



Linking root traits to plant physiology and growth in *Fraxinus angustifolia* Vahl seedlings under soil compaction conditions

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ABSTRACT

Roots are immersed in a soil matrix with different and contrasting conditions that may affect their anatomy, structure and function. Soil chemical factors, texture and compaction are some of the main factors experienced by roots. This study investigates how soil compaction and soil type can modify root anatomy and morphology, and, thus, plant physiology and growth. Seedlings of a woody species (*Fraxinus angustifolia* Vahl.) were grown in a greenhouse with two types of soil (loam and sandy-loam) under a wide range of soil compaction. Despite intrinsic differences in the chemical properties of the two soils, soil compaction explained much of the variation in root morphology and anatomy. We found, in a single species, a high root plasticity in different key root traits as a response to soil compaction. Soil compaction affected negatively the proportion of fine roots, specific root length and proportion of the root xylem vessels. These effects on root traits had an influence on plant physiology and growth. A causal model (by d-sep method) found that soil compaction affected specific root length, and this affects transpiration and photosynthesis rates. Also, the effect of soil compaction on xylem vessel proportion and xylem vessel diameter affected plant area and plant biomass. In conclusion, soil compaction shows an important effect on root anatomy and morphology during the seedling stage, with consequences on plant physiology and growth.

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1. Introduction

Roots explore the soils to acquire nutrients and water. Roots under different soil conditions or belonging to different species show contrasting root traits in anatomy and morphology. Anatomical root traits have been related to root functioning; for example, cortex thickness and the presence of a suberised exodermis are the best characteristics to explain hydraulic conductivity properties (Rieger and Litvin, 1999). Some recent studies (Hummel et al., 2007) have found that root anatomy and structure of grass species are related to plant physiology, whole plant traits and ecological aspects. Also, Wahl and Ryser (2000) found that the presence of small xylem vessels and in a low number was associated with slow growth and strategies of embolism avoidance or resistance against cavitation. Therefore, it seems that there is a strong causal relationship between root anatomy, morphology and physiology, which may explain traits at whole-plant level such as plant physiology, plant height and growth, and ecological aspects (Eissenstat, 2000; Wahl and Ryser, 2000; Tubeileh et al., 2003). However, although there are many studies about root structure and functioning, there

are still many gaps in some aspects (for example, variations in specific root length with soil conditions, the different response with different abiotic factors, the relationships of root anatomy to root and plant physiology or the ecological significance of variation in root morphology) (Ryser, 2006; Hummel et al., 2007; Alameda et al., 2011).

Soils show very different chemical and physical conditions that may affect root anatomy, structure and function (Ryser, 2006; Bengough et al., 2006; Bejarano et al., 2010). Soil physical properties such as bulk density, penetration resistance or porosity are descriptors of a complex variable called soil compaction. Soil compaction is defined as the increase in the amount of soil particles per volume unit. Soil compaction depends on the magnitude of disturbance and the size and proportion of soil particles, and, therefore, it depends on soil texture. Soil characteristics of a great biological importance like porosity, water capacity or penetration resistance are affected by compaction and they are also dependent on soil types (Whalley et al., 1995; Gómez et al., 2002a). Soil compaction effects are mediated by mechanical impedance and/or water and air availability (Bengough and Mullins, 1990; Atwell, 1993; Gómez et al., 2002a). In fact, under natural conditions, it is difficult to separate the effects of physical factors affecting root growth from the effects of water limitation (Gómez et al., 2002a; Cortina et al., 2008; Pérez-Ramos et al., 2010).

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In general, it is assumed that soil compaction affects negatively seedling establishment and development and processes mediated by root such as anchorage, water and nutrient uptake, or symbiotic relations (Kozłowski, 1999). Thus, soil compaction is one of the principal causes of yield reduction for a wide species spectrum (Wolkowski, 1990; Unger and Kaspar, 1994; Whalley et al., 2008). In woody species, it has been shown how soil compaction has many different effects on different plant traits, namely: a decrease in the length of primary and lateral roots, an increase in root diameter, a decrease in water absorption, which leads to increases in leaf water deficits, a decrease in absorption of nutrients and photosynthesis rate, and in general a reduced growth (Misra and Gibbons, 1996; Kozłowski, 1999; Tubeileh et al., 2003; Pérez-Ramos et al., 2010). However, these effects vary as a function of the species and the compaction range studied. Thus, Alameda and Villar (2009) found that increasing soil compaction from low to moderate values are in general, positively associated with growth and negatively with root biomass allocation. These positive responses on growth may be explained by an increase in nutrient transport, determining in both cases a higher mineral nutrition (Arvidsson, 1999) and water absorption (Kooistra et al., 1992). On the contrary, under high soil compaction, the effects on growth and plant performance are in general negative (Kozłowski, 1999, 2000; Tubeileh et al., 2003; Pérez-Ramos et al., 2010).

Although there are many studies on the response of plants to soil compaction, detailed studies on the effects of soil compaction on root anatomy and morphology together with plant physiology and growth are rare. Moreover, soil compaction studies are mainly done in agricultural and forestry species, whereas studies with wild woody species are not common.

In the present study, we test the response to soil compaction of *Fraxinus angustifolia* Vahl. seedlings in two soil types under greenhouse conditions. We used *F. angustifolia* as a model plant, as it is a woody species with a fast growth (Antúnez et al., 2001). We studied four categories of variables: root morphology, root anatomy, plant physiology and plant growth and architecture. The first objective of this study was to find out which of these groups of variables are affected by soil compaction and/or soil type and to what degree by each one. The second objective was to build a causal model which aims to explain how soil compaction affects root traits and how these changes explain whole-plant level functioning.

2. Materials and methods

2.1. Soil types and compaction treatments

The two natural soils used in the study were selected based on their different texture, allowing to obtain a wide compaction range. Soil type I has a loam texture and was collected along the banks of the Guadalquivir river ("La Golondrina" area, Córdoba, Spain). Soil type II has a sandy-loamy texture and comes from a forest of the Sierra Morena mountains ("Los Arenales" area, Córdoba, Spain). Soils were sifted by a 1 cm sieve to eliminate stones and plant remains. Soil texture, pH and nutrient concentrations were completely different (Table 1). Thus, the loam soil (type I) has more nutrients (N, P, K) than the sandy-loam soil (type II).

Compaction treatments were carried out at 5.6 and 4.3% of volumetric water content for loam and sandy-loam, respectively. Volumetric water content was measured with a TDR (Time Domain Reflectometry) (FieldScout TDR 100, Spectrum Inc. Technologies). The pots were PVC cylinders 50 cm tall and 10.5 cm in diameter (4.33 L), to prevent space limitation for root growth.

Three soil compaction treatments: no compaction (NC), moderate (MC) and high compaction (HC) were applied with an identical procedure for both soil types. Five replicates per treatment and

Table 1
Physical and chemical characteristics of the two natural soils used in the experiment.

Textural class	Soil type	
	I Loam	II Sandy-loam
Gravel < 10 mm > 2 mm (%)	25.5 ± 0a	60.4 ± 0b
Sand (%)	46.23 ± 0.38a	72.36 ± 1.22b
Silt (%)	36.36 ± 0.38a	16.43 ± 1.22b
Clay (%)	17.4 ± 0a	11.2 ± 0b
pH 1/2.5	8.24 ± 0.02a	6.94 ± 0b
pH (CIK)	7.75 ± 0.02a	6.22 ± 0.03b
Cation exchange (mequiv./100)	14.16 ± 0.05a	14.2 ± 0.27a
Organic matter (%)	1.07 ± 0.02a	1.16 ± 0.03b
N total (%)	0.06 ± 0a	0.03 ± 0b
P (Olsen) (ppm)	51.5 ± 0.7a	3.03 ± 0.19b
K (ppm)	370 ± 7.07a	111.67 ± 1.7b
Bulk density (g cm ⁻³)	1.36 ± 0.12a	1.54 ± 0.09b
Penetration resistance (Mpa)	1.87 ± 1.32a	3.37 ± 0.87b

Mean values ± SD, n = 3 for texture and chemical analyses, n = 15 for bulk density and penetration resistance. Significant differences between soil types are shown with different letters (P < 0.05). Texture and chemical analyses were made on the soil previously sieved at 2 mm.

soil type were made. The reference to create these treatments was the soil bulk density; therefore, by increasing the soil mass for the same volume, we increased soil compaction. The procedure for the NC treatments consisted of filling the pot with the aid of another equal-sized pot as a guide up to the total height of 100 cm, after which the column formed by both pots was shaken slightly. For the two remaining treatments, we used an electrical hammer (GSH 11 E, Bosch, Germany) with a modified piston to compact the soil (Supplementary Material, Fig. S1). MC treatments were made with the same procedure as the NC treatment, and then the hammer was applied to compact the soil to the height of 75 cm with an estimated force of 5150 N or 0.52 Tm (percussion frequency 1030 per minute, time of percussion 5 s, 15 J per percussion). After that, the guide was withdrawn and the spare soil was discarded when the pot was full. HC treatments were made by filling the guide plus the pot only up to approx. 75 cm, the height from which the electrical hammer was applied at an estimated force of 23,750 N or 2.42 Tm (percussion frequency 1900 per minute, time of percussion 10 s, 15 J per percussion). Once an approximate height of 55 cm was attained, the guide was removed where a soil scab was found caused by the hammer percussion. The objective of this methodology was to obtain a vertical compaction in a natural gradient, as happens in the trampling process.

2.2. Growth conditions and planting

Seeds of *Fraxinus angustifolia* were germinated in trays in a mixture of sand and potting soil in a growth chamber at 25 °C and 60% of relative humidity. When seedlings had 2 leaves, about 100 seedlings of a similar size were selected. A subsample of 15 seedlings was harvested to obtain the following: fresh and dry biomass of leaves, stems and roots. Dry weight was obtained after at least 2 days in an oven at 70 °C. Each seedling selected for the experiment was freshly weighed and then planted in one pot. The experiment was conducted in a greenhouse at the University of Córdoba (Spain) between July and November 2008. Due to the natural conditions under which ash seedlings grow (under an adults' canopy in wet habitats where plant cover is high and light availability is low), the experiment was conducted under a shadow cloth (transmission of 10% of the total radiation available). Thus, the photosynthetically active radiation (PAR) was 84 ± 13 μmol photon m⁻² s⁻¹ (mean ± standard deviation) measured with a canopy transmission meter (EMS-7, PP-system, UK) on a clear day and the total daily mean radiation during the experiment

was $2.4 \text{ mol m}^{-2} \text{ day}^{-1}$. The average temperature of the growth period inside the greenhouse was $17 \pm 7^\circ\text{C}$ and the time of growth was 124 days. The irrigation system was by dripping with a plastic bottle of 0.5 L (model 011, S. A. R. L. IRISO, France). Altogether 6.5 L of water was added to each pot during the first 86 days, after which watering was suppressed forcing the plants to use soil water reserves.

2.3. Ecophysiological measurements

Photosynthesis and transpiration rates were measured with a gas-exchange analyser (CIRAS-2, PP System, UK). The conditions during the measurements were: $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ of PAR provided artificially by leds, 400 ppm of referential CO_2 , $150 \text{ cm}^3 \text{ min}^{-1}$ of air flow and 23°C temperature. When leaves did not cover the entire window of the leaf cuvette, a digital photo was taken to correct the photosynthesis and transpiration rates. The digital photo was analysed with image analysis software (Image Pro Plus 4.5, Average Cybernetic, Inc., Silver Spring, MD, USA). Leaf water potentials were measured after photosynthesis measurements, using a pressure bomb (range 0–15 MPa; Manofrigido, Lisbon, Portugal) (Scholander et al., 1965). The same leaf used for photosynthesis (the final leaflet from one of the second whorl leaves) was used for water potential measurements. The measurement schedule was aimed at avoiding day-time effects on photosynthesis and leaf water potential; thus, in 30 min, the six different treatments were measured (3 compaction levels \times 2 soil types) between 11 a.m. and 2 p.m. (solar time). These physiological variables were carried out 20 days after the watering had been suppressed to determine the plant's ability to use water reserves.

2.4. Growth, biomass allocation and soil measurements at final harvest

In order to describe the compaction treatment of each pot at harvest time, penetration resistance and bulk density were measured. Penetration resistance was measured by penetrometer (Penetrologger, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) using a 60 cm long cylindrical probe with a 1 cm^2 , 30° angle cone into the soil, taking at least two measurements for each pot. This equipment provides measurements at 1 cm depth intervals. The data obtained were used to calculate the mean soil penetration resistance for the whole soil profile in each pot. After the harvest of each plant (see below), the soil mass water content (MWC) was measured in a soil sample for each pot. To do that, one sample for each pot was weighed, and then dried in a stove to 105°C for three days. Bulk density was calculated as the ratio between dry soil mass and the soil volume occupied in the pot (a cylinder with known radius and height). Penetration resistance and bulk density were highly correlated ($r=0.78$, $P<0.001$). Mean values of bulk density and penetration resistance were lower for loam soil (type I) than for sandy-loam soil (type II) (Table 1).

Each plant was harvested dissecting it in leaves, stems and roots. Roots were cleaned and put in a cool box ($7\text{--}10^\circ\text{C}$) until their use in anatomic and morphological analysis (less than four days). Fresh leaves were scanned and leaf area was measured by image analysis software (Image-Pro Plus v4.5; Media Cybernetic, Bethesda, MD, USA). Stem and leaves were dried in a stove at 70°C for at least 3 days to obtain the dry biomass. Relative growth rate (RGR) was calculated following the classical approach (Hunt, 1990) as: $(\ln M_2 - \ln M_1)/(t_2 - t_1)$, being M_2 and M_1 the final and initial dry mass of the seedling, respectively, and $t_2 - t_1$ the growth period (124 days). Initial dry mass (M_1) for each plant was obtained as the product of initial fresh mass and dry matter content of the first

harvest (see above). Specific leaf area (SLA), biomass fraction dedicated to leaves (LMR, Leaf Mass Ratio), stem (SMR, Stem Mass Ratio) and roots (RMR, Root Mass Ratio) was calculated as the ratio of biomass of the fraction and total biomass (Hunt, 1990). Leaf area ratio (LAR) was calculated as the product of SLA and LMR.

Nitrogen and carbon concentration of leaves, stems and roots was measured in order to evaluate the nutritional state of the plants after the growth period. Dry samples were ground with an electric mill (IKA, model A10, Germany) and then they were dried again at 70°C for at least 1 day. Nitrogen and carbon concentrations were obtained by an element analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy).

2.5. Root morphology

A fraction of root biomass of each plant was taken to analyse the morphological traits. This fraction represented about $20 \pm 5\%$ of total root biomass (mean \pm SD), and, in morphological terms, this fraction represents one of the main roots (first order) linked to the root-stem connection. This approach prevents the underestimation of root length caused by the root base, which has a high biomass and short length. Roots were scanned with a high resolution scanner at 600 dpi in a transparent tray with water. Root analysis was carried out with image analysis software (WhinRHIZO ver. 2004a, Regent Instruments Inc., Quebec, Canada). The output of the software gives the following: mean root diameter, total root length, root volume and length of each diameter class (between 0 and 4.5 mm). This software calculates the root volume based on the root diameter distribution and not only on mean root diameter, so therefore, the measurements are precise. After scanning, roots were dried at 70°C to obtain dry biomass. The variables calculated were as follows: specific root length (SRL) as a ratio of root length and dry mass; total root length as a product of SRL and dry mass of the whole root (excluding root base); root tissue mass density (TMD_R) was calculated as a ratio of root dry mass and root volume and root length ratio (RLR) as a ratio of total root length and plant mass. Length of each diametric class was expressed as a percentage of total length considering only the roots' length of diameter as being less than 1.5 mm (which represents about 98% of total length for all cases).

2.6. Root anatomy

Root samples for anatomy were taken using the same criterion for all samples: we selected 2 cm of the main root or one of the larger diameters (in the case of there not being any main root) at approximately 5 cm from stem-root connection. Samples were fixed with FAA (acetic formaldehyde:alcohol:acetic acid:distilled water; 10:50:5:35) until its processing in laboratory. Fixed material was cut by hand with razor blades raised under a binocular magnifying glass (Olympus SZ61). The slides were made on a cross section to longitudinal root axis taking about $100\text{--}200 \mu\text{m}$ in thickness. Cross section samples were stained for 1 min with toluidine blue solution (0.5% in distilled water), which stains lignin blue and cellulose purple, and they were then washed in water to eliminate colorant excess. Stained samples were placed on a glass slide with water. Images for analysis were photographed using a camera (Olympus Altra 20) connected to a microscope (Olympus BX41). Measurements were made over a subsection along a symmetry axis so that relative values refer to the surface measured (50%). The variables calculated were: the percentage of xylem per cross section area (CSA), the number and proportion of xylem vessels over total xylem CSA and the diameter of the xylem vessels.

2.7. Statistical analysis

The statistical analysis was made using ANCOVA analysis considering soil type (categorical variable) and soil compaction in terms of bulk density (continuous variable) with the General Linear Model (GLM) procedure (homogeneity of slopes) in Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA). According to the results in [Supplementary material \(Table S1\)](#) we could not perform a two-way factorial ANOVA (with soil type and compaction treatment as categorical factors) due to the different responses of each soil to the same perturbation system. For instance, a high compaction level in loam soil is comparable to a low compaction level in sandy-loam soil. So, compaction was considered to be a continuous variable using bulk density data instead of a categorical factor with three levels. Linear correlations were made to find out the relationships between the variables.

To determine the causal relations between the variables studied we carried out a multivariate analysis, using Shipley's d-sep method (Shipley, 2000). The d-sep method has successfully described causal patterns in plant biology and ecology (Shipley, 2004; Villar et al., 2004; Quero et al., 2006, 2008; Aponte et al., 2011). To perform the d-sep test we used a compiled Fortran program (dgraph.exe) written by Bill Shipley and freely available in The Causal Toolbox (<http://pages.usherbrooke.ca/jshipley/recherche/book.htm>). We tested different models to explain how differences in soil compaction may affect root morphology and anatomy with consequences to plant physiology and growth. The models tested were based both on biological hypotheses, derived from previous knowledge of the effect of soil compaction on the studied plant variables, and exploratory correlations of soil compaction with the structural and physiological variables. We present three models, each one with a different causal structure. We combined the data for the two soil types ($n = 26$) in order to present a general model. In those models where the chi- P value was not significant ($P > 0.05$), the model was accepted (Shipley, 2000). For the accepted model we add for each causal effect the standardized path coefficients, which were calculated using multiple regressions analyses. In Statistica v 7.1 they are called the beta coefficients and they are the regression coefficients you would have obtained with the standardized variables. Thus, these standardized path coefficients allow to compare the relative contribution of each independent variable in the prediction of the dependent variable.

3. Results

3.1. Root morphology

Mean root diameter, specific root length (SRL), root length and diameter class distribution were heavily influenced by soil bulk density but the soil type did not have any significant effect (Table 2). Mean root diameter was positively correlated with soil bulk density (Table 2) and SRL negatively correlated with soil bulk density (Fig. 1a), showing a 64% reduction with a 25% bulk density increment. The length of different root orders was strongly affected by soil compaction. Thus, an increase in soil compaction decreases the proportion of fine roots (diameter between 0 and 0.5 mm) whereas the proportion of medium and high diameter roots (between 0.5 and 1.5 mm) was increased with soil compaction (Fig. 1b). These changes in root size class distribution with soil compaction were visually evident (Fig. 2), where a reduction of second and third root order can be seen at the same time as the first root order was incremented with soil compaction. Despite these changes in the general root morphology pattern, root tissue mass density (TMD_R) was not affected by soil compaction or soil type (Table 2).

3.2. Root anatomy

The proportion of xylem was not affected by bulk density or soil type (Table 2). However, the proportion of xylem cross section area (CSA) dedicated to vessels, which is related to percentage area for water conduction, showed a decrease with soil compaction (Fig. 1c) and was not affected by soil type (Table 2). The number of xylem vessels per unit area of xylem CSA was not affected by bulk density or soil type, but the xylem vessel diameter was negatively affected by soil compaction in sandy-loam soil (Table 2). Visually we can see how soil compaction affects the xylem vessel diameter (Fig. 3).

3.3. Plant growth and architecture

Growth variables such as total biomass and relative growth rate were not modified by soil compaction or by soil type (Table 2). In general, most of the growth and morphology variables showed a significant interaction between soil compaction and soil type, which means that the effect of soil compaction varies depending on soil type. Specific leaf area, leaf mass ratio and leaf area ratio showed an increase with bulk density in the richer soil (loam soil, type I) (Table 2). However, the same variables showed a decrease with soil compaction in the poorer soil (sandy-loam soil, type II). Root mass ratio was affected by soil type with a significant interaction with bulk density due to a different response depending on the soil type. For sandy-loam soil, bulk density produced a significant increase in root mass ratio, whereas in loam soil there was no effect of bulk density (Table 2). The ratio of root length to plant biomass (root length ratio, RLR) was negatively affected by bulk density, but, also, soil type showed a significant interaction with bulk density. This is because, in loam soil, RLR was negatively affected by compaction while in sandy-loam it was not significantly affected (Table 2).

Chemical composition of some tissues was also affected by soil compaction. Leaf N concentration was slightly incremented with bulk density in loam soil ($r = 0.50$; $P = 0.06$), and root C was negatively affected by bulk density in loam soil ($r = -0.65$; $P = 0.01$).

3.4. Ecophysiology and whole plant functioning

Photosynthesis and transpiration rates were not affected by soil compaction or soil type (Table 2). However, leaf water potential was affected by soil compaction, resulting in higher water stress with higher soil compaction conditions (Table 2, Fig. 1d).

The four groups of variables considered (root morphology, root anatomy, plant growth and architecture, and plant physiology) showed different relationships with each other (Table S2, [Supplementary Material](#)). First, root morphology was correlated mostly with plant physiology. For example, SRL was positively related to photosynthesis and transpiration rates (Table S2, [Supplementary Material](#)), and SRL had a significant positive effect on leaf water potential (Fig. 4b). On the contrary, root morphology had only a few significant effects on growth and architecture variables; i.e., root length was positively related to total biomass (Table S2, [Supplementary Material](#)), and negatively to the specific leaf area.

Secondly, root anatomy showed relationships mainly with growth and architecture variables. For example, the xylem vessel diameter seems to be closely associated with growth, i.e., total plant biomass, leaf mass ratio and total plant area were positively associated with the xylem vessel diameter (Fig. 4d and Table S2, [Supplementary Material](#)), whereas root mass ratio was negatively associated.

To sum up, we tested different causal models using the d-sep method (Shipley, 2000) aiming to describe different causal relationships of the effects of soil compaction (Fig. 5). The objective of this was to understand the effects of soil compaction from

Table 2

Results of ANCOVA analysis for different variables studied using soil type as categorical factor and bulk density as a continuous predictor. The values represent the percentage of explained variance (SS_x/SS_{total}) for each factor and the interaction. ns, non significant. Below the column r sign is shown the correlation sign of the variable studied against bulk density in each soil type (I and II) and considering all data together.

		Soil type	Bulk density	Soil type \times bulk density	r sign		
		(I and II)			I	II	All
Root morphology							
MRD	Mean root diameter (mm)	1 ns	51***	0 ns	+	+	+
SRL	Specific root length (m g^{-1})	2 ns	24***	3 ns	–	–	–
RL	Root length (cm)	1 ns	24***	1 ns	–	–	–
TMD _R	Tissue mass density of root (g cm^{-3})	6 ns	5 ns	4 ns	ns	ns	ns
LDC _{0.5}	Length of diametric class $> 0 < 0.5$ mm (%)	0 ns	22***	0 ns	–	–	–
LDC _{1.0}	Length of diametric class $> 0.5 < 1$ mm (%)	0 ns	40***	0 ns	+	+	+
LDC _{1.5}	Length of diametric class $> 1 < 1.5$ mm (%)	2 ns	16*	2 ns	+	+	+
Root anatomy							
X _{CSA}	Xylem CSA (%)	0 ns	0 ns	0 ns	ns	ns	ns
PXV _{CSA}	Proportion of xylem CSA dedicated to vessels (%)	4 ns	15**	4 ns	–	–	–
NXV _{CSA}	Number of xylem vessels per mm^2 of xylem CSA	4 ns	2 ns	3 ns	ns	ns	ns
DXV _{CSA}	Diameter of xylem vessels (μm)	8 ^a	6 ^a	7 ^a	ns	–	ns
Plant growth and architecture							
B	Biomass (g)	5 ns	0 ns	6 ns	ns	ns	ns
RGR	Relative growth rate ($\text{mg g}^{-1} \text{day}^{-1}$)	11 ^a	0 ns	13*	ns	ns	ns
LA	Total leaf area (cm^2)	18*	0 ns	19**	ns	–	ns
SLA	Specific leaf area ($\text{m}^2 \text{kg}^{-1}$)	17**	1 ns	14**	+	–	+
LMR	Leaf mass ratio (kg kg^{-1})	22**	1 ns	24**	+	–	ns
SMR	Stem mass ratio (kg kg^{-1})	0 ns	1 ns	0 ns	ns	ns	ns
RMR	Root mass ratio (kg kg^{-1})	14*	2 ns	15*	ns	+	ns
LAR	Leaf area ratio ($\text{m}^2 \text{kg}^{-1}$)	28***	1 ns	28***	+	–	ns
RLR	Root length ratio (root length/plant mass) (m g^{-1})	6*	21***	6*	–	ns	–
Plant physiology							
Pn	Net photosynthesis rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	2 ns	0 ns	3 ns	ns	ns	ns
E	Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	0 ns	1 ns	0 ns	ns	ns	ns
Ψ_L	Leaf water potential (Bar)	1 ns	21*	1 ns	–	ns	–

^a $0.05 < P < 0.10$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

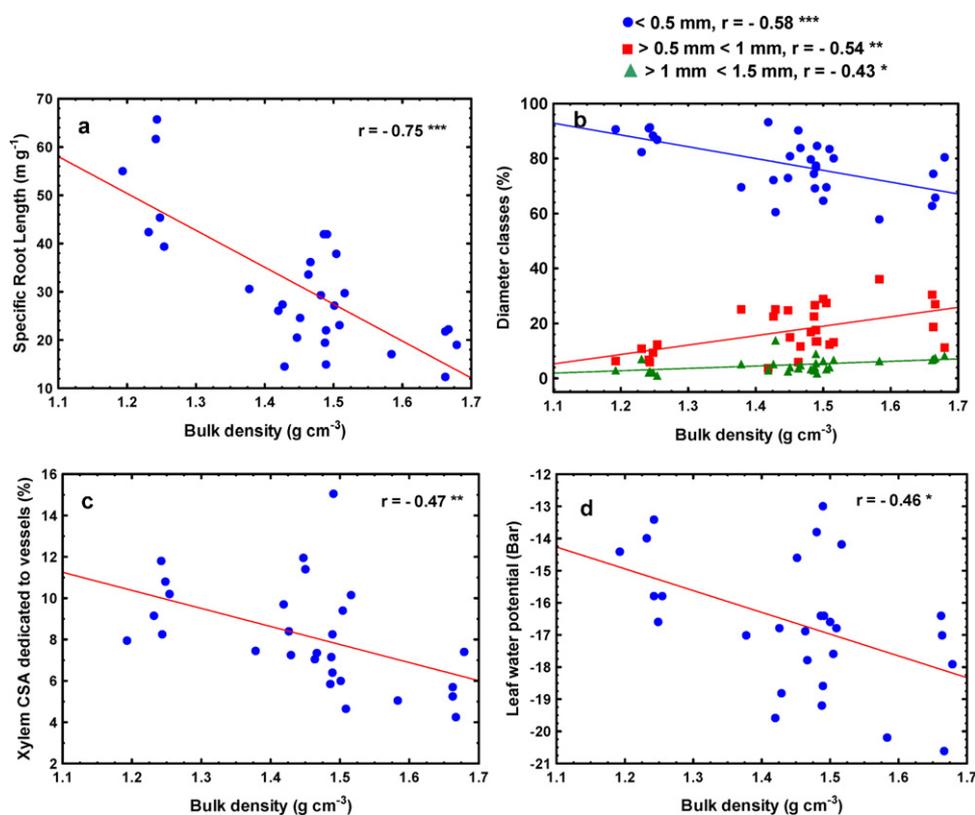


Fig. 1. Relationships of bulk density with (a) specific root length (SRL), (b) percentage of length of different diameter classes of roots, (c) percentage of xylem cross sectional area (CSA) dedicated to vessels, and (d) leaf water potential. The lines represent the significant relationships ($P < 0.05$).

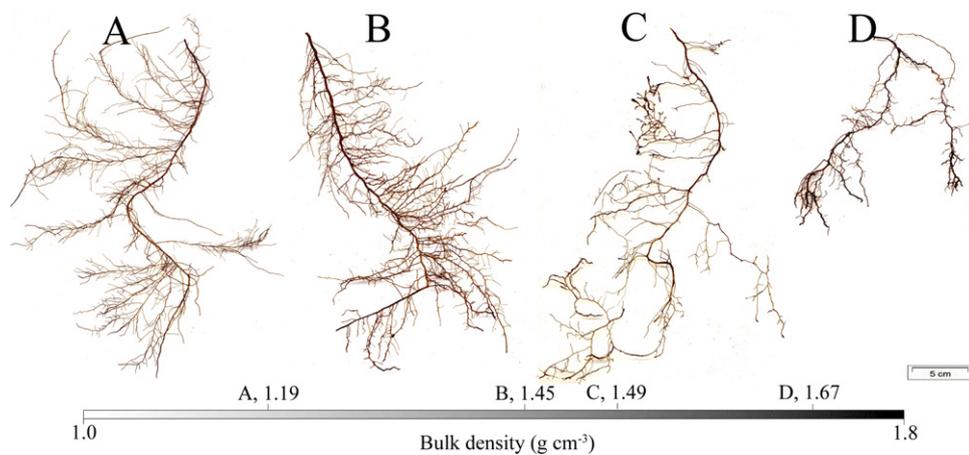


Fig. 2. Pictures of root morphology of *Fraxinus angustifolia* seedlings under different soil treatments. (A) No compaction of loam soil, (B) high compaction of loam soil, (C) no compaction of sandy-loam soil and (D) high compaction of sandy-loam soil. For each root, the value of soil bulk density is shown.

roots to whole plant functioning. We present three causal models, each one with a different causal structure. Model 1 states that soil compaction affects directly and independently different variables: root morphology (SRL) and anatomy (xylem vessel proportion and xylem vessel diameter); plant physiology (transpiration rate, the latter affecting the photosynthesis rate) and plant area (this affecting the plant biomass). This model was rejected, as the data did not agree with the proposed causal relationships ($P=0.021$, Fig. 5 and Table S3 in Supplementary Material). Model 2 states that soil compaction affects directly root variables: root morphology (SRL) and anatomy (xylem vessel proportion and xylem vessel diameter) but not plant physiology variables, which are independent of the changes in root characteristics caused by soil compaction. This model was also rejected ($P=0.014$, Fig. 5 and Table S3 in Supplementary Material).

In contrast, model 3 was accepted ($P=0.777$, Fig. 5 and Table S3 in Supplementary Material). In this model we consider that soil compaction produces changes in the root traits that have consequences on plant physiology and growth. Soil compaction has an effect on root morphological variables, such as a decrease in SRL (specific root length). SRL positively affects the transpiration rate and leaf water potential (less negative). Transpiration rate affects positively the photosynthesis rate. Soil compaction also has an effect on root anatomy variables, decreasing the xylem vessel proportion. Xylem vessel proportion has a positive effect on xylem vessel diameter, which influences positively the plant area and this affects positively the plant biomass. Therefore, plant biomass can

be affected through two paths: one due to the effects of soil compaction on SRL and the other through the effects of soil compaction on xylem vessels proportion (Fig. 5, model 3). The results of the combined effect of the standardised path coefficients (the multiplication of the path coefficients along each path) indicate that the effect of soil compaction through the path of xylem vessels proportion is more important than that caused by changes in SRL (-0.10 versus -0.03). In summary, our data support the model that soil compaction affects different root traits (morphology and anatomy) which have consequences for plant physiology and growth.

4. Discussion

4.1. Effects of soil compaction and soil type

Our first objective was to find out how soil compaction and soil type affect different root traits and plant functioning. Interestingly, we found that most of the variables studied were affected by soil compaction variables instead of chemical ones. The coefficient of variation in soil compaction variables (bulk density and penetration resistance) and chemical properties of the soils was similar (Fig. S2, Supplementary Material), indicating that the higher effect of soil compaction in comparison to chemical variables is due to a higher response of *Fraxinus* seedlings to soil compaction. However, variables related to plant growth and morphology were more affected by soil type, which could be due to the role of the nutrients

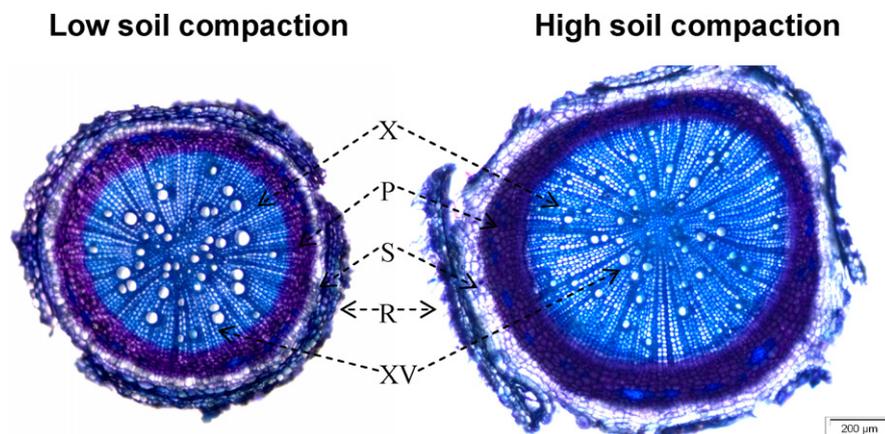


Fig. 3. Pictures of root histology of *Fraxinus angustifolia* seedling under compaction treatments: no compaction treatment of loam soil is shown in the left; high compaction treatment of sandy-loam soil on the right. X, xylem; P, phloem; S, suber; R, rhizodermis; XV, xylem vessels.

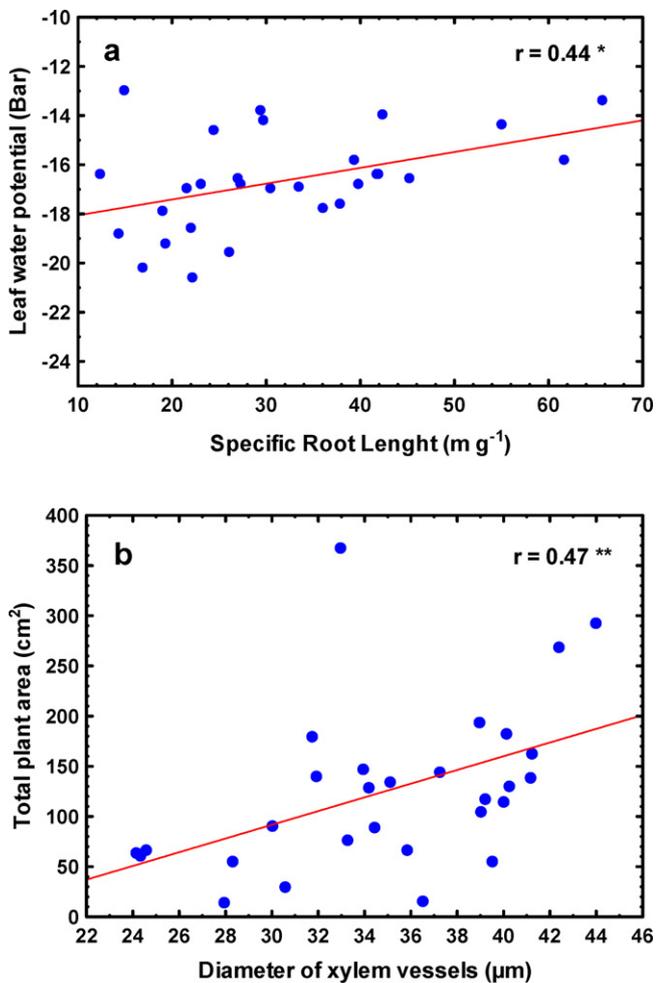


Fig. 4. Relationship between (a) leaf water potential and specific root length and (b) total plant area and diameter of xylem vessels.

in potential growth. Interestingly, most of the growth and morphology variables showed a significant interaction between soil compaction and soil type, indicating that the effect of soil compaction varies depending on soil type. For example, specific leaf area, leaf mass ratio and leaf area ratio showed an increase with soil compaction in the richer soil (loam soil, type I) (Table 2). This could be due to the fact that an increase in bulk density (soil mass per volume) is related to an increase in the amount of nutrients per volume unit (Arvidsson, 1999), and, therefore, soil compaction may have a positive effect on these variables. In this sense, we found that leaf N concentration was slightly incremented with compaction in loam soil, which could be because of a better root-soil contact allowing higher nutrient transport rates (Wolkowski, 1990; Arvidsson, 1999; Gómez et al., 2002b). However, the same growth and morphology variables showed a decrease with soil compaction in the poorer soil (sandy-loam soil, type II), which could be due to the fact that, because of this poor soil, any increase in soil compaction does not increase nutrient availability but it has a negative effect on growth and morphology variables.

Our results stress the importance of soil physical properties in root morphology and anatomy, and, thus, in root functioning, so that soil physical properties may act as a first filter in seedling establishment (Kozłowski, 1999). As Bejan et al. (2008) points out, plants could be considered as being a physical flow architecture that evolves to fulfill two objectives: maximum mechanical strength against the wind, and maximum access for the water flowing through the plant, from the ground to the atmosphere.

Thus, during the first stages of development, seedlings need to reach a place where they anchor themselves with a minimum of light, water and nutrient requirements, and, therefore, soil compaction seems to be an important factor in the initial stages of development.

4.2. Linking root traits to plant physiology and growth

Our second objective was to find out how changes in root traits due to soil compaction can be linked to plant functioning. Soil compaction had a negative effect on root elongation, which is translated into a root length decrease, a root diameter increase and changes in root class diameter distribution. Similarly, Whalley et al. (1995), Mósena and Dillenburg (2004), and Bejarano et al. (2010) have found shorter and thicker roots in highly compacted soils. The decrease in SRL with soil compaction is also commonly reported in the literature (Bengough and Mullins, 1990; Bejarano et al., 2010). Atwell (1993) explained this change as a mechanical impedance effect, because longitudinal growth is reduced in favor of a radial one. This is the cause of changes in root cell growth, as soil compaction produces reductions in both root cell elongation and production rate accompanied by a radial cell extension (Dexter, 1987). It has been suggested that ABA and ethylene activity lead this signal caused by soil compaction (Hussain et al., 2000; Roberts et al., 2002; Whalley et al., 2006). Considering the whole root system, soil compaction decreased the growth of the second order root, accompanied by an increase in the growth of the first root order. Subsequently, when the normal development of the root system is impeded, processes such as anchorage, water absorption and nutrient uptake can be affected. In this sense, difficulties have been reported in isolating soil compaction effects from water stress (Taylor and Ratliff, 1969; Whalley et al., 2007). Soil compaction and water stress can be considered as being cause-and-effect, due to the former reducing the exploring capacity of roots, thus, resulting in a lower water uptake. Moreover, soil compaction increases the soil matrix potential (Taylor and Ratliff, 1969), which makes it difficult to extract water from the soil. This may explain why leaf water potential was negatively correlated with bulk density (Fig. 1d), which means a higher water stress (more negative values) with a higher soil compaction (Liang et al., 1996). SRL was positively correlated with the leaf water potential (Fig. 5, model 3), indicating that longer and thinner roots can reduce the probabilities of reaching a water stress situation. Similarly, SRL was positively correlated with the transpiration rate, which could mean that evaporation demands were not interrupted thanks to the uptake throughout the thinner roots. Hund et al. (2009) found a root diameter reduction under water stress conditions, which could be explained by a restriction of a lateral expansion of root stele and cortex in the apical zone (Liang et al., 1997). We also found that the transpiration rate was positively related to the photosynthesis rate (Fig. 5, model 3), as this has been found in many studies (Jones, 1998; Quero et al., 2006).

Moreover, soil compaction also exerts some effects on root anatomy. For instance, soil compaction was negatively related to the proportion of xylem CSA dedicated to vessels and this was positively related to xylem vessels diameter (Fig. 5, model 3). These effects can be explained as a response to a water deficit situation (Holste et al., 2006). These changes in root anatomy has consequences on plant growth as the xylem vessel diameter was positively correlated with total plant area and the latter with the plant biomass (Fig. 5, model 3). According to Wahl and Ryser (2000) and Hummel et al. (2007), xylem vessel diameter is closely related to plant height (a proxy of plant biomass) in a wide spectrum of herbaceous species, which can be associated with an important aspect of the trade-offs between anatomical structures, and plant growth.

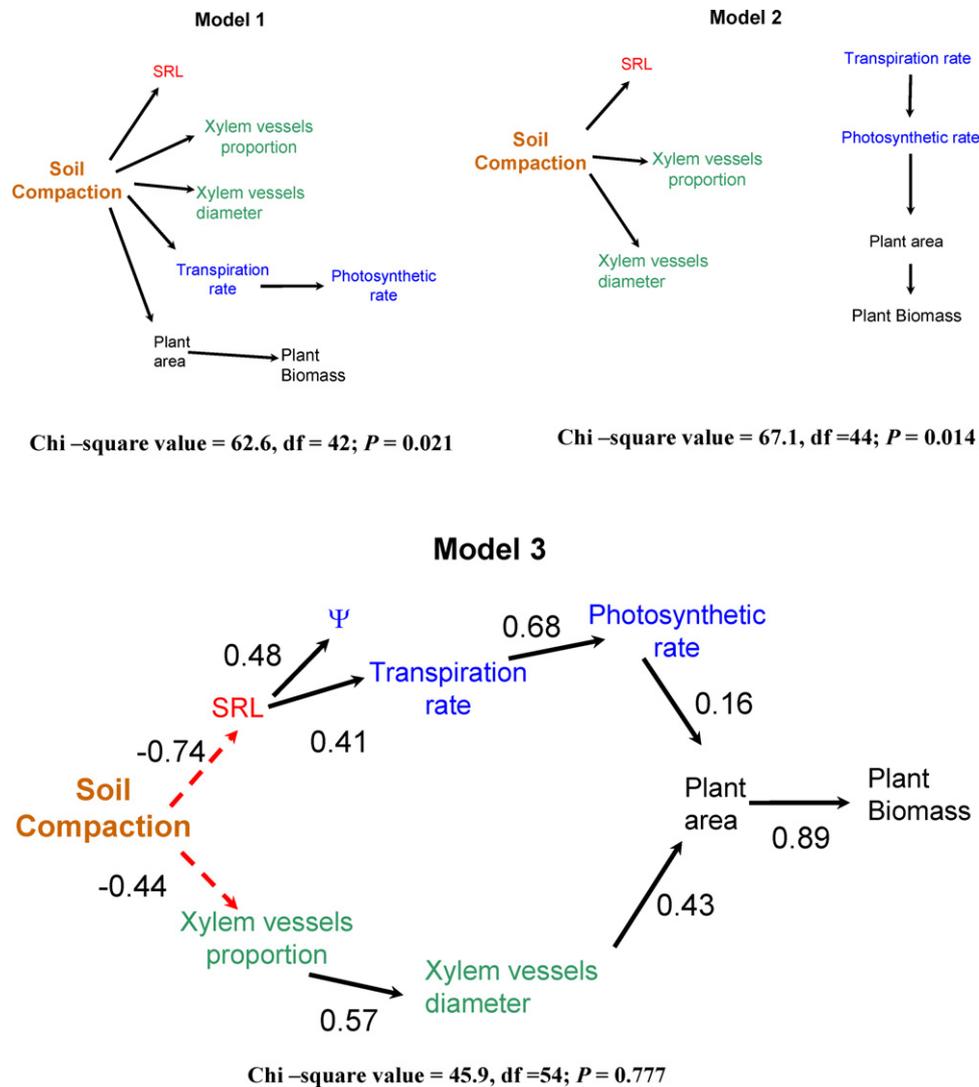


Fig. 5. Causal models based on d-sep analysis (Shibley, 2000). Model 1 states that soil compaction affects directly and independently different variables: root morphology and anatomy; plant physiology and plant growth. This model was rejected ($P=0.021$). The model 2 states that soil compaction affects directly root variables: root morphology and anatomy but not plant physiology and plant growth, which is independent of the changes in root characteristics. This model was also rejected ($P=0.014$). Model 3 considers that soil compaction produces changes in root traits that have consequences on plant physiology and growth. Model 3 was accepted ($P=0.777$). The numbers near the arrows indicate the standardized path coefficients of each relationship. Dashed and red lines indicate a negative relationship; continuous and black lines indicate positive relationship. SRL, specific root length; ψ , leaf water potential. The colours represent different types of variables: soil compaction (brown), physiology (blue), root anatomy (light green), root structure (red) and plant growth (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

Soil compaction modifies root morphology and anatomy as a result of mechanical impedance. These modifications are translated into changes in physiology and architecture at whole-plant level. It seems that root morphology leads to changes in plant physiology which may be due to the root's exploring capacity (nutrient and water uptake) being limited by soil compaction. *Fraxinus angustifolia* seedlings appear to be more sensitive to soil physical properties than to chemical ones. It would be necessary to find out if this is a general response in woody plants, and, therefore, more studies including a wide set of species are required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.envexpbot.2012.01.004](https://doi.org/10.1016/j.envexpbot.2012.01.004).

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Supplementary Material

Figure S1. Equipment used to compact soils.



Figure S2. Coefficient of variation ($100 \times \text{standard deviation} / \text{mean}$) for different soil chemical and physical variables.

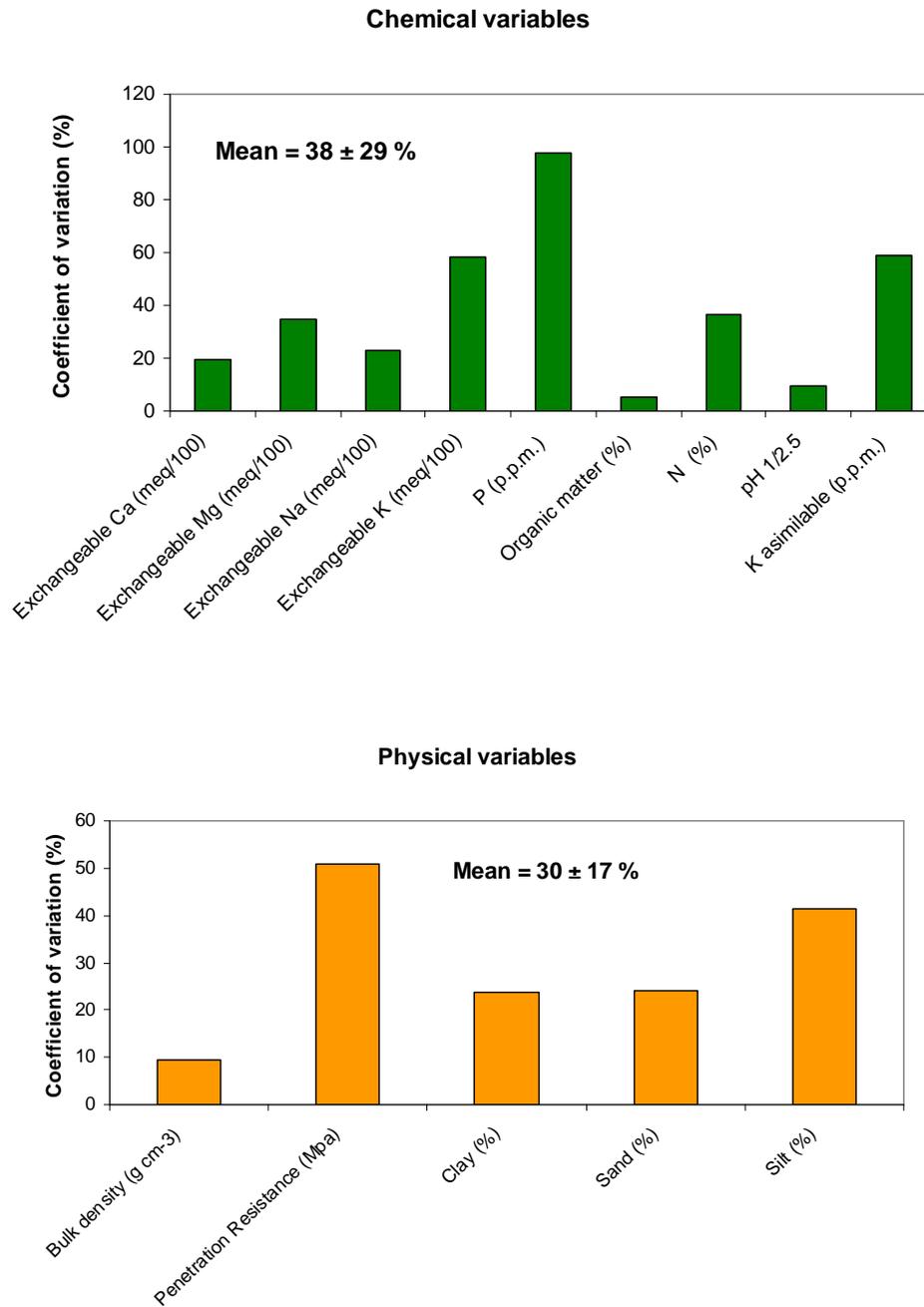
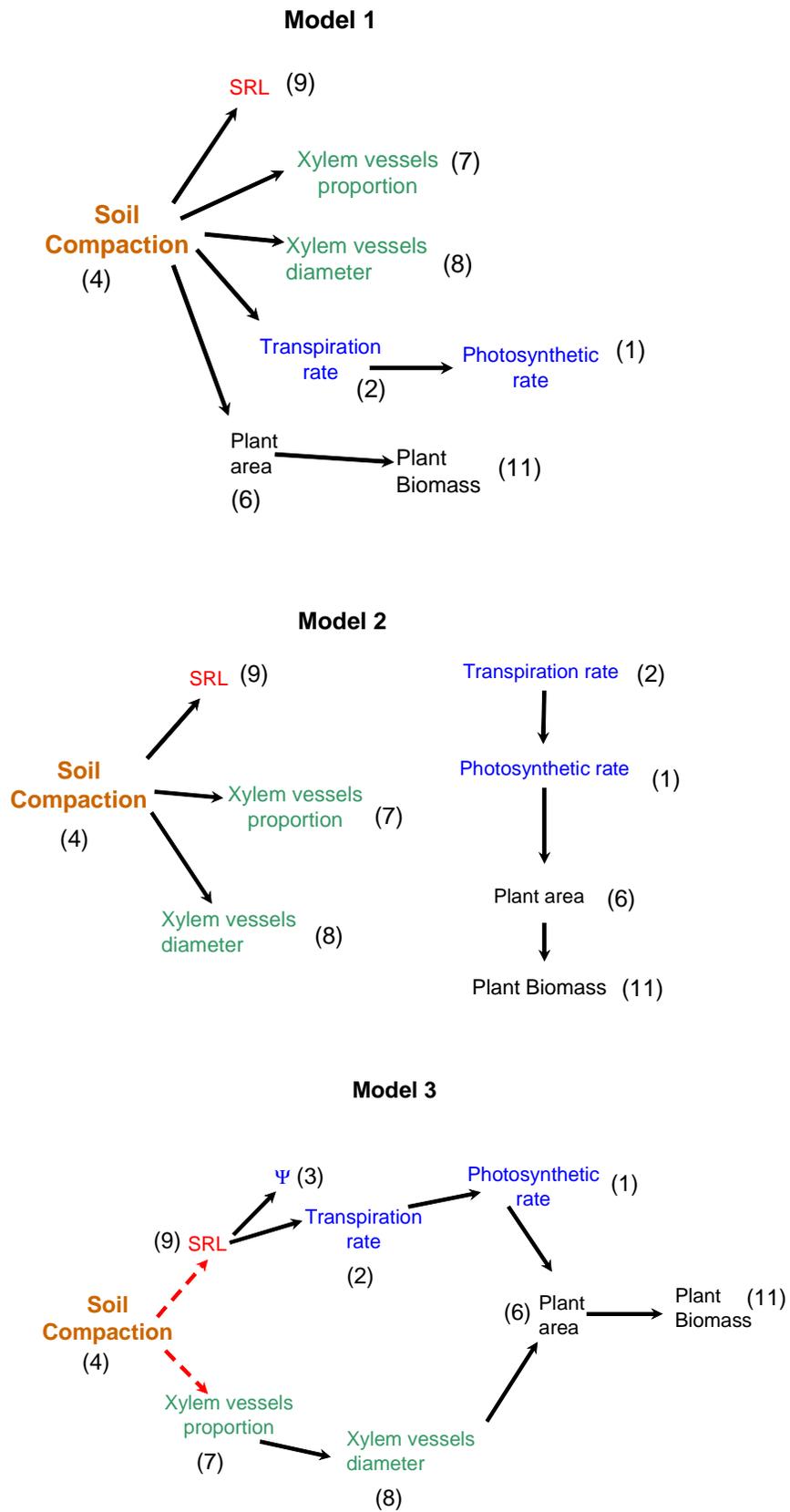


Figure S3. Diagram of the three models with the codes of the variables in brackets. See Table S3 for the basis set of independence statements.



Supplementary material

Table S1. Properties of two soils used in the experiment under different compaction treatments. Factorial ANOVA was made to evaluate significant differences (shown with different letter).

Soil type	N	Compaction treatment	Bulk density (g cm ⁻³)	Penetration resistance (Mpa)	Mass Water Content (%)
Loam (I)	5	1	1.23 ± 0.02 a	0.65 ± 0.43 a	12.63 ± 3.49 a
	5	2	1.37 ± 0.08 b	1.98 ± 0.62 b	10.81 ± 2.87 a
	5	3	1.49 ± 0.02 c	3.00 ± 1.44 c	10.11 ± 3.41 a
Sandy-loam (II)	5	1	1.46 ± 0.03 c	3.32 ± 0.23 c	3.85 ± 0.63 b
	5	2	1.50 ± 0.01 c	2.48 ± 0.67 bc	5.28 ± 0.69 b
	5	3	1.65 ± 0.04 d	4.32 ± 0.21 d	5.61 ± 1.30 b

Table S3. Basis sets for the partial independence constraints implied by each of the three models shown in Fig. 5. The notation ‘(X,Y)|{A,B,...}’ means that variables X and Y are hypothesized to be probabilistically independent, conditional on the set of variables {A,B,...} and the ‘ φ ’ represents the null (empty) set. Pearson’s partial correlation coefficient (r), and probability (P) are given for each conditional independence statement. Values in bold have a probability below 0.05. The overall model is tested with Fisher’s C statistic and the probability is shown in brackets (below each model). In those models where the C value was not significant ($P > 0.05$), the model was accepted. Model 1 and 2 are rejected ($P < 0.05$) and model 3 is accepted ($P > 0.05$). Codes of the variables are below the table, see also Fig. S3.

Model 1			Model 2			Model 3		
Basis set	r	P	Basis set	r	P	Basis set	r	P
(1,4) {2}	-0.213	0.309	(1,4) {2}	-0.213	0.309	(1,3) {2, 9}	-0.052	0.811
(1,6) {2,4}	0.409	0.045	(1,7) {2,4}	-0.061	0.779	(1,4) {2}	-0.213	0.309
(1,7) {2,4}	-0.061	0.779	(1,8) {2,4}	-0.016	0.939	(1,7) {2,4}	-0.061	0.779
(1,8) {2,4}	-0.016	0.939	(1,9) {2,4}	-0.106	0.625	(1,8) {2,7}	0.011	0.960
(1,9) {2,4}	-0.106	0.625	(1,11) {2,6}	-0.021	0.924	(1,9) {2,4}	-0.106	0.625
(1,11) {2,6}	-0.021	0.924	(2,4) { φ }	-0.330	0.099	(1,11) {2,6}	-0.021	0.924
(2,6) {4}	-0.303	0.141	(2,6) {1}	-0.468	0.017	(2,3) {9}	-0.022	0.916
(2,7) {4}	-0.033	0.875	(2,7) {4}	-0.033	0.875	(2,4) {9}	-0.047	0.826
(2,8) {4}	-0.134	0.527	(2,8) {4}	-0.134	0.527	(2,6) {1,8,9}	-0.456	0.026
(2,9) {4}	0.260	0.210	(2,9) {4}	0.260	0.210	(2,7) {4,9}	-0.080	0.713
(2,11) {4,6}	0.028	0.895	(2,11) {6}	0.089	0.676	(2,8) {7, 9}	-0.063	0.773
(4,11) {6}	-0.165	0.432	(4,6) {1}	-0.094	0.657	(2,11) {6,9}	0.180	0.402
(6,7) {4}	0.123	0.561	(4,11) {6}	-0.165	0.432	(3,4) {9}	-0.224	0.282
(6,8) {4}	0.416	0.037	(6,7) {1,4}	0.132	0.541	(3,6) {1,8,9}	0.087	0.695
(6,9) {4}	-0.324	0.113	(6,8) {1,4}	0.431	0.033	(3,7) {4,9}	0.092	0.669
(7,8) {4}	0.556	0.003	(6,9) {1,4}	-0.337	0.106	(3,8) {7,9}	-0.008	0.970
(7,9) {4}	0.163	0.439	(7,8) {4}	0.556	0.003	(3,11) {6,9}	-0.020	0.925
(7,11) {4,6}	-0.355	0.087	(7,9) {4}	0.163	0.439	(4,6) {1,8}	-0.003	0.990
(8,9) {4}	-0.132	0.533	(7,11) {4,6}	-0.355	0.087	(4,8) {7}	0.092	0.665
(8,11) {4,6}	-0.179	0.403	(8,9) {4}	-0.132	0.533	(4,11) {6}	-0.165	0.432
(9,11) {4,6}	-0.492	0.013	(8,11) {4,6}	-0.179	0.403	(6,7) {1,4,8}	-0.143	0.518
			(9,11) {4,6}	-0.492	0.013	(6,9) {1,4,8}	-0.316	0.139
						(7,9) {4}	0.163	0.439
						(7,11) {4,6}	-0.355	0.087
						(8,9) {4,7}	-0.271	0.200
						(8,11) {6,7}	-0.019	0.931
						(9,11) {4,6}	-0.492	0.013

42 df, C= 62.62 (0.021)

44 df, C=67.09 (0.014)

54 df, C= 45.86 (0.777)

The codes of the variables of the models are: 1, Photosynthetic rate; 2, Transpiration rate; 3, ψ , leaf water potential; 4, Soil compaction (soil bulk density); 5, Plant height (not used in the models); 6, Plant area; 7, Xylem vessels proportion; 8, Xylem vessels diameter; 9, SRL, specific root length; 10, Root tissue mass density (TMD_R) (not used in the models); 11, Plant Biomass.