Soil compaction effects on growth and root traits of tobacco depend on light, water regime and mechanical stress

David Alameda [a,b,*], Niels P.R. Anten [a], Rafael Villar [b]

[a] Ecology and Biodiversity, Institute of Environmental Biology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands
[b] Área de Ecología, Dpto. Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias, Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, Spain

ABSTRACT

Soil compaction can strongly affect plant performance as many other stress factors. In nature, many combinations of different stress factors may be found. We expect that the effects of soil compaction may be different depending of the occurrence of other stress. This has not been fully investigated; most studies have included only one stress factor together with soil compaction. In this study, we combine soil compaction with the interaction of shade, low water availability and mechanical stress. We use as a model system tobacco plants (Nicotiana tabacum), in which the effects of the combination of these factors in a greenhouse experiment were studied on their growth, biomass allocation, root morphology and anatomy. Soil compaction effects on growth and root traits depended strongly on the other factors. In unstressed conditions, plant growth increased with compaction up to 1.4 g cm^{-3} bulk density and then declined. However, at low water and under mechanical stress plant growth declined monotonically with compaction, while under shade, soil compaction had no effect on growth. Soil compaction reduced fine root proportion in all treatments except in shade condition, while it increased root diameter and xylem area only under mechanical stress. These results indicate that analyses of soil compaction effects on plant performance should take the levels of other stress factors into account. More generally, they illustrate the difficulty of interpreting effects of a given stress factor on plants as these effects tend to interact with presence of other stressors.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Soil compaction is commonly considered to have a negative effect on plant growth and agricultural yields (Wolkowski, 1990; Hansen, 1996; Kozlowski, 1999). It is also considered to be an ecological factor that for instance plays a role in hampering forest succession after land clearance for pasture use (Small and McCarthy, 2002; Lawrence, 2003) while in (semi)arid regions it may aggravate the impact of overgrazing and contribute to desertification (Rietkerk et al., 2000, 2004; Castellano and Valone, 2007).

The direct impact of soil compaction is an increase of the mechanical resistance to root penetration, which makes it more difficult for plants to exploit a large soil volume. However, a major complication in understanding the effects of compaction is that its occurrence tends to be correlated with the presence of other environmental factors with which it may interact. For example, it

may decrease (more negative) the soil matric potential (Whalley et al., 2006), thus reducing water availability to plants, and can thus aggravate the effects of drought (Taylor and Ratliff, 1969). Soil compaction also tends to increase under grazing (Drewry et al., 2008), logging in forests (Small and McCarthy, 2002) or tillage in agricultural systems (Diaz-Zorita et al., 2002), all of which tend to increase light availability as well wind exposure, while grazing is also associated with mechanical stress through trampling. Agricultural and silvicultural treatments entail disturbances whereby a set of factors that can influence plant growth occur simultaneously (changes in soil compaction, light and water availability, wind, etc.) (Godefroid and Koedam, 2008). Overall this raises the question to what extent the effects of soil compaction depend on light and water availability and on the magnitude of mechanical stress (i.e. wind or trampling) to which plants are exposed.

Light is probably the most important factor affecting plant growth as it supplies the energy for photosynthesis. High light availability tends to increase the demand of plants for soil resources, so plants at high light availability could be more susceptible to changes in soil compaction, due to its key role in root–soil interaction.

* Corresponding author at: Área de Ecología, Dpto. Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias, Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, Spain. Tel.: +34 666720100.
E-mail address: dammad4@msn.com (D. Alameda).

On the other hand, in many environments plant growth is limited by water availability, which is strongly determined by the soil physical characteristics. However, under natural conditions it may be difficult to separate the effects of water limitations from other physical factors affecting root growth (Cortina et al., 2008). Soil compaction entails changes in various soil properties such as penetration resistance, porosity and bulk density. Since these properties have different and interactive effects on plants, controlled greenhouse and lab experiments are needed in which they can be quantified separately.

Mechanical stress is another stress factor affecting plant growth. Plants typically respond to mechanical stress through reduced stem elongation and increased allocation to root growth, a set of responses commonly denoted as thigmomorphogenesis (Jaffe and Forbes, 1993; Anten et al., 2005). It has also been suggested that responses to soil compaction (i.e., mechanical impediment to root growth) and externally applied mechanical force (wind, flexing or rubbing) on the plant shoot involve at least partly the same signal transduction pathway (Anten et al., 2006). It could thus be hypothesized that externally applied mechanical stress may aggravate the effects of soil compaction.

Plant responses to soil compaction are probably in the first place mediated by changes in root traits and functioning. Yet studies that combine growth analysis and measurements of root anatomy and morphology in this respect are rare. It is commonly reported that specific root length (SRL; root length per unit root mass) decreases with soil compaction (Bengough and Mullins, 1990). The activity of the phytohormones ABA and ethylene seems to be involved in regulating the responses to compaction (Hussain et al., 1999; Roberts et al., 2002; Anten et al., 2006; Whalley et al., 2006), having two main effects: changes in root morphology and shoot growth inhibition. Although plants are able to regulate shoot growth in response to a mechanical impedance signal, it is difficult to avoid side effects on water uptake. Due to compaction, root length and the amount of fine roots are reduced, while soil matric potential is decreased (more negative); both of which reduce the ability of plants to take up water. A decrease in root diameter has been suggested (Hund et al., 2009) in response to water stress. This is coherent with the theoretical considerations about hydraulic conductivity and resistance (Steudle, 2000), which assumes that roots with a smaller cortex width have lower resistances for water transport from soil to xylem vessels. Xylem vessel diameter may also decrease in order to maximize hydraulic conductivity under safety margins (Martinez-Vilalta et al., 2002). Therefore, we would expect two types of root anatomy changes in response to water stress: a cortex reduction and xylem vessels diameter reduction. Iijima and Kato (2007) addressed the difficulty to distinguish soil compaction and water stress effects on root anatomical changes because of their high interdependence and the specific response of the species studied.

In this paper we address the following questions: (i) what are the effects of soil compaction on plant performance in terms of growth, (ii) to what extent do these effects depend on light and water availability and on the degree of mechanical stress to which plants are exposed and (iii) to what extent can the effects on growth be explained by changes in root characteristics? To this end we exposed tobacco plants (*Nicotiana tabacum* L.), as a model system, to a continuous range of soil compaction together with four different conditions: control, shade, low water availability and mechanical stress. Tobacco is an extensively cultivated species with a high economic value. Soil compaction is an important factor reducing yield in this species (Tursic et al., 2008). This work attempts to use tobacco as a model system in order to describe the interaction between soil compaction and other environmental factors (light, water and mechanical stress) under an ecophysiological point of view.

2. Materials and methods

2.1. Growth facility and plant material

This experiment was carried out in a greenhouse at Utrecht University (The Netherlands; 52° 30′ N, 5° 45′ E) between June and August of 2009. We used the annual plant, tobacco (*N. tabacum* L., cv. Samson N × N), this particular cultivar reaching a maximum height of about 2.5 m. On May 18th, seeds were sown in trays in a mixture of sand and potting soil. Seedlings were grown at 30% of natural daylight, a level achieved with neutral-density shade cloth and shading by the greenhouse roof. When plants reached a fresh mass of 1.4 ± 0.6 g, they were fresh weighed and transplanted into pots. These were PVC tubes of 30 cm in height and 10.5 cm in diameter, whose base was drilled to get a good drainage of the irrigation water. At the same time, a subsample of plants ($n = 10$) were harvested, fresh weighed, dried out at 70 °C for at least three days and the dry mass was obtained. Initial dry mass ratio was calculated as the ratio of dry and fresh mass to be used to estimate the dry mass of each seedling (see Section 2.4). For the description of soil type and soil compaction treatment see Section 2.3.

The day/night temperatures in the greenhouse were set to 22 and 18 °C, respectively. For the control plants (CO) the light and water availability was the following. The light level during the experiment was 50% of natural day light and was created by the shading of the greenhouse roof. Watering was done by hand using 100 ml each time and 3 times a week for a total of 2900 ml during the experiment. This irrigation system forces plants to use soil water reserve as a function of soil compaction treatment.

2.2. Experimental design: water, light and mechanical treatments

The experiment was set up as an incomplete factorial design focusing in soil compaction as main factor, thus only compaction × other stress factors (shade; low water; mechanical stress) interactions were studied. This allows to answer our questions keeping the number of replicates under a reasonable number. Soil compaction treatments were made in order to create a continuous range of bulk density (see Section 2.3). A total amount of 15 plants, covering the whole range, were assigned to each treatment: control (CO), shade conditions (SH), low water (LW) and mechanical stress (MS). For the control treatment the plants were grown in optimal conditions of light, water (see Section 2.1) and without mechanical stress. The shade treatment was created using a cloth which transmitted just 10% of total day light. The low water treatment was established by applying 40% less water than under optimal conditions (60 ml 3 times a week). The mechanical stress treatment consisted of gently grasping the plants at the base and flexing them no more than 45° for a total of 50 flexures (see Anten et al., 2005). This treatment was done daily during the last 30 days prior to the final harvest.

2.3. Soil compaction treatments

The soil substrate was a mixture of sand, silt and gravel in a proportion 3:2:1 resulting in a sandy soil texture. This type of soil was chosen as it is most easily compacted. We added a 7% of NPK fertilizer (7:7:7) to each pot to avoid nutritional deficiencies. Different levels of soil compaction were made considering three bulk density ranges having as reference the increment of soil mass per volume unit (a cylindrical pot of 10.5 cm in diameter and a known soil height). No compaction treatment (NC) was made just filling the whole volume of the pot with soil without compacting. For the other two compaction levels, we used an electric hammer (GSH 11 E, Bosch, Germany) with a modified piston to compact with the purpose of being able to increase the soil mass per

volume. Medium compaction (MC) treatments were made applying the hammer from the top of a soil column (formed by two equal pots, one as soil receptacle and the other as "guide") of 60 cm in height 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 pot was retired and the excess soil removed resulting in a completely filled pot. The high compaction (HC) treatment was created in the same way but applying 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). The result of these three treatments was a continuous range of soil compaction.

In order to describe each compaction-treatment within each factor-treatment at harvest time it was measured for each pot: bulk density (the amount of dry soil mass per unit pot volume, estimated as a cylinder volume), penetration resistance with cone of $30°$ and $1 cm^2$ of surface (by penetrologger, Eijkelcamp, The Netherlands) and soil water content (MWC, mass water content: measured as the difference between wet soil sample weight and its dried weight in stove at 105 °C) (see Table A1).In addition, soil matric potential of each pot was calculated by a regression equation (see Fig. A1) obtained in an experiment following the filter-paper technique (Deka et al., 1995). To this purpose compaction treatment pots without plants were used. The soil matric potential values were obtained to a different water conditions over the same replicates: pots were dried by stove at 105 °C, then were added 300 ml of water to each treatment in order to obtain the Ψ_m under slight watering (SW) and finally each pot was saturated of water to obtain the Ψ_m at field capacity (see Fig. A1).

2.4. Growth and biomass allocation measurements

After 70 days of growth, height of each plant was measured from soil level to the top meristem and the number of leaves was counted. Plants were subsequently harvested and separated into stems, leaves and roots (the latter were carefully washed with water) of which fresh masses were determined. Leaf area was measured using a LICOR leaf-area meter (LI3100 LiCor, Lincoln, USA). Then stems and leaves were dried at 70 °C for at least three days to obtain leaf and stem dry biomass. A root sample was taken on fresh material for anatomy analysis (see Section 2.6), and then roots were frozen until morphological analysis (see Section 2.5), after what were dried to get the root dry biomass. Relative growth rate (RGR) was calculated as (Hunt, 1990):

$$RGR = \frac{(\text{Ln } M_2 - \text{Ln } M_1)}{(t_2 - t_1)} \quad (1)$$

$$M_1 = FM_1 \times DM_1 \quad (2)$$

where M_2 and M_1 are the final and initial dry mass of the whole seedling, respectively; $t_2 - t_1$ is the growth period (70 days); FM_1 is the initial fresh mass and DM_1 is the initial dry mass ratio.

Initial plant dry mass was obtained by the product of initial fresh mass and initial dry mass ratio (Eq. (2)). 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 following Hunt (1990).

2.5. Root morphology

After defrosting the roots, we selected for the analysis approximately 66% of the root excluding the first 5 cm, which corresponds with the stem–root connection (root base). This was done to avoid an underestimation of specific root length, as this portion has very low length per unit biomass. Then, every root sample was scanned to high resolution (600 dpi) using the Winrhizo analysis system (Winrhizo ver. 2004a, Regent Instruments Inc., Quebec, Canada). The output gives: mean root diameter, total root length, root volume and length of each diametric class distribution between 0 and 4.5 mm. The calculated variables were: specific root length (SRL) which is the ratio between root length and its dry mass; total root length as the product of SRL and root biomass (excluding the root base); tissue mass density of roots (TMD_R) as the ratio between root dry mass and root volume; and length of each diametric class. The last was expressed in percentage of total length considering only roots' length of diameter less than 1.5 mm (which represents 99% of total length).

2.6. Root anatomy

Before roots were frozen, a root sample was taken to be used for histological analysis. Approximately 1 cm of primary root from 5 cm to stem–root connection was selected and fixed in a FAA solution (formaldehyde:alcohol:acetic acid:distilled water, 10:50:5:35). Then, fixed material was cut by hand with a razor blade raised under binocular magnifying glass (Olympus SZ61, Tokyo, Japan). The slides were made on cross section of the longitudinal root axis taking about 100–200 μm in thickness. Cross section samples were stained during 1 min with astra blue–safranine solution (distilled water:astra blue:safranine, 20:1:0.5). 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. Xylem cross sectional area (CSA), proportion of xylem CSA dedicated to vessels, mean radius of xylem vessels and xylem vessels frequency (VF) as number of vessels with diameter higher than 10 μm mm^{-2} were all measured using an image analysis software (Image Pro Plus 4.5, Average Cybernetic, Inc., Silver Spring, MD, USA). Taking into account only the vessels higher than 10 μm avoids confusion with radial cells, which are often about 10 μm in diameter, and focuses in those vessels which mainly contribute to total hydraulic conductance.

2.7. Data analysis

The result of the compaction method used was a continuous range of soil compaction that allows us to use bulk density as covariable in an ANCOVA analysis. The reason of using a continuum of compaction instead a categorical approach is due to the great predictive power of regressions which can also be used to obtain predictors in ecological models (Cottingham et al., 2005). The statistical analysis was made following an ANCOVA considering the CO, SH, LW and MS treatments as categorical variables and soil compaction in terms of bulk density as the continuous predictor. A GLM (general linear model) of homogeneity of slopes procedure was made in Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA). Although the compaction treatments were made in a categorical manner, bulk density exhibited a continuous range, which does not allow us to use it as a categorical factor. In the cases in which bulk density showed a significant effect, we tested the significance of the second order polynomial term using the multiple regression module in order to determine whether relationships were linear or not. The model for each variable's response was selected in function to the highest multiple r coefficient.

3. Results

3.1. Plant growth and biomass allocation

Soil compaction had significant effects in all growth variables studied except SLA (Table 1), but interestingly, for most of the

Table 1
Results of ANCOVA analysis for the different variables studied under different treatments (categorical factor: CO, control; SH, shade; LW, low water; MS, mechanical stress) and bulk density (continuous predictor) and the interaction between treatment and bulk density.

		Treatment (CO, SH, LW, MS)	Bulk density	Treatment × bulk density	Coefficient sign			
					CO	SH	LW	MS
Plant growth and biomass allocation								
B	Biomass (g)	11.50**	15.11***	7.26*	+−	ns	−	−
H	Height (cm)	6.85*	16.80***	5.22 ns	+−	ns	−	−
DM	Dry matter (%)	13.20**	13.53***	8.21*	+−	ns	−	−
RGR$_B$	Relative growth rate on biomass (mg g^{-1} day^{-1})	13.19**	10.50***	9.75*	+−	ns	−	−
LA	Leaf area (m^2)	1.11 ns	13.14***	0.73 ns	+−	ns	−	−
LMR	Leaf mass ratio (kg kg^{-1})	8.45^a	16.68***	7.49 ns	+	ns	+	+
SMR	Stem mass ratio (kg kg^{-1})	4.80^a	14.56***	5.06^a	+−	ns	−	−
RMR	Root mass ratio (kg kg^{-1})	17.87**	5.23*	14.81**	ns	ns	+	ns
S/R	Shoot/root (kg kg^{-1})	10.95*	6.32*	9.43*	ns	ns	−	ns
SLA	Specific leaf area (m^2 kg^{-1})	26.34***	0.22 ns	8.87*	+−	ns	ns	ns
Root anatomy and morphology								
XCSA	Xylem CSA (mm^2)	7.16 ns	4.14 ns	6.19 ns	ns	ns	ns	+
RXV	Mean radius of xylem vessels (μm)	2.96 ns	0.46 ns	2.93 ns	ns	ns	ns	ns
PXV$_{CSA}$	Proportion of xylem CSA dedicated to vessels (%)	6.16 ns	3.10 ns	6.10 ns	ns	ns	−	ns
XVF	Xylem vessels frequency (vessels mm^{-2})	12.70**	7.31**	11.83*	ns	−	ns	ns
RL	Root length (cm)	21.07***	13.57***	15.54***	−	ns	−	ns
SRL	Specific root length (m g^{-1})	10.94*	3.78^a	8.65^a	ns	ns	−	ns
TMD$_R$	Tissue mass density of root (g cm^{-3})	26.26***	6.71**	20.57***	ns	ns	ns	−
LDC$_{0.5}$	Fine roots (length diameter class < 0.5 mm) (%)	2.10 ns	6.08***	2.76^a	−	ns	−	−
MRD	Mean root diameter (mm)	8.00 ns	9.97**	10.41^a	ns	ns	ns	+

The values represent the percentage of explained variance by each factor calculated as (SS$_x$/SS$_{total}$) for each model: ns, non significant; ^{a}0.05 < P < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001. The coefficient sign means the sign of the coefficient of the linear equation of the *y* variable with respect to bulk density for each treatment. For CO, the two signs mean the signs of the linear and quadratic term of a polynomial equation, respectively.

variables the pattern of the response to soil compaction depended on the treatment considered (i.e. a significant interaction term "Treatment × Bulk density" was found) (Table 1, Fig. 1). In the CO treatment, the responses of biomass, height, leaf area and dry matter content to soil compaction showed an optimum curve, with values increasing, up to a bulk density of about 1.4 g cm^{-3} and then declining (Fig. 1a–d). In the SH treatment there was no significant effect of soil compaction on any of the growth variables (Fig. 1e–h). Finally, under LW and MS treatments all growth variables declined linearly with soil compaction (Fig. 1i–p).

Soil compaction showed significant effects on biomass allocation in all treatments but these effects again tended to differ between the CO, SH, LW and MS treatments (Table 1). The leaf mass ratio (LMR) increased while the stem mass ratio (SMR) declined with soil compaction in all treatments except the SH treatment (Table 1, Fig. 2). The root mass fraction (RMR) showed a significant increase with soil compaction only in the LW treatment.

3.2. Root morphology and anatomy

Soil compaction had significant effects in all root morphology variables (Table 1). As in the case of the plant growth variables, the response of root morphology to soil compaction depended on the treatment considered (i.e. a significant interaction term was found) (Table 1, Fig. 3). For example, in the CO and LW treatments, root length decreased linearly with soil compaction (Fig. 3). However, in the SH and MS treatments this effect was not observed. Specific root length showed a negative trend with soil compaction, which was only significant in the LW treatment (Table 1). Root tissue mass density decreased linearly with soil compaction in the MS treatment, while in the other treatments there was no significant relationship (Table 1). On average, MS plants had considerable higher root mass density and lower SRL values than the CO, SH and LW plants [Appendix A, Table A1]. The proportion of fine roots and the mean root diameter were negatively affected by soil compaction independent in all categorical treatments (Table 1). In general, soil compaction

resulted in plants producing relatively fewer roots in the smaller diameter classes and more in the larger ones.

Surprisingly, in general, none of the factors in our experiment exerted a strong effect on root anatomy (Table 1). Xylem cross section area (XCSA), mean radius of xylem vessels (RXV) and proportion of XCSA dedicated to vessels (PXV$_{CSA}$) were not significantly affected by any of the experimental conditions. However, xylem vessels frequency (XVF) was smaller in MS plants than in those of the other treatments. The effect of soil compaction on XVF differed between treatments, being negative in the shaded plants and non significant in the others treatments (Fig. 4).

4. Discussion

This study shows that the effect of soil compaction on plant growth and related traits strongly depends on the levels of light, water availability and mechanical stress to which plants are exposed. Most studies on soil compaction consider this factor in isolation, and only a few studies (Small and McCarthy, 2002; Bejarano et al., 2010) have analyzed its interaction only with light availability. As noted in the introduction, in nature soil compaction can be strongly correlated with other environmental factors. Disturbances that commonly bring about soil compaction such as logging, grazing and agriculture often entail reductions in water storage and availability, and increased light exposure (logging and grazing) and mechanical stress (trampling and wind exposure). Therefore, the effects of soil compaction on plant performance and ecosystem functioning will vary both in time and space and will be hard to elucidate by means of field studies alone.

4.1. The positive effect of moderate soil compaction

In the control treatment (i.e., no limitation of water, nutrient or light and absence of mechanical stress) growth increased with soil compaction up to bulk density values of about 1.4 g cm^{-3} and then declined. This indicates that at low to intermediate values, soil compaction can stimulate growth. This result is contrary to the general notion that soil compaction negatively affects plant
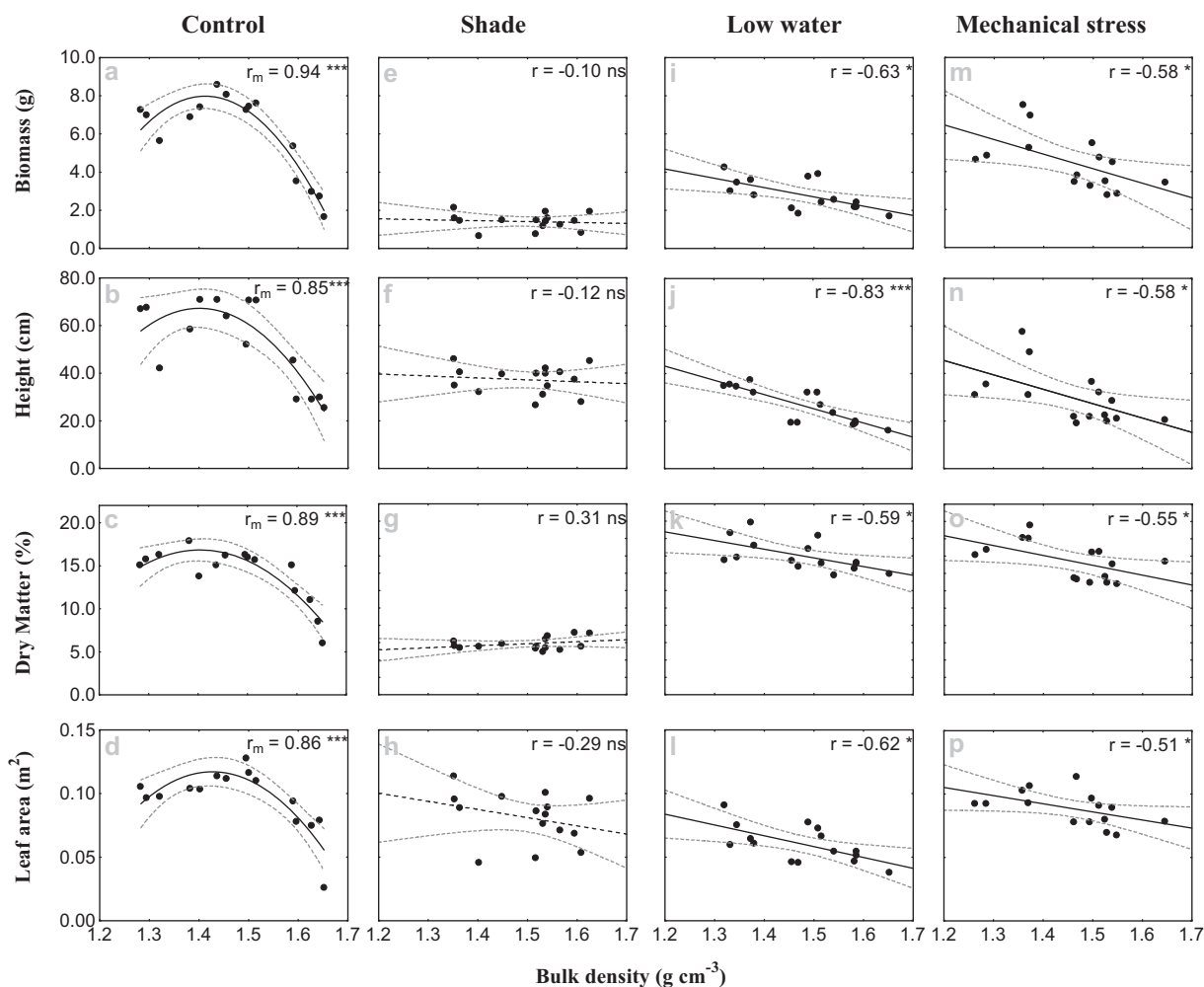
Fig. 1. Soil bulk density effects on growth variables of tobacco seedlings under different treatments: control (a–d), shade (e–h), low water (i–l) and mechanical stress (m–p). Regression coefficients are shown with their signification expressed by: ns, non significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. r_m in panels (a)–(d) means multiple regression coefficient for polynomial models. Dry matter percentage is dry mass/fresh mass × 100 ($n = 15$).

performance (Kozlowski, 1999), but it is consistent with some other studies (Carter, 1990; Håkansson, 1990; Alameda and Villar, 2009; Bejarano et al., 2010). This positive effect could be due to a better root–soil contact, which can improve nutrient uptake (Arvidsson, 1999; Kooistra et al., 1992). Moreover, compaction may increase mass flow transport by increasing the hydraulic conductivity (Kemper et al., 1971). However, these effects may depend on soil texture (Whalley et al., 2008) and the species studied (Godefroid and Koedam, 2004; Alameda and Villar, 2009).

On the other hand, negative effects of compaction on growth at higher bulk densities values can be explained as the results of root morphology distortion (Chassot and Richner, 2002). Our results show that high compaction levels diminish total root length and the proportion of fine roots, and that these changes are associated with an increase in mean root diameter. So, negative effects of soil compaction could follow a logical sequence: root distortion, above growth inhibition, architecture changes and root anatomic acclimatization (Holste et al., 2006).

4.2. Soil compaction under shade conditions

In our study, shade induced a strong reduction in plant growth compared with control conditions (−76%) and a change in biomass allocation (i.e., an increase in specific leaf area), a result that is commonly observed (Quero et al., 2006; Poorter, 2009).

Despite our shading treatment was extreme and may not simulate shade conditions on arable lands, this was intended rather to know the physiological response of a plant model (tobacco). This severe shading can be found in the understory of natural forest where soil compaction is commonly present; Small and McCarthy (2002) found similar values of light reduction under close canopy. Intriguingly, among the shade plants, soil compaction did not have a significant effect on growth. This could be explained by the fact that under shaded conditions growth shifts from being limited by belowground resources to being limited by light. Effects of soil compaction are largely mediated through their effects on root growth affecting water and nutrient uptake. Under extreme shade conditions, water and nutrients demands are strongly reduced which summed to reduced carbohydrate supply might explained the lack of soil compaction effects. Consistent with our results, Bejarano et al. (2010) found that under high light conditions growth of seedlings of a woody species (Quercus pyrenaica) increased with soil compaction but that at low light there was no significant relationship of soil compaction on growth. Similarly, Small and McCarthy (2002) reported interactive effects between light availability and soil compaction in some forest herb species although not in all species. The direction of the interactive effect also differed between species: e.g. in Geum canadense soil compaction exerted a stronger negative on growth at high than at low light but the reverse held for Osmoryza claytonii.
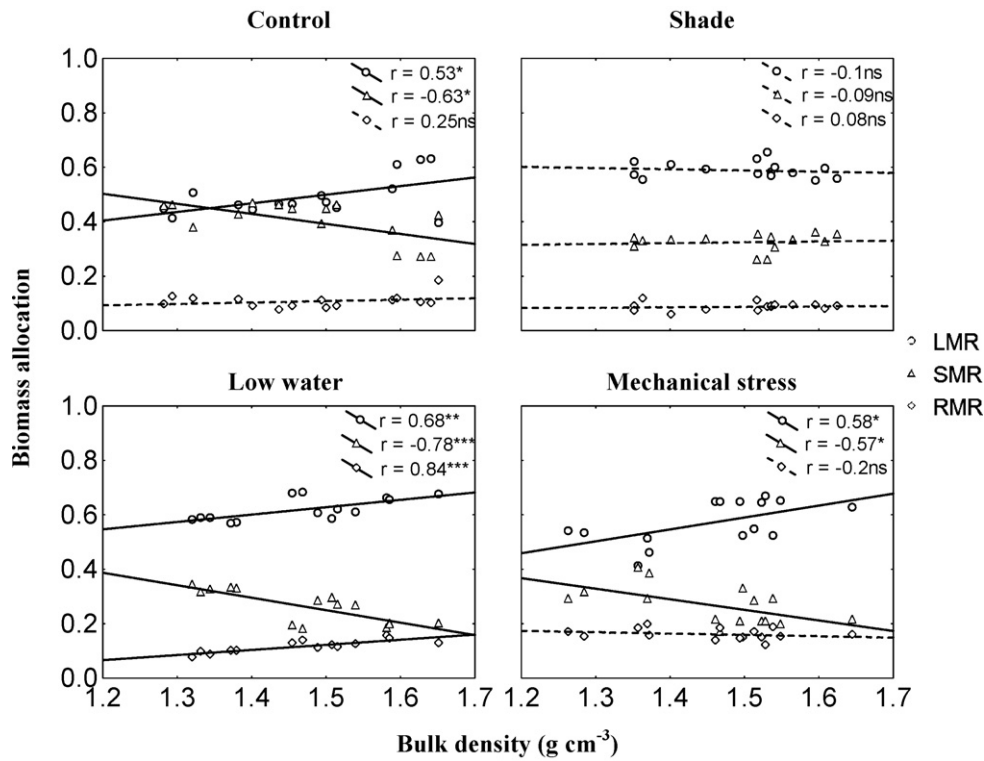
Fig. 2. Biomass allocation for tobacco seedlings under control, shade, low water and mechanical stress conditions at different levels of soil compaction (expressed as bulk density). LMR, leaf mass ratio. SMR, stem mass ratio. RMR, root mass ratio. r, regression coefficient. Signification level is expressed by: ns, non significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ ($n = 15$).

Disturbances such as logging and grazing tend to increase both light availability and soil compaction (Lawrence, 2003), and together our results and those of Small and McCarthy (2002) and Bejarano et al. (2010) indicate that more research is needed to clearly elucidate the degree to which these two factors interact.

4.3. Low water and mechanical stress aggravated the negative effects of soil compaction

As expected, low water availability negatively affected growth. Interestingly, low water aggravated the negative effects of soil
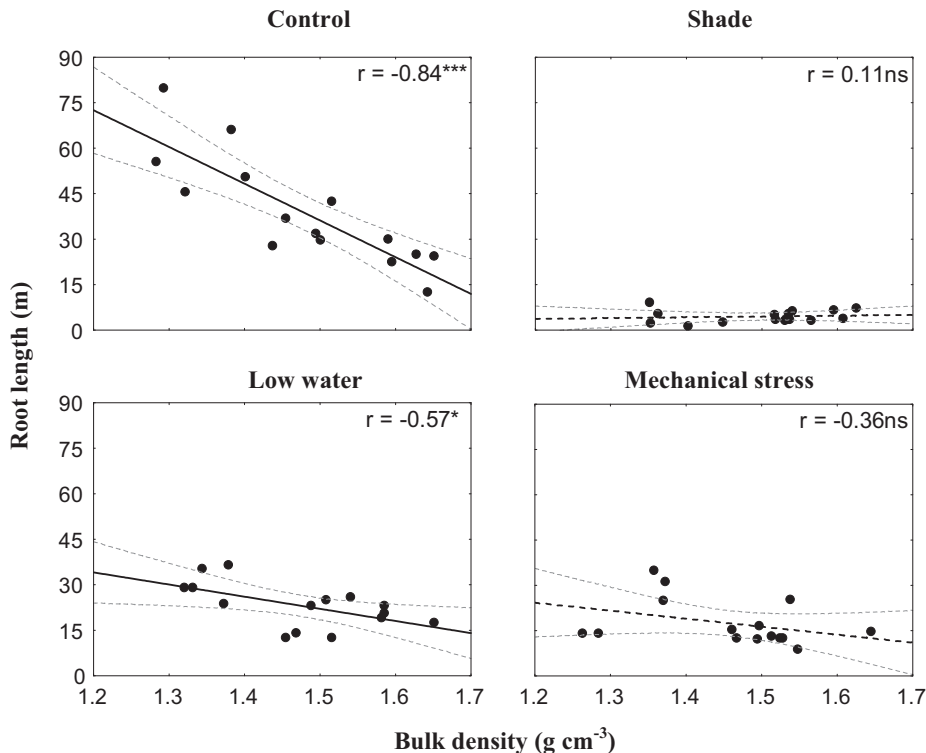

Fig. 3. Root length as a function of soil compaction (expressed as bulk density) under control, shade, low water and mechanical stress treatment conditions. r, regression coefficient. Signification level is expressed by: ns, non significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ ($n = 15$).
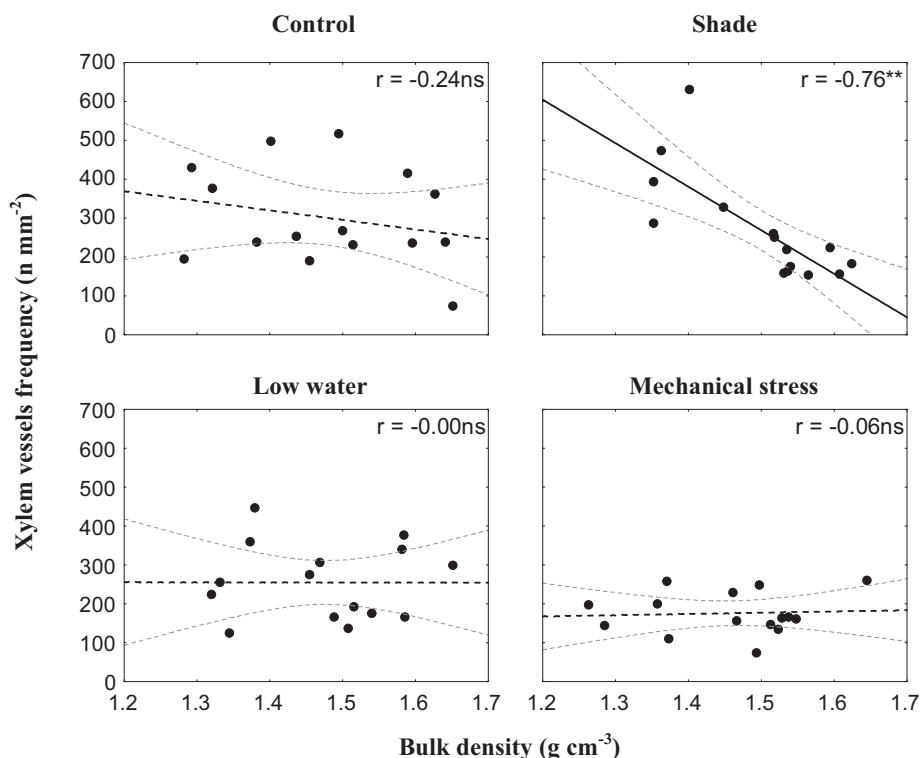
Fig. 4. Xylem vessel frequency as a function of soil compaction (expressed as bulk density) under control, shade, low water and mechanical stress treatment conditions. *r*, regression coefficient. Signification level is expressed by: ns, non significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ ($n = 15$).

compaction; the relationship between growth and soil compaction shifted from an optimum curve to a linearly declining function. The relationship between soil water content and water availability to the plant is strongly determined by physical properties of the soil and thus also by the degree of compaction: soil compaction usually decreases the soil matric potential under low water availability condition (see Fig. A1) (Taylor and Ratliff, 1969; Fekete et al., 1975). In addition, soil compaction entails a greater mechanical impediment to root penetration, which was reflected in the reduced root length and specific root length with soil compaction (Atwell, 1993; Bejarano et al., 2010). As a result plants can explore smaller soil volumes which are particularly detrimental when soil resources are already limiting (Zegada-Lizarazu et al., 2006). The negative effect of soil compaction on root length, which can negatively affect survivorship of plants in drought period (Lloret et al., 1999; Padilla and Pugnaire, 2007), should also be considered.

Under dry conditions, narrower xylem vessels might be favorable as they are less prone to cavitation, though they also have lower water conductance (e.g. Martinez-Vilalta et al., 2002). However, we found that root anatomy traits were not much affected by either low water availability or compaction. Plasticity in root anatomy differs considerably between species (Hernandez et al., 2009). For example, Cortina et al. (2008) found almost no changes in root characteristics of *Pistacia lentiscus* plants under contrasting water supply. It is possible that tobacco exhibits a similar low plasticity in these traits.

The mechanical stress treatment produced a small but significant growth reduction. Biomass, height and relative growth rate were lower than control plants. Mechanical stress also induced a reduction in biomass allocation to stems in favor of root and leaf investment. These responses are consistent with previous studies (Niklas, 1992; Cipollini, 1999), and it is generally considered that shorter plants with relatively large root systems are resistant to mechanical failure (Goodman and Ennos, 1996; Niklas et al., 2002; Anten et al., 2006).

Mechanical stability can be further affected by the mechanical properties of roots. In our study, plants subjected to mechanical stress had thicker roots with larger xylem cross sectional area, a lower xylem vessel frequency and a higher dry mass density than plants from the other treatments. Consistent with this pattern, Scippa et al. (2008) found a lignin increase in roots of plants subjected to mechanical stress, which could be explained by a higher xylem proportion which is based mainly on lignin. All these factors may contribute to a larger resistance to rupture of individual roots in plants subjected to mechanical stress as observed by Niklas (1992).

As with low water, the presence of mechanical stress aggravated the negative effects of soil compaction, the relationship between growth and soil compaction shifting from an optimum curve in the control plants to a linear declining relationship in the plants subjected to mechanical stress. Responses of plants to mechanical impediments to root growth as occurs as a result of soil compaction and externally applied mechanical stress to the shoots (wind, trampling or flexure) may entail at least partially overlapping signal transduction pathways (Anten et al., 2006). This is evident from studies whereby wild type (WT) and ethylene insensitive transgenic plants were exposed to either soil compaction (tomato, Hussain et al., 1999) or flexing (tobacco, Anten et al., 2006). In both cases shoot growth was inhibited in WT but not in the ethylene insensitive transgenics, suggesting an involvement of ethylene in both responses.

5. Conclusion

This study shows that plant growth responses to soil compaction interact with the effects of shading, water limitation and

mechanical stress. Both natural and anthropogenic factors that induce soil compaction tend to also change the levels of these other factors. For example, overgrazing and trampling in semi arid regions results in reduced vegetation cover, more wind exposure, greater water surface run off and thus lower water availability; model analyses indicate that this feedback mechanism contributes to vegetation patchiness and collapse. Our result that mechanical stress or water limitations aggravate the negative effects of soil compaction may strengthen this feedback mechanism. Similarly, our results suggest that potential interactive effects between soil compaction and light and water availability should be considered when analyzing forest secondary succession in relation to prior land use.

Acknowledgements
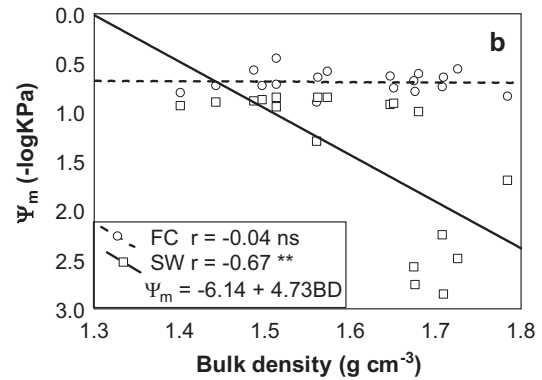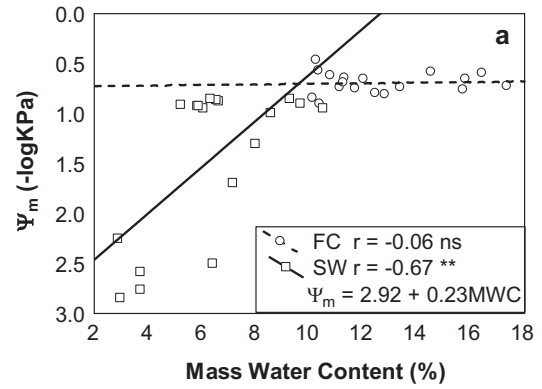
Appendix A

Fig. A1. Relation between soil matric potential, mass water content and bulk density under two water conditions: field capacity (FC) and slight watering (SW). Signification level is expressed by: ns, non significant; * $P < 0\,05$; ** $P < 0.01$.

Table A1

Mean ± SD values of plant and soil variables for each treatment ($n = 15$). Due to some variables are affected by soil compaction the mean value has to be taken in relation to the ANCOVA analysis shown in Table 1.

	Control	Shade	Low water	Mechanical stress
Plant variables				
Biomass (g)	5.96 ± 2.2	1.41 ± 0.43	2.82 ± 0.81	4.47 ± 1.41
Height (cm)	52.87 ± 17.79	37.2 ± 5.86	26.77 ± 7.6	29.86 ± 11.26
Dry matter (%)	14.06 ± 3.27	5.87 ± 0.68	16.01 ± 1.78	15.42 ± 2.19
RGR_b (mg g^{-1} day^{-1})	68.28 ± 10.6	48.64 ± 4.47	56.9 ± 6.34	63.41 ± 5.97
Leaf area (m^2)	0.1 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.09 ± 0.01
LMR	0.49 ± 0.07	0.59 ± 0.03	0.62 ± 0.04	0.57 ± 0.08
SMR	0.4 ± 0.07	0.32 ± 0.03	0.26 ± 0.06	0.27 ± 0.07
RMR	0.11 ± 0.03	0.09 ± 0.01	0.12 ± 0.02	0.16 ± 0.02
S/R	8.7 ± 1.86	10.8 ± 2.09	7.84 ± 1.92	5.3 ± 0.8
SLA (m^2 kg^{-1})	34.61 ± 4.46	99.9 ± 8.71	35.02 ± 3.23	36.33 ± 3.07
XCSA (mm^2)	0.08 ± 0.07	0.09 ± 0.04	0.07 ± 0.03	0.12 ± 0.08
Mean radius XV (μm)	23.37 ± 6.71	22.28 ± 3.35	20.34 ± 7.88	19.91 ± 4.95
XCSA dedicated to XV (%)	26.25 ± 11.35	32.59 ± 5.87	25.7 ± 6.15	27.7 ± 7.16
XVF (number mm^{-2})	300 ± 125	269 ± 136.83	255 ± 97.41	176 ± 54.77
RL (m)	38.65 ± 18.13	4.5 ± 2.12	23.05 ± 7.38	17.44 ± 7.78
SRL (m kg^{-1})	109.39 ± 27.83	101.93 ± 44.97	110.52 ± 43.83	30.81 ± 6.56
TMD_R (g cm^{-3})	0.1 ± 0.02	0.18 ± 0.05	0.1 ± 0.04	0.33 ± 0.19
Fine roots < 0.5 mm (%)	86.36 ± 4.85	90.75 ± 2.04	83.66 ± 7.14	83.4 ± 6.39
Mean root diameter (mm)	0.35 ± 0.06	0.28 ± 0.04	0.38 ± 0.09	0.39 ± 0.09
Soil variables				
Bulk density (g cm^{-3})	1.48 ± 0.13	1.5 ± 0.09	1.48 ± 0.11	1.46 ± 0.11
Mass water content (%)	3.5 ± 1.09	7.26 ± 1.52	2.65 ± 0.34	3.56 ± 0.96
Penetration resistance (MPa)	3.14 ± 0.6	2.24 ± 1.23	–[b]	3.18 ± 1
Matric potential[a] (−log kPa)	2.12 ± 0.25	1.24 ± 0.35	2.31 ± 0.08	2.1 ± 0.22

Abbreviations: RGR_b: relative growth rate on biomass basis. SLA: specific leaf area. LMR: leaf mass ratio. SMR: stem mass ratio. RMR: root mass ratio. S/R: shoot–root ratio. LAR: leaf area ratio. XCSA: xylem cross section area. XV: xylem vessels. XVF: xylem vessels frequency. RL: root length. SRL: specific root length. TMD_R: tissue mass density of roots.

[a] Matric potential values were obtained by a regression equation between mass water content and soil matric potential values measured in a sub sample of pots without plants (see Section 2.3).

[b] Penetration resistance in low water treatments raised values not measurable (>5 MPa).

References

Alameda, D., Villar, R., 2009. Moderate soil compaction: implications on growth and architecture in seedlings of 17 woody plant species. Soil & Tillage Research 103, 325–331.

Anten, N.P.R., Casado-Garcia, R., Nagashima, H., 2005. Effects of mechanical stress and plant density on mechanical characteristics, growth, and lifetime reproduction of tobacco plants. American Naturalist 166, 650–660.

Anten, N.P.R., Casado-Garcia, R., Pierik, R., Pons, T.L., 2006. Ethylene sensitivity affects changes in growth patterns, but not stem properties, in response to mechanical stress in tobacco. Physiologia Plantarum 128, 274–282.

Arvidsson, J., 1999. Nutrient uptake and growth of barley as affected by soil compaction. Plant and Soil 208, 9–19.

Atwell, B.J., 1993. Response of roots to mechanical impedance. Environmental and Experimental Botany 33, 27–40.

Bejarano, M.D., Villar, R., Murillo, A.M., Quero, J.L., 2010. Effects of soil compaction and light on growth of Quercus pyrenaica Willd. (Fagaceae) seedlings. Soil & Tillage Research 110, 108–114.

Bengough, A.G., Mullins, C.E., 1990. Mechanical impedance to root-growth – a review of experimental-techniques and root-growth responses. Journal of Soil Science 41, 341–358.

Carter, M.R., 1990. Relative measures of soil bulk density to characterize compaction in tillage studies on fine sandy loams. Canadian Journal of Soil Science 70, 425–433.

Castellano, M.J., Valone, T.J., 2007. Livestock, soil compaction and water infiltration rate: evaluating a potential desertification recovery mechanism. Journal of Arid Environments 71, 97–108.

Chassot, A., Richner, W., 2002. Root characteristics and phosphorus uptake of maize seedlings in a bilayered soil. Agronomy Journal 94, 118–127.

Cipollini, D.F., 1999. Costs to flowering of the production of a mechanically hardened phenotype in Brassica napus L. International Journal of Plant Sciences 160, 735–741.

Cortina, J., Green, J.J., Baddeley, J.A., Watson, C.A., 2008. Root morphology and water transport of Pistacia lentiscus seedlings under contrasting water supply: a test of the pipe stem theory. Environmental and Experimental Botany 62, 343–350.

Cottingham, K.L., Lennon, J.T., Brown, B.L., 2005. Knowing when to draw the line: designing more informative ecological experiments. Frontiers in Ecology and the Environment 3, 145–152.

Deka, R.N., Wairiu, M., Mtakwa, P.W., Mullins, C.E., Veenendaal, E.M., Townend, J., 1995. Use and accuracy of the filter-paper technique for measurement of soil matric potential. European Journal of Soil Science 46, 233–238.

Diaz-Zorita, M., Duarte, G.A., Grove, J.H., 2002. A review of no-till systems and soil management for sustainable crop production in the subhumid and semiarid Pampas of Argentina. Soil & Tillage Research 65, 1–18.

Drewry, J.J., Cameron, K.C., Buchan, G.D., 2008. Pasture yield and soil physical property responses to soil compaction from treading and grazing – a review. Australian Journal of Soil Research 46, 237–256.

Fekete, A., Baganz, K., Helbig, W., 1975. Some observations on soil compaction under a tire. Journal of Terramechanics 12, 217–223.

Godefroid, S., Koedam, N., 2004. Interspecific variation in soil compaction sensitivity among forest floor species. Biological Conservation 119, 207–217.

Godefroid, S., Koedam, N., 2008. Using high resolution mapping of disturbance indicator species to assess the sustainability of silviculture activities. Forest Ecology and Management 255, 3416–3423.

Goodman, A.M., Ennos, A.R., 1996. A comparative study of the response of the roots and shoots of sunflower and maize to mechanical stimulation. Journal of Experimental Botany 47, 1499–1507.

Håkansson, I., 1990. A method for characterizing the state of compactness of the plow layer. Soil & Tillage Research 16, 105–120.

Hansen, S., 1996. Effects of manure treatment and soil compaction on plant production of a dairy farm system converting to organic farming practice. Agriculture Ecosystems and Environment 56, 173–186.

Hernandez, E.I., Milagrosa, A., Luis, V.C., Llorca, M., Chirino, E., Vallejo, V.R., 2009. Root hydraulic conductance, gas exchange and leaf water potential in seedlings of Pistacia lentiscus L. and Quercus suber L. grown under different fertilization and light regimes. Experimental and Environmental Botany 67, 267–276.

Holste, E.K., Jerke, M.J., Matzner, S.L., 2006. Long-term acclimatization of hydraulic properties, xylem conduit size, wall strength and cavitation resistance in Phaseolus vulgaris in response to different environmental effects. Plant Cell and Environment 29, 836–843.

Hund, A., Ruta, N., Liedgens, M., 2009. Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. Plant and Soil 318, 311–325.

Hunt, R., 1990. Basic Growth Analysis. Unwin Hyman Ltd., London, p. 112.

Hussain, A., Black, C.R., Taylor, I.B., Roberts, J.A., 1999. Soil compaction: a role for ethylene in regulating leaf expansion and shoot growth in tomato. Plant Physiology 121, 1227–1237.

Iijima, M., Kato, J., 2007. Combined soil physical stress of soil drying, anaerobiosis and mechanical impedance to seedling root growth of four crop species. Plant Production Science 10, 451–459.

Jaffe, M.J., Forbes, S., 1993. Thigmomorphogenesis: the effect of mechanical perturbation on plants. Journal of Plant Growth Regulation 12, 313–324.

Kemper, W.D., Stewart, B.A., Porter, L.K., 1971. Effects of compaction on soil nutrient status. In: Barnes, K.K., Charleton, W.M., Taylor, H.M., Throckmorton, R.I., Vanden Berg, G.E. (Eds.), Compaction in Agricultural Soils. Am. Soc. Agr. Eng., St. Joseph, MI, pp. 178–189.

Kooistra, M.J., Schoonderbeek, D., Boone, F.R., Veen, B.W., Vannoordwijk, M., 1992. Root–soil contact of maize, as measured by a thin-section technique. 2. Effects of soil compaction. Plant and Soil 139, 119–129.

Kozlowski, T.T., 1999. Soil compaction and growth of woody plants. Scandinavian Journal of Forest Research 14, 596–619.

Lawrence, D., 2003. The response of tropical tree seedlings to nutrient supply: meta-analysis for understanding a changing tropical landscape. Journal of Tropical Ecology 19, 1–12.

Lloret, F., Casanova, C., Peñuelas, J., 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. Functional Ecology 13, 210–216.

Martinez-Vilalta, J., Prat, E., Oliveras, I., Pinol, J., 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia 133, 19–29.

Niklas, K.J., 1992. Plant Biomechanics. University of Chicago Press, Chicago, IL, USA.

Niklas, K.J., Molina-Freaner, F., Tinoco-Ojanguren, C., Paolillo, D.J., 2002. The biomechanics of Pachycereus pringlei root systems. American Journal of Botany 89, 12–21.

Padilla, F.M., Pugnaire, F.I., 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Functional Ecology 21, 489–495.

Poorter, L., 2009. Leaf traits show different relationships with shade tolerance in moist versus dry tropical forests. New Phytologist 181, 890–900.

Quero, J.L., Villar, R., Marañon, T., Zamora, R., 2006. Interactions of drought and shade effects on seedlings of four Quercus species: physiological and structural leaf responses. New Phytologist 170, 819–834.

Rietkerk, M., Ketner, P., Burger, J., Hoorens, B., Olff, H., 2000. Multiscale soil and vegetation patchiness along a gradient of herbivore impact in a semi-arid grazing system in West Africa. Plant Ecology 148, 207–224.

Rietkerk, M., Dekker, S.C., de Ruiter, P.C., van de Koppel, J., 2004. Self-organized patchiness and catastrophic shifts in ecosystems. Science 305, 1926–1929.

Roberts, J.A., Hussain, A., Taylor, I.B., Black, C.R., 2002. Use of mutants to study long-distance signalling in response to compacted soil. Journal of Experimental Botany 53, 45–50.

Scippa, G.S., Trupiano, D., Rocco, M., Di Iorio, A., Chiatante, D., 2008. Unravelling the response of poplar (Populus nigra) roots to mechanical stress imposed by bending. Plant Biosystems 142, 401–413.

Small, C.J., McCarthy, B.C., 2002. Effects of simulated post-harvest light availability and soil compaction on deciduous forest herbs. Canadian Journal of Forest Research 32, 1753–1762.

Steudle, E., 2000. Water uptake by plant roots: an integration of views. Plant and Soil 226, 45–56.

Taylor, H.M., Ratliff, L.F., 1969. Root elongation rates of cotton and peanuts as a function of soil strength and soil water content. Soil Science 108, 113.

Tursic, I., Husnjak, S., Zalac, S., 2008. Soil compaction as one of the causes of lower tobacco yield in the Republic of Croatia. Cereal Research Communications 36 (Part 2, Suppl. S), 687–690.

Whalley, W.R., Clark, L.J., Gowing, D.J.G., Cope, R.E., Lodge, R.J., Leeds-Harrison, P.B., 2006. Does soil strength play a role in wheat yield losses caused by soil drying? Plant and Soil 280, 279–290.

Whalley, W.R., Watts, C.W., Gregory, A.S., Mooney, S.J., Clark, L.J., Whitmore, A.P., 2008. The effect of soil strength on the yield of wheat. Plant and Soil 306, 237–247.

Wolkowski, R.P., 1990. Relationship between wheel-traffic-induced soil compaction, nutrient availability and crop growth – a review. Journal of Production Agriculture 3, 460–469.

Zegada-Lizarazu, W., Izumi, Y., Iijima, M., 2006. Water competition of intercropped pearl millet with cowpea under drought and soil compaction stresses. Plant Production Science 9, 123–132.