisms in the soil in the feedback phase. In our experiment, the 10% of the total volume of pots in the feedback phase belonged to the soil conditionate in the conditioning phase. Low symbionts and pathogens density could reduce the strength of feedbacks (Brinkman *et al.*, 2010). Specifically pathogens effects on plant growth are more density dependence (Laliberté *et al.*, 2015). Therefore, low density of root feeding nematodes in the soil could explain the low infection rates as well as the lack of effects on plant biomass. This study represents the first comprehensive multi-crop assessment on the consequences of crop domestication for soil microorganism recruitment and PSF.

Conclusions

Our results showed that domesticated plants were less colonized by arbuscular mycorrhizal fungi but more infected by root feeding nematodes irrespective of the soil conditioning identity. Moreover, plants grown in soils previously trained by domesticated plants showed less mycorrhizal colonization and more nematodes infection. Altogether, these results revealed an impact of crop domestication on the recruitment of rhizosphere organisms through an overall reduction of plant resistance to herbivores and mycorrhizal mutualism. However, in spite of such legacies, plants grown in soils previously trained by domesticated plants did not show a generalized growth depression. This knowledge highlights the importance to undertake plant breeding strategies to optimize the profitable functions from the plant-soil interactions towards a sustainable agriculture.

Acknowledgments

We thank José Margalet and Diana Íñigo for assistance with data gathering and Erica Seco for assistance with mycorrhizal quantification. We also thank all seed providers that provided seeds for the project (complete list in Supplementary Table S1). This work was supported by MINECO (grants CGL2014-56567-R, CGL2017-83855-R, BES-2012-054356, PCIN-2014-053), and the European Union (Eco-serve project, 2013-2014 BiodivERsA/FACCE-JPI, with the national funders ANR, NWO, FCT, MINECO, FORMAS, and SNSF).

References

Abawi GS, Widmer TL. 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* **15**: 37–47.

Baon JB, Smith SE, Alston AM. 1993. Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant and Soil* **157**: 97–105.

Bardgett RD. 2017. Plant trait-based approaches for interrogating belowground function. *Biology and Environment* **117B**: 1–13.

Bates D, Sarkar D, Bates MD, Matrix L. 2007. The lme4 package. *R package version*, *2*, 74.

Barton K. 2014. MuMIn: multi-model inference. R package ver. 1.10. 0.

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD. 2014. Are plant-soil feedback responses explained by plant traits? *Journal of Physiology* **204**: 408–423.

Bell T, Tylianakis JM. 2016. Microbes in the Anthropocene: spillover of agriculturally selected bacteria and their impact on natural ecosystems. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20160896.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181–184.

Bever JD. 2003. Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical tests. *New Phytologist* **157**: 465–473.

Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC, Schulze-Lefert P. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host and Microbe* **17**: 392–403.

Campbell CD, Chapman SJ, Cameron CM, Davidson M S, Potts JM. 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and environmental microbiology* **69:** 3593-3599.

Cardinale M, Grube M, Erlacher A, Quehenberger J, Berg G. 2015. Bacterial networks and co-occurrence relationships in the lettuce root microbiota.

Environmental Microbiology **17**: 239–252.

Chagnon PL, Bradley RL, Maherali H, Klironomos JN. **2013**. A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science* **18**: 484–491.

Chen YH, Welter SC. **2007**. Crop domestication creates a refuge from parasitism for a native moth. *Journal of Applied Ecology* **44**: 238–245.

Coley PD, Bryant JP, Chapin FS. **1985**. Resource Availability and Plant Antiherbivore Defense. *Science* **230**: 895–899.

Cortois R, Schröder-Georgi T, Weigelt A, van der Putten WH, De Deyn GB. **2016**. Plant–soil feedbacks: role of plant functional group and plant traits. *Journal of Ecology* **104**: 1608–1617.

Dávila-Flores AM, DeWitt TJ, Bernal JS. **2013**. Facilitated by nature and agriculture: Performance of a specialist herbivore improves with host-plant life history evolution, domestication, and breeding. *Oecologia* **173**: 1425–1437.

Faucon M-P, Houben D, Lambers H. **2017**. Plant Functional Traits: Soil and Ecosystem Services. *Trends in Plant Science* **22**: 385–394.

García-palacios P, Bowker MA, Maestre FT, Valladares F, Papadopoulos J, Escudero A. **2011**. Ecosystem development in roadside grasslands : biotic control , plant – soil interactions and dispersal limitations. *Ecological Applications* **21**: 2806–2821.

García-Palacios P, Milla R, Delgado-Baquerizo M, Martín-Robles N, Álvaro-Sánchez M, Wall DH. **2013**. Side-effects of plant domestication: Ecosystem impacts of changes in litter quality. *New Phytologist* **198**: 504–513.

Giovanetti, M.; Mosse B. **1980**. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New phytologist* **84**: 489–500.

Graham JH, Eissenstat DM. **1994**. Host genotype and the formation and function of VA mycorrhizae. *Plant and Soil* **159**: 179–185.

Graham JH, Eissenstat DM, Drouillard DL. **1991**. On the Relationship Between a Plant's Mycorrhizal Dependency and Rate of Vesicular-Arbuscular Mycorrhizal Colonization. *Functional Ecology* **5**: 773.

Hale IL, Broders K, Iriarte G. **2014**. A Vavilovian approach to discovering crop-

associated microbes with potential to enhance plant immunity. *Frontiers in Plant Science* **5**: 1–7.

Van der Heijden MGA, Bardgett RD, Van Straalen NM. **2008**. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Helgason T, Daniell TJ, Husband R. **1998**. Ploughing up the wood-wide web ? *Nature* **394**: 431.

Herms DA, Mattson WJ. **1992a**. The Dilemma of Plants: To Grow or Defend. *The Quarterly Review of Biology* **67**: 283–335.

Herms D a., Mattson WJ. **1992b**. The Dilemma of Plants: To Grow or Defend. *The Quarterly Review of Biology* **67**: 283.

Hetrick B a. D, Wilson WT, Cox TS. **1993**. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. : 512–518.

Hoeksema JD, Chaudhary VB, Gehring C a, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, *et al.* 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology letters* **13**: 394–407.

Hoogsteen MJJ, Lantinga EA, Bakker EJ, Groot JCJ, Tittonell PA. **2015**. Estimating soil organic carbon through loss on ignition: Effects of ignition conditions and structural water loss. *European Journal of Soil Science* **66**: 320–328.

Iannucci A, Fragasso M, Beleggia R, Nigro F, Papa R. **2017**. Evolution of the Crop Rhizosphere: Impact of Domestication on Root Exudates in Tetraploid Wheat (Triticum turgidum L.). *Frontiers in Plant Science* **8**.

Johnson NC. **1993**. Can Fertilization of Soil Select Less Mutualistic Mycorrhizae ? *Ecological Applications* **3**: 749–757.

Johnson NC. **2010**. Tansley review Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. : 631–647.

Johnson NC, Pfleger FL. **1992**. Vesicular-arbuscular mycorrhizae and cultural stresses. *Asa special publication; mycorrhizae in sustainable agricultureASA Special Publication*: 71–99.

Johnson PC. 2014. Extension of Nakagawa & Schielzeth's R^2GLMM to random

slopes models. *Methods in Ecology and Evolution* **5**: 944-946.

Kardol P. **2007**. *Plant and soil community assembly in secondary succession on ex-arable land. Fundamental and applied approaches*.

Kardol P, De Deyn GB, Laliberté E, Mariotte P, Hawkes C V. 2013. Biotic plant-soil feedbacks across temporal scales. *Journal of Ecology* **101**: 309–315.

Kardol P, Veen GF (Ciska), Teste FP, Perring MP. **2015**. Peeking into the black box: a trait- based approach to predicting plant – soil feedback. *New Phytologist* **206**: 1–4.

Kempel A, Schadler M, Chrobock T, Fischer M, van Kleunen M. **2011**. Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proceedings of the National Academy of Sciences* **108**: 5685–5689.

Kiers ET, Denison RF. **2014**. Inclusive fitness in agriculture. *Phil. Trans. R. Soc. B* **369**.

Kiers ET, Heijden MGA van der. **2006**. Mutualistic Stability in the Arbuscular Mycorrhizal Symbiosis: Exploring Hypotheses of Evolutionary Cooperation. *Ecology* **87**: 1627–1636.

Kiers ET, West S a., Denison RF. **2002**. Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. *Journal of Applied Ecology* **39**: 745–754.

Klironomos JN. **2002**. Feedback with soil biota contributes to plants rarity and. *Nature* **417**: 67–69.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. **2008**. Plant-soil feedbacks: A meta-analytical review. *Ecology Letters* **11**: 980–992.

Laliberté E. **2017**. Below-ground frontiers in trait-based plant ecology. *New Phytologist* **213**: 1597–1603.

Laliberté E, Lambers H, Burgess TI, Wright SJ. **2015**. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* **206**: 507–521.

Leff JW, Lynch RC, Kane NC, Fierer N. **2016**. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. *New Phytologist*.

Lehmann A, Barto EK, Powell JR, Rillig MC. **2012**. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. *Plant and Soil* **355**: 231–250.

Lemmermeyer S, Lörcher L, van Kleunen M, Dawson W. **2015**. Testing the Plant Growth-Defense Hypothesis Belowground: Do Faster-Growing Herbaceous Plant Species Suffer More Negative Effects from Soil Biota than Slower-Growing Ones? *The American Naturalist* **186**: 264–271.

Lenth RV. 2016. Least-squares means: the R package lsmeans. *J Stat Softw* **69**: 1-33.

Luo S, De Deyn GB, Jiang B, Yu S. **2017**. Soil biota suppress positive plant diversity effects on productivity at high but not low soil fertility. *Journal of Ecology* **105**: 1766–1774.

Macfadyen S, Bohan DA. **2010**. Crop domestication and the disruption of species interactions. *Basic and Applied Ecology* **11**: 116–125.

Mäder P, Edenhofer S, Boller T, Wiemken A, Niggli U. **2000**. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic , biological) and high-input (conventional) farming systems in a crop rotation. : 150–156.

Mao L, Liu Y, Shi G, Jiang S, Cheng G, Li X, An L, Feng H. **2013**. Wheat cultivars form distinctive communities of root-associated arbuscular mycorrhiza in a conventional agroecosystem. *Plant and Soil* **374**: 949–961.

Mariotte P, Mehrabi Z, Bezemer TM, De Deyn GB, Kulmatiski A, Drigo B, Veen GF, van der Heijden MGA, Kardol P. **2017**. Plant-Soil Feedback: Bridging Natural and Agricultural Sciences. *Trends in Ecology and Evolution* **xx**: 1–14.

Maron JL, Marler M, Klironomos JN, Cleveland CC. **2011a**. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* **14**: 36–41.

Maron JL, Marler M, Klironomos JN, Cleveland CC. **2011b**. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* **14**: 36–41.

Martín-Robles N, Lehmann A, Seco E, Aroca R, Rillig MC, Milla R. **2018**. Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop

species. *New Phytologist* **218**: 322–334.

Massei G, Hartley SE. **2000**. Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. *Oecologia* **122**: 225–231.

McGonigle T, Miller. **1990**. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi.

Milla R, Osborne CP, Turcotte MM, Violle C. **2015**. Plant domestication through an ecological lens. *Trends in Ecology and Evolution* **30**: 463–469.

Miller ZJ, Menalled FD. **2015**. Impact of species identity and phylogenetic relatedness on biologically-mediated plant-soil feedbacks in a low and a high intensity agroecosystem. *Plant and Soil* **389**: 171–183.

Moora M, Davison J, Öpik M, Metsis M, Saks Ü, Jairus T, Vasar M, Zobel M. **2014**. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. *FEMS Microbiology Ecology* **90**: 609–621.

Nijjer S, Rogers WE, Siemann E. **2010**. The Impacts of Fertilization on Mycorrhizal Production and Investment in Western Gulf Coast Grasslands. *Am. Midl. Nat* **163**: 124–133.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A, Ma P. **2003**. Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. *Applied and Environmental Microbiology* **69**: 2816–2824.

Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LFV, De Hollander M, Garcia AAF, Ramírez CA, Mendes R, Raaijmakers JM. **2017**. Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. *ISME Journal* **11**: 2244–2257.

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. **2016**. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology* **90**: 635–644.

Pernilla Brinkman E, Van der Putten WH, Bakker E-J, Verhoeven KJF. **2010**. Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* **98**: 1063–1073.

Pieterse CMJ, de Jonge R, Berendsen RL. 2016. The Soil-Borne Supremacy. *Trends in Plant Science* **21**: 171–173.

Pinheiro J, Bates D, DebRoy S, Sarkar D. 2015. nlme: Linear and Nonlinear Mixed Effects Models R package version 3.1–117.

Postma-Blaauw MB, De Goede RGM, Bloem J, Faber JH, Brussaard L. 2010. Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology* **91**: 460–473.

Singmann H, Bolker B, Westfall J. (2015). Afex: analysis of factorial experiments. R package version 0.13–145.

Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, *et al.* 2013. Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* **101**: 265–276.

Van der Putten WH, Cook R, Costa S, Davies KG, Fargette M, Freitas H, Hol WHG, Kerry BR, Maher N, Mateille T, *et al.* 2006. Nematode Interactions in Nature: Models for Sustainable Control of Nematode Pests of Crop Plants? *Advances in Agronomy* **89**: 227–260.

van der Putten WH, Kowalchuk GA, Brinkman EP, Doodeman GTA, van der Kaaij RM, Kamp AFD, Menting FBJ, Veenendaal EM. 2007. Soil Feedback of Exotic Savanna Grass Relates to Pathogen Absence and Mycorrhizal Selectivity. *Ecology* **88**: 978–988.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Remigi P, Zhu J, Young JPW, Masson-Boivin C. 2016. Symbiosis within Symbiosis: Evolving Nitrogen-Fixing Legume Symbionts. *Trends in Microbiology* **24**: 63–75.

Rivera MJ, Rodriguez-Saona C, Egizi A, Fonseca DM, Jennings DE, Koppenhöfer AM. 2016. Cultivation and domestication of highbush blueberry (Vaccinium corymbosum) alters abundance, diversity and virulence of entomopathogenic nematodes. *Agriculture, Ecosystems and Environment* **222**: 148–

155.

Roberts PA, Waines JG, Van Gundy SD. **1981**. Reaction of Wild and Domesticated Triticum and Aegilops Species To Root-Knot Nematodes (Meloidogyne). *Nematologica* **28**: 182–191.

Rodriguez-Saona C, Vorsa N, Singh AP, Johnson-Cicalese J, Szendrei Z, Mescher MC, Frost CJ. **2011**. Tracing the history of plant traits under domestication in cranberries: Potential consequences on anti-herbivore defences. *Journal of Experimental Botany* **62**: 2633–2644.

Rosenthal JP, Dirzo R. **1997**. Effects of life history, domestication and agronomic selection on plant defence against insects: Evidence from maizes and wild relatives. *Evolutionary Ecology* **11**: 337–355.

Roucou A, Violle C, Fort F, Roumet P, Ecarnot M, Vile D. **2018**. Shifts in plant functional strategies over the course of wheat domestication. *Journal of Applied Ecology* **55**: 25–37.

Schmidt JE, Bowles TM, Gaudin ACM. **2016**. Using Ancient Traits to Convert Soil Health into Crop Yield: Impact of Selection on Maize Root and Rhizosphere Function. *Frontiers in plant science* **7**: 373.

Sheedy JG, Thompson JP. **2009**. Resistance to the root-lesion nematode Pratylenchus thornei of Iranian landrace wheat. *Australasian Plant Pathology* **38**: 478–489.

Simpson KJ, Wade RN, Rees M, Osborne CP, Hartley SE. **2017**. Still armed after domestication? Impacts of domestication and agronomic selection on silicon defences in cereals. *Functional Ecology* **31**: 2108–2117.

Stevens CJ, Dise NB, Mountford O, Gowing DJ. **2004**. Impact of Nitrogen Deposition on the Richness of Grasslands. **1876**.

Szoboszlay M, Lambers J, Chappell J, Kupper J V., Moe LA, McNear DH. **2015**. Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. *Soil Biology and Biochemistry* **80**: 34–44.

Tawaraya K. **2003**. Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition* **49**: 655–668.

Thiele-Bruhn S, Bloem J, Vries FT de, Kalbitz K, Wagg C. **2012**. Author â€™s personal copy Linking soil biodiversity and agricultural soil management § So. *Environmental Sustainability* **4**: 523–528.

Treseder KK. **2004**. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO 2 in field studies. *New Phytologist*: 1–9.

Treseder KK. **2008**. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters* **11**: 1111–1120.

Trudgill DL, Blok VC. **2001**. Apomitic, Poliphagous, Root knot Nematodes: Exceptionally Successful and Damaging Biotrophic Root Pathogens. *Annual Review of Phytopathology* **39**: 53–77.

Turcotte MM, Lochab AK, Turley NE, Johnson MTJ. **2015**. Plant domestication slows pest evolution. *Ecology Letters* **18**: 907–915.

Turcotte MM, Turley NE, Johnson MTJ. **2014**. The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytologist* **204**: 671–681.

Turrini A, Giordani T, Avio L, Natali L, Giovannetti M, Cavallini A. **2016**. Large variation in mycorrhizal colonization among wild accessions, cultivars, and inbreds of sunflower (Helianthus annuus L.). *Euphytica* **207**: 331–342.

Vargas MAT, Mendes IC, Hungria M. **2000**. Response of field-grown bean (phaseolus vulgaris l.) to Rhizobium inoculation and nitrogen fertilization in two cerrados soils. *Biology and Fertility of Soils* **32**: 228–233.

Verbruggen E, Toby Kiers E. **2010**. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evolutionary Applications* **3**: 547–560.

Verbruggen E, Xiang D, Chen B, Xu T, Rillig MC. **2015**. Mycorrhizal fungi associated with high soil N:P ratios are more likely to be lost upon conversion from grasslands to arable agriculture. *Soil Biology and Biochemistry* **86**: 1–4.

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. **2004**. Ecological linkages between aboveground and belowground biota. *Science (New York, N.Y.)* **304**: 1629–33.

Weese DJ, Heath KD, Dentinger BTM, Lau JA. **2015**. Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution* **69**: 631–642.

Whitehead SR, Turcotte M, Poveda K. **2016**. Domestication impacts on plant-herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B*.

Wissuwa M, Mazzola M, Picard C. **2009**. Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil* **321**: 409–430.

Xing X, Koch AM, Jones AMP, Ragone D, Murch S, Miranda M, Maxwell AP, Hart MM. **2011**. Mutualism breakdown in breadfruit domestication Subject collections Mutualism breakdown in breadfruit domestication.

Yan J, Han XZ, Ji ZJ, Li Y, Wang ET, Xie ZH, Chen WF. **2014**. Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. *Applied and Environmental Microbiology* **80**: 5394–5402.

Zachow C, Müller H, Tilcher R, Berg G. **2014**. Differences between the rhizosphere microbiome of Beta vulgaris ssp. maritima-ancestor of all beet crops-and modern sugar beets. *Frontiers in Microbiology* **5**: 1–13.

Zhu YG, Smith SE, Barritt AR, Smith FA. **2001**. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil* **237**: 249–255.

Zuur, A. F. EN leno, NJ Walker, AA Saveliev, and GM Smith. **2009**. *Mixed effects models and extensions in ecology with R*. New York, USA: Springer.

Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DFB. **2014**. Selection for niche differentiation in plant communities increases biodiversity effects. *Nature* **515**: 108–111**.**

Supporting Information

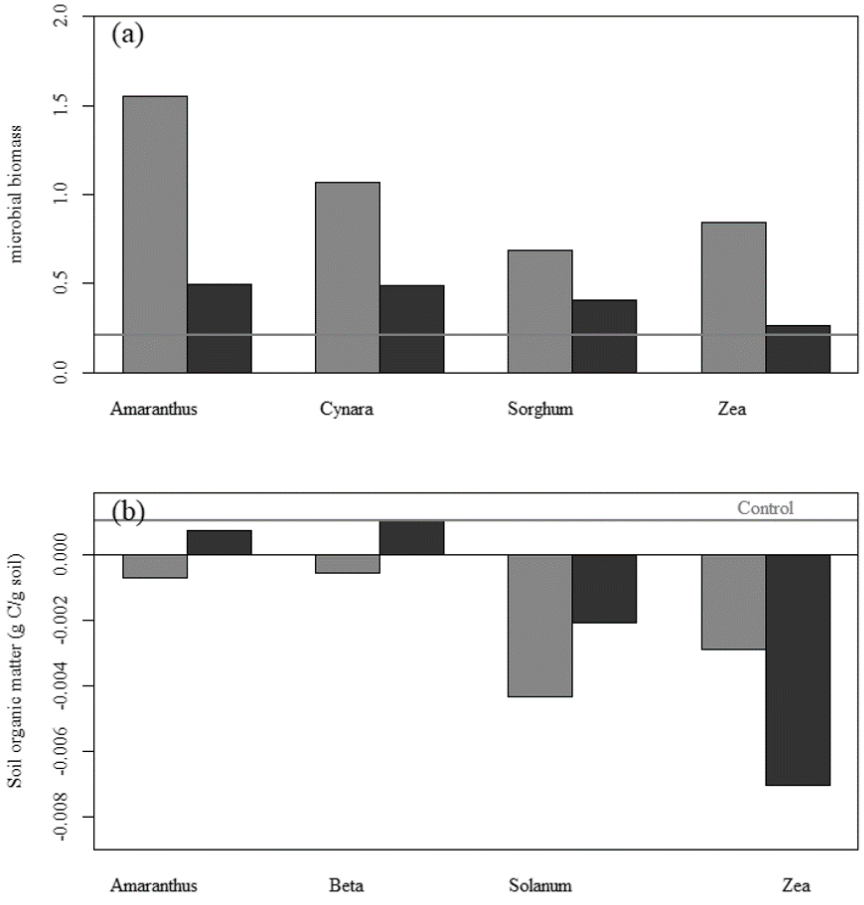


Figure S1. Effect of soil conditioning on microbial biomass (a) and soil organic matter enrichment (b). The bars showed the mean value of the soil variables belonging to wild progenitors (light grey) and domesticated plants (dark grey) of the four crops in comparison with the control (red line).

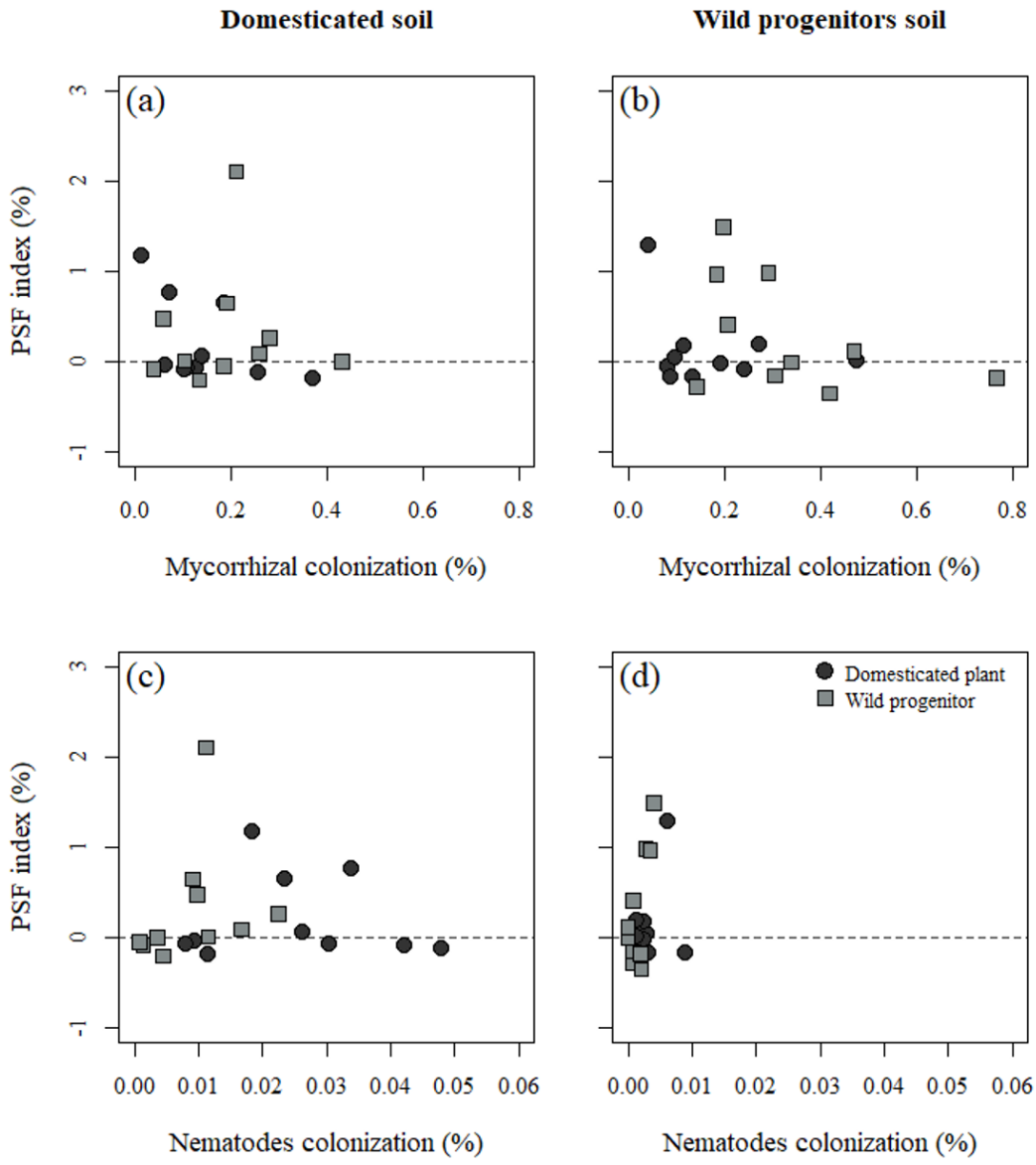


Figure S2. Correlation plot of mycorrhizal (a,b) and nematodes (c, d) colonization with plant soil feedback index (PSF index). Points represent the mean values of each accession of domesticated (dark circles) or wild progenitor (light circles).

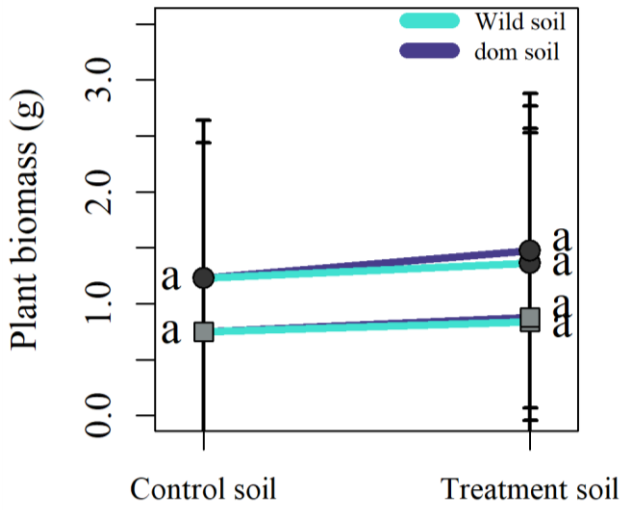


Figure S3. Plant biomass reaction of wild progenitors (squares) and domesticated plants (circles) to soil conditioned by wild progenitors (light blue) and domesticated plants (dark blue). The symbols, error bars (standard error) and letters show the least squares means and 95% confidence interval of domesticated plants (circles) and wild progenitors (squares), obtained by mixed models.

	Microbial biomass		Organic matter enrichment (gC/gsoil)	
	F	*P*	*F*	*P*
Domestication status	3.56	0.08	0.07	0.792
Crop identity	0.51	0.7	5.61	**0.003**
Dom x crop identity	0.21	0.88	1.91	0.144
R^2	0.01		0.25	

Domestication and crop identity were factors. Interactions are indicated by x.

Table S3. Results of linear model testing the effect wild progenitors and domesticated plants on soil parameters during the conditioning phase. The models tested whether soil microbial biomass and organic matter enrichment were affected by domestication status, crop identity and the interaction. The table shows the *F* and *P* scores of each variable, and the R^2 of the models.

Botanic family	Common name	Botanical name	Dom status	Accession identifier	Seed donor	collected in	Time under dom	Ref. dom
Alliaceae	leek	*Allium porrum* L.	D	N.A.	Clause	commercial	4500	1
		Allium ampeloprasum L.	W	CGN20776	CGN	Turkey	4500	1
Amaranthaceae	amaranth	*Amaranthus cruentus* L.	D	AMA 169	IPK	Nepal	4000	2
		Amaranthus hybridus L.	W	PI652417	grin	Brazil	4000	2
	chard	*Beta vulgaris L.*	D	N.A.	Clause	commercial	2360	3
		Beta vulgaris L.	W	1582	IPK	Italy	2360	3
Asteraceae	cardoon	*Cynara cardunculus* L.	D	N.A.	Rocalba	Spain	750	4
		Cynara cardunculus L.	W	ES-01-14-0256	S. Silvestres	Spain	750	4
Fabaceae	white clover	*Trifolium repens* L.	D	N.A.	Intersemillas	commercial	1650	5
		Trifolium repens L.	W	CGN22513	CGN	Kyrgystan	1650	5
Poaceae	barley	*Hordeum vulgare* L.	D	BGE000214	CRF	commercial	10000	2
		Hordeum spontaneum K.Koch	W	BGE025385	CRF	Morocco	10000	2
	maize	*Zea mays* L.	D	Ames26252	grin	Brazil	8000	6
		Zea mexicana (Schrad.) Kuntze	W	PI566674	grin	Mexico	8000	6
	sorghum	*Sorghum drummondii* (Nees ex Steud.) Millsp. & Chase	D	N.A.	Rocalba	commercial	4000	2
		Sorghum bicolor (L.) Moench	W	PI524718	grin	Sudan	4000	2

Botanic family	Common name	Botanical name	Dom status	Accession identifier	Seed donor	collected in	Time under dom	Ref. dom
Solanaceae	pepper	*Capsicum annuum L.*	D	N.A.	Mascarell	Spain	6000	2
		Capsicum annuum var. *glabriusculum* (Dunal) Heiser & Pickersgill	W	PI631137	grin	Guatemala	6000	2
	tomato	*Solanum esculentum* Dunal	D	N.A.	Clause	commercial	600	2
		Solanum pimpinellifolium L.	W	LA1383	grin	Peru	600	2

Table S1. Detailed information of each of the 10 domesticated-wild progenitor used in this experiment, and reference sources for wild progenitor assignment. Common and botanical names of each crop, the domesticated and progenitor identities, and botanical family. Domestication status (dom: domesticated; wild: wild ancestor). Seed donor (CGN: Center for Genetic Resources, The Netherlands; IPK: Germplasm bank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; CRF: Centro Nacional de Recursos Fitogenéticos-INIA, Spain; NPGS: National Plant Germplasm System-USDA, U.S.A.). Accession identifier refers to the code assigned by each seed donor excepting the commercial companies. Accession country refers to the country where the seeds were collected. Time under domestication refers to the years since domestication started. Ref. dom: reference source for wild ancestor assignment. N.A.: data not available.

References Table S1

1. De Clercq H, Van Bockstaele E. 2002. Leek: Advances in Agronomy and Breeding. In: Rabinowitch HD, Currah L, eds. Allium crop science: recent advances. Wallingford, UK: CABI, 431.
2. Sauer JD. 1993. Historical geography of crop plants. A select roster. Boca Raton, USA: CRC Press.
3. Hancock, JF. 2004. Plant Evolution and the origin of crop species. CABI Publishing, NY, USA.
4. Sonnante G, Pignone D, Hammer K. 2007. The domestication of artichoke and cardoon: from Roman times to the genomic age. Annals of Botany 100(5): 1095-1100.
5. De Vries IM 1997. Origin and domestication of Lactuca sativa L. Genetic Resources and Crop Evolution 44(2): 165-174.
6. Fuller DQ. 2003. Further evidence on the prehistory of sesame. Asian Agri-History 7(2): 127-137.

	Conditioning phase soil
Coordinates (U.T.M.)	30T 0424133 / 4469923 N
Successional stage	Late (> 20 yrs.)
pH	7.15 ± 0.17
Organic C (mg C g soil^{-1})	23.02 ± 1.28
NO_3^- - N (mg N Kg soil^{-1})	13.80 ± 0.09
NH_4^+ - N (mg N Kg soil^{-1})	5.34 ± 0.10
C-Hex:N	0.45 ± 0.06
C-Phe:N	0.20 ± 0.01
Microbial functional diversity[1]	2.627 ± 0.84
Bacteria (DNA copies g^{-1} soil)[2]	3.56 109 ± 1.31 109
Fungi (DNA copies g^{-1} soil)[2]	9.27 108 ± 4.40 108
Relative fungal:bacterial ratio	0.26 ± 0.02

1 The functional diversity of the soil microbial communities was quantified using a carbon substrate diversity index or modified Shannon index from the data gathered in García-Palacios et al. (2011) with the MicroResp system: H' = - Σ [pi ln (pi)], where: pi is the ratio of the CO2 rate for a carbon source to the sum of CO2 rates for all substrates.

2 The relative abundance of bacterial 16S and fungal 18s rRNA genes were measured using quantitative PCR (García-Palacios, unpublished data). The bacterial and fungi genes were amplified with the Eub 338-Eub 518 and ITS 1-5.8S primer sets, respectively following Fierer et al. (2005).

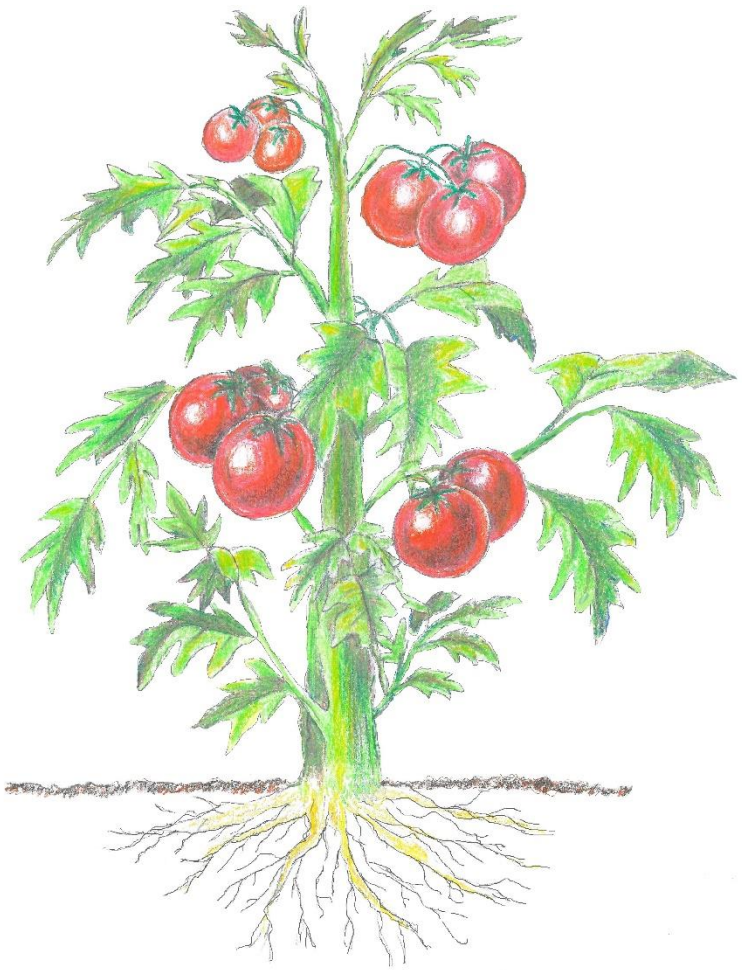
Table S2. Field location and characteristics of the soil employed in the conditioning phase. Data are means ± 1 SE (n = 5).

References Table S2

Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiol*ogy 71: 4117–4120.

García-Palacios P, Bowker MA, Maestre FT, Soliveres S, Valladares F, Papadopoulos J, Escudero A. 2011. Ecosystem development in roadside grasslands: biotic control, plant–soil interactions and dispersal limitations. *Ecological Applications* 21: 2806–2821.

Discusión general



Discusión general

El estudio comparado de los rasgos de raíces y sus interacciones con microorganismos del suelo en un amplio grupo de cultivos, nos ha permitido extraer algunos mensajes claves. En primer lugar, la adaptación del fenotipo de las raíces de plantas domesticadas a las condiciones fértiles típicas en agricultura podría estar determinada en gran parte por la elección temprana de los ancestros silvestres, en lugar de por la evolución bajo la domesticación (capítulo 1). En segundo lugar, la domesticación afectó negativamente a la eficiencia de la simbiosis con micorrizas arbusculares, bajo las condiciones de alta disponibilidad de nutrientes típicas de los sistemas agrícolas (capítulo 2) así como a la colonización dentro de la raíz (capítulo 3). Además, la domesticación debilitó la resistencia frente a la infección de nemátodos que penetran y se alimentan de la raíz (capítulo 3). La reducción de la simbiosis con la micorriza y menor inversión en defensa desarrolladas bajo la domesticación, están en consonancia con una evolución hacia estrategias mas adquisitivas de los recursos. En tercer lugar, a través de un efecto global negativo sobre el mutualismo con la micorriza y la resistencia frente a herbívoros, la domesticación habría alterado la capacidad de reclutamiento de organismos de la rizosfera. Como consecuencia del cambio en el reclutamiento de microorganismos, el legado que dejan las plantas domesticadas en el suelo diferiría del legado de sus ancestros (capítulo 3). El legado en el suelo de las plantas domesticadas promovería la aparición de efectos negativos en el crecimiento y desarrollo de las plantas que ocupan esos suelos. Estos tres mensajes tienen implicaciones teóricas y prácticas importantes para elaborar estrategias de mejora de plantas dirigidas a optimizar las funciones de las plantas con los microorganismos del suelo, necesarias para una agricultura más sostenible.

Basándonos en las teorías ecológicas, predecimos que la morfología y el patrón de asignación de biomasa a la raíz evolucionarían hacia estrategias adquisitivas de recursos durante la domesticación. No obstante, encontramos una gran variedad de respuestas de los rasgos radiculares a la domesticación entre los cultivos muestreados. Esta variabilidad en la respuesta a la domesticación según cultivos se ve reflejada también en la literatura existente sobre el tema (Gaudin *et al.*, 2011; Burton *et al.*, 2013; Nakhforoosh *et al.*, 2014; Szoboszlay *et al.*, 2015; Pérez-Jaramillo *et al.*, 2017). Aunque la evolución de los cultivos bajo domesticación ejerció un impacto sobre los rasgos de la raíz modesto y diverso según cultivos, los primeros agricultores ya mostraron un sesgo en los fenotipos de las raíces de las plantas agrícolas. Las raíces de los ancestros silvestres poseen raíces menos densas y más gruesas, rasgos típicos de especies con estrategias adquisitivas de recursos (Ryser, 1996; Reich, 2014; Kramer-Walter *et al.*, 2016). El hecho de que los progenitores silvestres exhiban un fenotipo de raíz adaptado a hábitats agrícolas está en línea con la hipótesis de Dump Heap, que sugiere que la domesticación comenzó con entre las especies próximas a los asentamientos humanos, ambientes que se caracterizan por una disponibilidad de nutrientes relativamente alta y frecuencias de perturbación (Sauer, 1952; Zeven, 1973; Hawkes, 1983). Estudios previos indican que los ancestros silvestres muestran valores de área foliar y contenido en nitrógeno típicos de estrategias adquisitivas (Cunniff *et al.*, 2014; Milla *et al.*, 2015; Roucou *et al.*, 2018). Por tanto, las plantas silvestres con estrategias de adquisición de nutrientes fueron candidatas más exitosas para la domesticación al adaptarse previamente a las condiciones de cultivo.

Los beneficios que la planta obtiene de la micorriza disminuyeron en las plantas domesticadas cuando las condiciones nutricionales aumentaron. Sin embargo, los beneficios que recibió el ancestro de la micorriza no variaron.

La simbiosis con los hongos micorrícicos arbusculares varía de mutualismo a parasitismo según factores como las especies de planta y hongo que entran en juego y las condiciones ambientales, principalmente la disponibilidad de fósforo (Johnson, 2010). La fertilización reduce los beneficios trasferidos por la micorriza, incluso a tasas negativas (Johnson *et al.*, 2015) y disminuye la colonización de la micorriza (Kaeppler *et al.*, 2000; Treseder, 2008; Nijjer *et al.*, 2010). Estudios previos en maíz y trigo mostraron un efecto negativo de la fertilización en el beneficio de la micorriza obtenido por los cultivos, pero no en sus variedades nativas (Manske, 1989; Wright *et al.*, 2005), sugiriendo que la respuesta de la simbiosis a la fertilización podría haberse visto alterada por la domesticación. Los mecanismos que regulan la transferencia de carbono entre planta y hongo podrían explicar por qué la fertilización redujo el beneficio de la micorriza en las accesiones domesticadas. Los hongos AM y las plantas hospedadoras pueden regular la cantidad de recursos que se ceden mutuamente (Kiers *et al.*, 2011). No todas las plantas tienen la habilidad de regular los recursos cedidos a la micorriza (Grman & Robinson, 2013). Especulamos que la regulación de la asignación de recursos entre planta y hongo podría verse afectada por la domesticación. La selección para obtener un mayor rendimiento podría haber cambiado el patrón de asignación de la biomasa en los cultivos, dando como resultado una menor translocación de carbono hacia las raíces y, por lo tanto, a sus hongos asociados. La disponibilidad reducida de carbohidratos podría conducir a la disminución de la colonización de la raíz fúngica AM, desestabilizando la capacidad de recompensa mutua y finalmente desestabilizando la cooperación de la simbiosis. Sin embargo, se necesitan evidencias empíricas para probar esta hipótesis.

La resistencia a la herbivoría por nemátodos disminuyó con la domesticación. Aunque evidencias de las consecuencias de la domesticación en la herbivoría subterránea están menos estudiadas, algunas evidencias también sugieren que los cultivares modernos están más infectados por los nemátodos de la raíz que los ancestros silvestres (Rivera *et al.*, 2016) o variedades locales (Sheedy & Thompson, 2009). Mas estudiadas son las consecuencias de la domesticación en la defensa de la parte aérea de la planta, que afirman igualmente, que la domesticación ha reducido la capacidad de defensa de la planta (Macfadyen & Bohan, 2010; Whitehead *et al.*, 2016). Esta reducción de la resistencia a la herbivoría es explicada por la teoría ecológica, que predice que las especies en entornos ricos en recursos invierten recursos en crecimiento en lugar de defensa (Coley *et al.*, 1985; Lemmermeyer *et al.*, 2015). Específicamente, la domesticación puede haber comprometido la capacidad de defensa de los cultivos modernos para aumentar el crecimiento y el rendimiento de las plantas (Rosenthal & Dirzo, 1997). La cría de alto rendimiento y palatabilidad puede tener consecuencias no deseadas que reducen la capacidad de resistencia contra los nematodos en los cultivos, lo que lleva a la proliferación de nematodos en la rizosfera.

Referencias de la discusión

Burton AL, Brown KM, Lynch JP. 2013. Phenotypic diversity of root anatomical and architectural traits in Zea species. *Crop Science* **53**: 1042–1055.

Coley PD, Bryant JP, Chapin FS. 1985. Resource Availability and Plant Antiherbivore Defense. *Science* **230**: 895–899.

Cunniff J, Wilkinson S, Charles M, Jones G, Rees M, Osborne CP. 2014. Functional traits differ between cereal crop progenitors and other wild grasses gathered in the neolithic fertile crescent. *PLoS ONE* **9**.

Gaudin ACM, McClymont SA, Raizada MN. 2011. The nitrogen adaptation strategy of the wild teosinte ancestor of modern maize, Zea mays subsp. parviglumis. *Crop Science* **51**: 2780–2795.

Grman E, Robinson TMP. 2013. Resource availability and imbalance affect plant – mycorrhizal interactions: a field test of three hypotheses. *Ecology* **94**: 62–71.

Johnson NC. 2010. Tansley review Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. : 631–647.

Johnson NC, Wilson GWT, Wilson JA, Miller RM, Bowker MA. 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist* **205**: 1473–1484.

Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF. 2000. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Science* **40**: 358–364.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *science* **333**: 880–882.

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC, Mommer L. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *Journal of Ecology* **104**: 1299–1310.

Lemmermeyer S, Lörcher L, van Kleunen M, Dawson W. 2015. Testing the Plant Growth-Defense Hypothesis Belowground: Do Faster-Growing Herbaceous Plant Species Suffer More Negative Effects from Soil Biota than Slower-Growing Ones? *The American Naturalist* **186**: 264–271.

Macfadyen S, Bohan DA. 2010. Crop domestication and the disruption of species interactions. *Basic and Applied Ecology* **11**: 116–125.

Manske GGB. 1989. Genetical Analysis of the Efficiency of VA Mycorrhiza with Spring Wheat. *Agriculture, Ecosystems and environment* **29**: 273–280.

Milla R, Osborne CP, Turcotte MM, Violle C. 2015. Plant domestication through an ecological lens. *Trends in Ecology and Evolution* **30**: 463–469.

Nakhforoosh A, Grausgruber H, Kaul HP, Bodner G. 2014. Wheat root diversity and root functional characterization. *Plant and Soil* **380**: 211–229.

Nijjer S, Rogers WE, Siemann E. 2010. The Impacts of Fertilization on Mycorrhizal Production and Investment in Western Gulf Coast Grasslands. *Am. Midl. Nat* **163**: 124–133.

Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LFV, De Hollander M, Garcia AAF, Ramírez CA, Mendes R, Raaijmakers JM. 2017. Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. *ISME Journal* **11**: 2244–2257.

Reich PB. 2014. The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology* **102**: 275–301.

Rivera MJ, Rodriguez-Saona C, Egizi A, Fonseca DM, Jennings DE, Koppenhöfer AM. 2016. Cultivation and domestication of highbush blueberry (Vaccinium corymbosum) alters abundance, diversity and virulence of entomopathogenic nematodes. *Agriculture, Ecosystems and Environment* **222**: 148–155.

Rosenthal JP, Dirzo R. 1997. Effects of life history, domestication and agronomic selection on plant defence against insects: Evidence from maizes and wild relatives. *Evolutionary Ecology* **11**: 337–355.

Roucou A, Violle C, Fort F, Roumet P, Ecarnot M, Vile D. 2018. Shifts in plant functional strategies over the course of wheat domestication. *Journal of Applied Ecology* **55**: 25–37.

Ryser P. 1996. The Importance of Tissue Density for Growth and Life Span of Leaves and Roots: A Comparison of Five Ecologically Contrasting Grasses. *Source: Functional Ecology British Ecological Society Functional Ecology* **10**: 717–723.

Sheedy JG, Thompson JP. 2009. Resistance to the root-lesion nematode Pratylenchus thornei of Iranian landrace wheat. *Australasian Plant Pathology* **38**: 478–489.

Szoboszlay M, Lambers J, Chappell J, Kupper J V., Moe LA, McNear DH. **2015**. Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. *Soil Biology and Biochemistry* **80**: 34–44.

Treseder KK. **2008**. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters* **11**: 1111–1120.

Whitehead SR, Turcotte M, Poveda K. **2016**. Domestication impacts on plant-herbivore interactions: a meta-analysis. *Philosophical Transactions of the Royal Society B*.

Wright DP, Scholes JD, Read DJ, Rolfe S a. **2005**. European and African maize cultivars differ in their physiological and molecular responses to mycorrhizal infection. *The New phytologist* **167**: 881–96.

General conclusions

1. None of the root traits reacted to domestication in accordance with evolution towards fast-growth strategies. Root traits changed during most of the domestication processes surveyed, but this occurred in diverse directions, depending on the crop species.

2. Root traits of domesticated plants and of their wild progenitors are biased towards trait scores indicative of acquisitive strategies, in the context of botanical trait variation. Thus, the good adaptation of crop root phenotypes to the fertile conditions of agricultural fields might be largely determined by early choices of wild species, rather than by further evolution under domestication.

3. The strength and direction of the response of arbuscular mycorrhizal symbiosis to domestication varied with soil P availability. Arbuscular mycorrhizal symbiosis provided growth benefits to wild progenitors irrespective of P availability, but the benefits turned negligible or costly to domesticated plants when P availability increased.

4. Domesticated plants were more infected by root feeding nematodes than wild progenitors, indicating a reduction in the resistance to belowground herbivory along crop domestication.

5. Thus, domestication would have impacted the recruitment of rhizosphere organisms through an overall negative effect on plant resistance to herbivores and mycorrhizal mutualism.

6. The strength and direction of some traits response to domestication were diverse among the several crop species. These variations were however unrelated to phylogeny or variability of each domestication event.

7. This thesis highlights the importance to optimize the profitable interactions with soil organisms, to undertake plant breeding strategies towards a sustainable agriculture.

8. Multi-species experiments are a powerful approach to address questions regarding the generality of patterns.