Winter cereal root growth and aboveground-belowground biomass ratios as affected by site and tillage system in dryland Mediterranean conditions

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Abstract

Background and Aims.

Understanding the interaction between crop roots and management and environmental factors can improve crop management and agricultural carbon sequestration. The objectives of this study were to determine the response of winter cereal root growth and aboveground-belowground biomass ratios to tillage and environmental factors in the Mediterranean region and to test an alternative approach to determine root surface area.

Methods

Winter cereal root growth and biomass ratios were studied in three sites with different yield potential according to their water deficit (high yield potential, HYP; medium yield potential, MYP; low yield potential, LYP) in the Ebro Valley (NE Spain). At all sites three tillage systems were compared (CT, conventional tillage; MT, minimum tillage; NT, no-tillage). Root surface density (RSD), soil water content, yield components and grain yield were quantified and shoot-to-root and grain-to-root ratios were calculated. RSD was measured with the use of image analysis software comparing its performance to a more common intersection method.

Results

Significant differences on RSD between sites with different yield potential were found being the greatest at the HYP site and the lowest at the LYP one. Shoot-to-root ratio was 2.7 and 4.6 times greater at the HYP site than at the MYP and LYP sites, respectively. Moreover, the grain-to-root ratio was significantly affected by site, with a ratio that increased with yield potential. Tillage had no significant effects on RSD at any of the sites studied; however, tillage did affect grain yield, with NT having the greatest yields.

Conclusions

This study shows that in the Mediterranean dryland agroecosystems, winter cereals relative above- and belowground biomass growth is strongly affected by the yield potential of each area. NT in the Mediterranean areas does not limit cereal root growth and leads to greater grain yields. A highly significant linear relationship ($P<0.001$; r^2: 0.77) was observed between the root surface values obtained with the free-software image analysis method and the most common intersection method, showing it to be a reliable method for quantifying root density.

Keywords

Plant biomass ratios; Mediterranean; Roots; Tillage.

Introduction

Roots play a major role in plant anchorage, water and nutrients uptake, storage of carbohydrates, synthesis of growth regulators and organic carbon input into the soil (Xu and Juma, 1992). However, roots are rarely evaluated in agronomic studies and are usually estimated from aboveground biomass based on ratios obtained from the literature (Campbell and de Jong 2001). As a consequence, there is a need for a better understanding of the interaction between crop root systems and the growing environment in order to increase crop performance (McMichael and Quisenberry 1993). Plant roots are the link between belowground resources and aboveground growth. Their plasticity in different environments is well recognized, being one of the most desirable traits of crop production (Benjamin and Nielsen 2006; von Arx et al. 2012). The spatial and temporal pattern of root growth determines the uptake of water and nutrients (Brown et al. 1987; Qin et al. 2004). Regarding the necessity of assessment ofglobal warming and the potential for C sequestration of the soil, the requirement of more accurate estimates of belowground C inputs through roots, exudates and other root-derived organic material have also increased the interest on root characterization and quantification (Bolinder et al. 2007). Environmental factors that impact the development of root systems include soil temperature, soil strength, soil atmosphere, water content, soil pH, nutrient status, photosynthesis rate and growth stage (Mc Michael and Quisenberry, 1993).

Shoot-to-root ratios vary widely with environment and management (Xu and Juma 1992; Bolinder et al. 1997; Álvaro-Fuentes et al. 2008) and better estimates of root growth are needed to evaluate the contribution of agriculture to the global C budget

(Prince et al, 2001). Also ontogeny and size plays a major role on C allocation within plant fractions (Andrews et al. 1999; Poorter and Nagel, 2000). As Poorter and Nagel (2000) pointed out, larger plants invest a larger fraction of their biomass in support structure, and have a larger leaf area due to the increase in self-shading. Passioura (1983) suggested that there is an optimum shoot-to-root ratio at which above-ground biomass is maximal for a given water supply. The identification of the agricultural management practices that optimize that ratio for every environmental condition is of a vital importance to increase crop productivity.

In the Mediterranean region water is the most limiting factor and strong efforts have been made to optimize management and maximize the efficiency of water use (Cantero-Martínez et al. 2007). The Ebro Valley (Northeast Spain) is representative of a wide area of the Mediterranean region, presenting a sharp difference in annual precipitation from the center (300 mm) to the surrounding northern and southern mountains (800 mm) (Vicente-Serrano and Beguería-Portugués 2003). Soil management and N fertilization practices have a strong influence on the use of water during crop growth (Cantero-Martínez et al. 2003; Morell et al. 2011b). Cantero-Martínez et al. (2007) tested the performance of no-tillage on crops yield and water use efficiency in different climatic conditions. They observed an increase in grain yields under NT in the driest conditions, but not in wetter conditions. While root development was likely an important factor in that study, it was not measured. In a tillage systems comparison, Lampurlanés et al. (2001) concluded that NT favors root growth by greater and deeper water accumulation in the soil profile. In the same area, Morell et al. (2011a) quantified the effects of tillage and N fertilization on root length density. They concluded that NT presents greater root growth than conventional intensive tillage during dry years due to higher soil water content, while in wet years opposite results were found. In the four year period that they measured, N fertilization affected slightly root length density. Also, Muñoz-Romero et al. (2010) studied the effect of tillage on root length density in the Southern-Spain Vertisols. They found greater root length under CT than under NT in the upper soil layer. However, they found higher grain yields under NT. The authors pointed out the role played by the higher penetration resistance found under NT in the Vertisols as the main cause of the reduced root growth in soil surface when this type of soil management was performed. Different authors have reported limitations on root growth when using soil management conservation techniques such as minimum tillage and no-tillage that could lead to a reduction in grain yield (Pietola 2005; Munkholm et al. 2008).

One reason root growth is rarely measured is that extraction and measurement is time and labor intensive. Much effort has been devoted to improve the quantification of root length, surface or architecture (Himmelbauer et al. 2004). A broad range of techniques have been proposed for root characterization and quantification (Böhm 1979), but most of the methods are still tedious and many are vulnerable to operator subjectivity (Benjamin and Nielsen 2004; Blouin et al. 2007). Once washed from soil, different methods have been used to measure root characteristics such as length, surface or diameter. Newman (1966) proposed a method to estimate total root length in a sample by counting the number of intercepts of roots in an area with randomly located lines. Newman’s method has been the most widely used, as evidenced by the large number of citations found in the scientific literature. However, the advances in image analysis systems have led to automated techniques to measure root length and area (Benjamin and Nielsen 2004). Although commercial image analysis systems specifically designed for root studies already exist, their cost can be prohibitive. License-free open source general purpose image analysis software, like ImageJ (Abramoff et al. 2004), can be customised for root measurements and offer an inexpensive alternative to commercial software. Kimura and Yamasaki (2001) developed an algorithm for the ImageJ software to estimate the length versus the diameter distribution of root samples. However, Kokko et al. (1993) indicated that the determination of total root surface area provides a better index for the assesment of the potential uptake of water and nutrients than root length due to the fact that area measurements integrate the diverse range of root diamenters found in root samples. Moreover, according to Varney and Canny (1993) and Wang and Smith (2004) root surface area rather than root length controls water uptake. Thus, there is a need to develop and test the performance of automated techniques to quantify root surface area.

The objectives of this study were (i) to determine the response of winter cereal root growth and aboveground-belowground biomass ratios along a yield potential gradient under three different types of tillage in the Mediterranean region and (ii) to test if the determination of root surface area with the use of a license-free image analysis software is acceptable for the determination of root length compared with the more common intersection method of Newman. We hypothesized that (i) C allocation within biomass will depend on the yield potential, with increased shoot-to-root ratio in the more favorable sites, and (ii) the new method for quantifying root surface area would be comparable to the more common intersection method.

Materials and Methods

Experimental sites

The study was carried out during the 2009-2010 cropping season in three rainfed tillage experimental sites located along a yield potential gradient due to different rainfall, potential evapotranspiration and soil characteristics located in the Ebro valley, Spain: Peñalba (LYP), Agramunt (MYP) and Selvanera (HYP). LYP, MYP and HYP indicate the yield potential in the gradient as: low yield potential, medium yield potential and high yield potential, respectively. The study focused on winter cereal root growth, as these crops are the most important in the dryland Mediterranean areas. Specific climatic and soil characteristics of the experimental sites are given in Table 1. The sites represent a decreasing water deficit from the site with lowest yield potential to the site with the highest (914, 425 and 325 mm water deficit for the LYP, MYP and HYP sites, respectively). The high evaporative demand at the LYP site is related to the prevailing wind in the area, from the northwest, locally called *Cierzo* (Ramos et al. 2009). Moreover, at this site soil salinity could also limit the yield. Also, soil texture becomes more silty from the site with the highest yield potential (HYP) to the site with the lowest yield potential (LYP) (Table 1). The average annual winter cereal grain yield at the LYP site is 800 kg ha^{-1}, 2800 kg ha^{-1} in the MYP and 4000 kg ha^{-1} in the HYP.

In Peñalba (LYP), prior to the set up of the experiment in 2005, agricultural management consisted in barley (*Hordeum vulgare L.*) – fallow rotation with intensive tillage with disk plough. After grain harvest, the straw was often grazed by sheep. Once the experiment was established, the cropping system changed to barley monocropping and not grazing with three tillage systems: conventional tillage (CT), minimum tillage (MT) and no-tillage (NT). The CT treatment consisted of one pass of disk plough to a depth of about 20 cm followed by one pass with a cultivator before seeding. The MT treatment consisted of one pass with a cultivator to 15 cm soil depth.

In Agramunt (MYP) the management prior the set-up of the experiment consisted of intensive tillage with moldboard plow and pig slurry applications. In 1990 a tillage experiment was established with three treatments: CT, MT and NT. The CT treatment consisted of one pass with a moldboard plow to 25 cm depth immediately followed by one or two passes with a cultivator to 15 cm, both in September. The MT treatment consisted of one pass with a cultivator. The cropping system consisted of a wheat (*Triticum aestivum L.*) – barley – triticale (*X triticosecale W.*) rotation. The campaign of the current experiment (2009-2010) covered the wheat phase of the rotation.

In Selvanera (HYP), prior to the set up of the experiment agricultural management consisted in barley – wheat rotation with intensive tillage with subsoiler. In 1987 a tillage experiment was established with three treatments: CT, MT and NT. The CT treatment consisted of one pass with a subsoiler to 40 cm depth in August followed by chisel plowing to 15 cm depth in October before seeding. The MT treatment consisted of one pass with a chisel plow to 15 cm depth in October. Once the experiment was established the cropping system consisted of a wheat-barley-wheat-rapeseed (*Brassica napus L.*) rotation. As at the MYP site, the campaign of the current experiment (2009-2010) covered the wheat phase of the rotation. For all the three experimental sites the NT treatment included a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. An overview of the species and cultivars grown in each experiment is shown in Table 2.

Tillage treatments were arranged in a randomized complete block design, with three replicates in LYP and HYP and four in the MYP experiment. Plot size was 7 by 50 m at the HYP experiment, 9 by 50 m at the MYP experiment and 34 by 175 m in the LYP experiment.

As has been mentioned above, all the cereals grown before and during the experiment were winter cereals. In all the experimental fields planting was performed in November with a direct drilling machine set to 2-4 cm depth. Seeding rate was set at 450 seeds m^{-2} and row spacing was 17 cm. The harvest was carried out with a medium-sized commercial combine. Crop residues were chopped and spread over the soil surface. Sowing and harvesting dates are shown in Table 2.

Root and aboveground biomass sampling and analyses

Soil samples were taken at tillering and at flowering in 2010 in the three tillage experiments (Table 2). In each plot for all experiments, representative areas of 2 x 2 m were identified and four soil samples per area were obtained with a mechanized soil corer and divided in 30 cm increments until 90 cm soil depth. In order to better characterize the spatial heterogeneity of the roots in the soil, half of the samples were taken on the seeding line and at the midpoint between seeding lines. Care was taken in order to avoid wheel track locations. Once in the laboratory the samples were kept in the refrigerator at 4ºC.

Additional soil samples were obtained in order to quantify the soil moisture of every depth increment at sowing, tillering, stem elongation, flowering and harvest. Soil moisture was obtained by drying the samples at 105ºC during 48 h. Soil moisture from the LYP experiment was quantified by drying the samples at 50ºC until constant weight due to the high gypsum content of the soil (Porta 1998). Bulk density was determined at

30 cm depth with the cylinder method and was considered constant for the whole soil profile.

Every fresh sample for root determination was weighed and dispersed with a 5% sodium hexametaphosphate solution in a reciprocal shaker during at least 30 minutes and washed by hand with a low-pressure shower jet through a 0.5-mm sieve to recover the roots, following the methodology proposed by Böhm (1979). Once washed, roots, debris and soil particles greater than 0.5 mm remained on the sieve. In order to separate roots from soil particles and debris, the sieve was submerged 10 cm in a plastic tray filled with water. The roots were skimmed with the use of one flat 0.1-mm sieve of 7 cm diameter. Care was taken to avoid the larger debris material. The roots obtained for each soil sample were placed in 1 L plastic bottles filled with a 15% ethanol solution and refrigerated at 4ºC until analysis.

To measure the root surface, each root sample was spread over a clear methacrylate tray filled by a solution of 15% ethanol covering all the roots in order to avoid meniscuses. Afterwards, the tray was covered with a blue tough paper (29, 30 and 51 of the red, green and blue primary colors, respectively). This color was chosen after an analysis of different possibilities. According to our results, blue was the background color that better distinguished root color from the rest of the tray. Once covered, roots were scanned with a conventional flatbed scanner with a 300 dpi resolution. Image files were analyzed with ImageJ (Rasband 2011), a public domain image processing software usually used for medical images analyses. Color threshold ranges from 0 to 70, from 0 to 60 and from 0 to 20, for the red, green and blue primary colors were established in order to better distinguish live roots among the rest of the sample (i.e. old roots, sand particles and some debris) and the blue-coloured background of the images. These values were chosen after analyzing which of the ranges of the three basic colors better distinguished the different materials present in the images (i.e. roots of the year, old roots, sand, etc). Then, the area of the image corresponding to fresh roots was selected. The scale of measure was set according to the resolution of the images (300 pixels for every inch of length; i.e. 2.54 cm) in order to transform the values to cm. Once the color threshold was applied to the images, root surface was quantified with the "analyze particles" command of the software. In order to avoid the selection of small areas or individual pixels that did not correspond to root areas, a measurement range from 10-pixels areas to the infinity was established. The 2-dimensional area measured by the software was multiplied by π in order to obtain the real surface of the roots assuming that their section was circular (Kokko et al. 1993). The soil bulk density and moisture were used to compute root surface density (RSD) as cm^2 root cm^{-3} dry soil. Finally, root samples were dried at 65ºC during 48 h and weighed to obtain the root biomass.

To test the validity of the method described above, 36 of the root samples quantified with ImageJ were also analyzed with the commonly used lineal intersection method proposed by Newman (1966) and modified by Tennant (1975). Roots were stained with a 1% solution of Congo red in order to distinguish between dead and live ones. Then, the roots were spread randomly on a filter paper and covered with a 1x1 cm grid. The number of intersections between the roots and the grid was counted on the sides of 25 non-adjoining squares randomly selected and the root length (L) was obtained applying the formula:

$$L = (\pi * A/2 * l) * i.$$

A is the area of the filter paper where the roots were spread, *l* is the total length of the grid and *i* is the number of intersections between the roots and the grid lines.

Root length density (RLD) was then obtained by dividing L by de volume of the soil sample. Finally, a linear regression was performed between the RSD and the RLD to test the validity of the root surface method.

In order to calculate the shoot-to-root ratio, an aboveground biomass sampling was performed at flowering (Table 2) by cutting a variable number of plants at the soil surface level along 0.5 m of the seeding line at 3 randomly selected locations per plot. The samples were dried at 65ºC during 48 h and weighed. The shoot-to-root ratio was calculated by dividing the above-ground biomass by the biomass of roots at the flowering stage, being both variables transformed previously in mass units per square meter.

At maturity another aboveground biomass sampling was performed in order to quantify the yield components of the crop for each treatment (Table 2). The ears were counted and threshed. The number of grains and their weight were recorded and the number of ears per square meter, the number of grains per ear and the harvest index were calculated. Moreover, the grain-to-root ratio was calculated by dividing the grain weight at maturity by the root biomass at flowering, when it is assumed to be at its maximum (Lampurlanés et al. 2001).

Yield of each treatment was measured by harvesting the plots with a commercial combine and weighing the grain. Harvest dates for each experiment are shown in Table 2. After determining grain moisture, the yields were corrected and expressed at 10% moisture.

RSD and root biomass were analyzed using the SAS statistical software (SAS institute 1990). A global ANOVA was performed with site, tillage, depth and growth stage (tillering and flowering) and their interactions as sources of variation. The effect of the same factors but stage were studied for root biomass. Block was nested within site. RSD and root biomass were transformed using the best Box-Cox transformation (equations shown in Table 3) in order to normalize the data and the variances. Shoot-root and grain-root ratios were also analyzed with site and tillage and their interactions as sources of variation nesting the block within site. When significant, differences among treatments were identified at the 0.05 probability level of significance using Tukey's test. The linear relationship between the RSD and RLD was determined with Sigmaplot 11 (Systat Software 2008).

Results

Rainfall distribution, grain yield and soil water content

Rainfall distributions during the 2009-2010 cropping season and the historic (30 yr.) averages of every experimental site are shown in Figure 1. In the year of the study, the rainfall of the LYP, MYP and HYP sites was 231, 702 and 416 mm, respectively. Those values represent a 69, 165 and 99% of the long-term average rainfall of each site. At the LYP site, the period of crop fastest development (February-May) was drier when compared to the average rainfall of the period (71 vs. 133 mm) (Fig. 1a). Differently, at the MYP site, the soil water recharge period (July-January) was much wetter than usual with 402 mm compared with the average of the last 30 years (238 mm) (Fig. 1b). Finally, at the HYP site, 82% of the season rainfall received occurred during the cropping period (November-June) (Fig. 1c).

The soil water content was at its maximum at tillering in the MYP and HYP sites, but at the LYP site the recharge of soil water was not complete until stem elongation (Fig. 2). Tillage only affected soil water content at the MYP and HYP sites (Fig. 2). At the MYP site, the NT treatment had greater soil water content than CT at tillering, stem elongation and flowering growth stages whereas the MT had intermediate values (Fig. 2). At the HYP site, significant differences between tillage systems were only observed at crop seeding with greater water content under MT when compared to NT and CT (Fig. 2). At tillering, when the fast growth of the crop starts due to the increase of the temperature, water content in the soil was 59, 227 and 270 mm at the LYP, MYP and HYP sites, respectively, as an average of the three tillage systems compared.

Yield potential site effects on RSD and root biomass

RSD and root biomass varied among experimental sites with different yield potential (Table 3). As an average of the two growth stages analyzed (tillering and flowering) and the three depths studied (0-30, 30-60 and 60-90 cm), RSD was greatest at the HYP site with 0.048 cm^2 cm^{-3} and lowest at the LYP site with 0.017 cm^2 cm^{-3} (Table 4). The MYP site was intermediate with 0.042 cm^2 cm^{-3}. There was a significant interaction of site and depth on RSD (Table 3). In the 0-30 cm depth, the HYP site had the highest RSD (0.098 cm^2 cm^{-3}), the MYP site was intermediate (0.066 cm^2 cm^{-3}) and the LYP site was least (0.036 cm^2 cm^{-3} dry soil) (Table 4). In the 30-60 cm depth, the HYP and the MYP sites was greater RSD than the LYP site (0.030 vs. 0.009 cm^2 cm^{-3} dry soil). Finally, in the 60-90 cm depth, the greatest RSD was found at the MYP site with 0.023 cm^2 cm^{-3} without significant differences between the HYP and LYP sites (Table 4).

Tillage system, growth stage and depth effects on RSD and on root biomass

Tillage had no significant effects on RSD in any of the growth stages studied (Table 3 and Figs. 3 and 4). Equally, tillage did not affect root biomass (Table 4). Although not significant ($p=0.72$), a trend of greater RSD under MT and CT when compared to NT was found in the 0-30 cm depth for the MYP site at tillering (Figure 3b). A similar trend was found at the same experimental site for the flowering stage (Figure 4b). The growth stage also affected significantly the RSD with greater values in the flowering period than for tillering (Tables 3 and 4). Moreover, the soil depth and the interaction between the growth stage and depth had a significant effect on RSD (Table 3). In the 0-30 cm depth greater RSD was found in the flowering stage when compared to the tillering, but no differences were found in the 30-60 and 60-90 soil depths (data not shown).

Comparison of root analysis methods

RLD values obtained with the most commonly used Newman's method ranged from 0.06 to 0.74 cm cm^{-3} while the RSD values obtained with image analysis ranged from 0.003 to 0.061 cm_2 cm^{-3}. A highly significant linear relationship ($P<0.001$; r^2: 0.77) between the two methods was observed (Fig. 5).

Shoot-to-root ratios

The shoot-to-root ratio ranged between 3.32 in the CT treatment at the MYP site and 9.11 under NT at the HYP site (Table 5). Significant differences between sites were found with greater shoot-to-root values at the HYP site (7.55 as an average of the three tillage systems studied) when compared to the LYP and MYP sites (4.86 and 4.12, respectively). However, no differences between tillage systems were found on shoot-to-root ratio.

Yield components and grain-to-root ratios

Significant differences were found between sites and tillage treatments on yield components (Table 6). Greater number of grains per ear and a higher harvest index were found at the MYP and HYP sites when compared to the LYP site. Moreover, an increasing grain weight was found when increasing the potential yield: 23.5, 32.5 and 44.3 mg per grain at the LYP, MYP and HYP sites, respectively. When comparing among tillage treatments and as an average of the three sites studied, greater amount of grains per ear were found under NT than under MT or CT. Also, the grain weight was higher under NT than under CT. The interaction between the site and the tillage treatment was significant for the number of grains per ear. Its values ranged between 9.9 for the CT treatment of the LYP site and 45.1 for the NT treatment at the MYP site (Table 6). The grain yield average (mean of the three tillage systems compared) for the LYP, MYP and HYP sites was 386, 3966 and 8230 kg ha^{-1}, respectively, with significant differences between yield potential sites. Also, the interaction between site and tillage for grain yield was significant. At the HYP site, no differences between tillage systems were found on grain yield. In contrast,, at both MYP and LYP sites significant differences between tillage systems were found: at the MYP site the CT and NT treatments presented greater grain yield than MT whereas at the LYP site the NT treatment presented the greatest grain yield when compared to CT and MT (Table 6). As an average for the three sites, grain yield was the greatest under NT and the lowest under MT, with intermediate values under CT (Table 6).

The grain-to-root ratio was significantly affected by experimental site, with an increasing ratio when increasing the yield potential (Table 5). The ratio was 2.7 and 4.6 times greater at the HYP site than at the MYP and LYP sites, respectively (Table 5).

Discussion

The rainfall distribution of the cropping season studied (2009-2010) differed from the normal pattern. In general, at the three sites studied, the summer and autumn (July-November 2009) were drier that the average record, compromising the period of water recharge of the soil which, under Mediterranean conditions, is a key factor to maximize crop yields (Cantero-Martínez et al. 2007). However, during active crop growth (January-May) rainfall was above the historic mean at the MYP and HYP sites.

Site effects on root surface density and biomass ratios.

At the MYP and HYP sites grain yields were higher than the historic average of each area and could be considered exceptional. The differences in grain yield found between experimental sites demonstrate the gradient in production potential among the experimental sites in the study. As it has been already explained in the Materials and Methods section the sites present a decreasing water deficit from the site with lowest yield potential to the site with the highest. That fact is also aggravated in the LYP site due to the presence of soil salinity that can limit the availability of water for the plant, affecting yield components (Maas and Grieve, 1990; Rasouli et al. 2013)

The highest grain yield production at the HYP site was accompanied by greater root growth in the first 30 cm of soil and greater shoot-to-root and grain-to-root ratios when compared with the other two sites. It has been widely documented that, when resources are scarce, plants allocate new biomass to the organs involved in the acquirement of those resources (Wild et al. 1987; Marschner 1995). Palta and Gregory (1997) studied the effect of water limitations on the fate of pulse-labelled ^{13}C in wheat plants and observed that, on a relative basis, more assimilates were allocated to the roots in plants under limited water conditions. In turn, Bolinder et al. (1997) suggested that climate and fertilization strategy affect significantly the shoot-to-root ratio. For instance, it has been found that under N or P deficiencies, shoot-to-root ratios usually decrease (Brown et al. 1987; Hermans et al. 2006). In line of last authors' suggestion, Poorter and Nagel (2000) point out the role played by water availability and its relationship to nutrient uptake in the C allocation shift to roots. Moreover, shoot:root ratios depend on the ontogeny and the size of the plant: larger plants will have to invest a larger fraction of their biomass in support structure, and have a larger leaf area so that self-shading increases (Poorter and Nagel 2000). Thus, in our results, treatment effects on plant size could also have influenced the shoot:root ratios that we obtained. A broad range of shoot-to-root ratios have been reported in the literature. Xu and Juma (1992) studied the growth of four barley cultivars during two consecutive years in a Mollisol in Alberta, Canada. They reported a range of shoot-to-root ratios from 9.4 to 12.5 in the ripening stage of the crop. In a broad review covering different winter cereal species and cultivars, Bolinder et al. (2007) reported a shoot-to-root ratio of 7.4 for small-grain cereals as the mean of 59 studies in Canada. In the Mediterranean region shoot-to-root ratios have also been reported. For instance, Álvaro-Fuentes et al. (2008) found a broad range in the shoot-to-root ratio varying from 1 to 12 when comparing different tillage and cropping systems. Also in the Mediterranean area, Muñoz-Romero et al. (2010) reported shoot-to-root ratios of spring wheat in a range from 4.7 to 7.0. In our study, the ratio ranged between 3.3 and 9.1, being greatly affected by the site, demonstrating the effect of soil and climate on resource allocation by plants. It has to be taken into account that different cereal species were grown at the different sites: barley was used in the LYP, while wheat was grown in the MYP and HYP sites, according to the cropping system of each area, fact that could have influenced our results (Bolinder et al. 1997). Campbell and de Jong (2001) quantified shoot-to-root ratios of wheat grown in lysimeters under natural rainfall and irrigated conditions. They found a greater shoot-to-root ratio under irrigation when compared with the natural rainfall conditions. Husain et al. (1990) concluded that, if water shortages occur for a prolonged period, an enhanced root growth in absolute terms can occur. In the same area of our study, Isla et al. (1999) quantified the effects of increasing soil salinity on barley root growth, above-ground biomass and grain yield. They found a significant reduction in root length and a more linear distribution (i.e. less sharp decrease with depth) of roots along the soil profile when increasing soil salinity. Thus, we could hypothesize that the lower RSD and the more linear root distribution along soil depth measured at the LYP site could be related with the presence of soil salinity induced by gypsum content.

According to the data obtained, no differences were found between the LYP and MYP sites on the shoot-to-root ratio. However, the MYP site presented a grain-to-root ratio 2.7 times higher than the LYP site. Also, as an average of the three tillage treatments studied, the grain weight was 1.4 times greater at the MYP site than in the LYP one. Passioura (1983) demonstrated that the allocation of C to grain (i.e. harvest index) is often related to the proportion of total water supply that is used after flowering. Loss and Siddique (1994) and Gonzalez et al. (2007) pointed out the importance of terminal drought on the limitation of winter cereal yields due to scarce rainfall and high temperatures during the grain filling period. Brown et al. (1987) also suggested that seasonal moisture stress, like for years when spring and summer rains are exceptionally low in the Mediterranean areas, can cause a reduction in kernel weight reducing the potential yield. Although we did not find differences between sites on the number of ears per square meter, we observed significantly fewer grains per ear and a lower grain weight at the LYP site, accompanied by a lower harvest index. Thus, a lack of enough water in the most extreme environment (LYP site) during the grain filling period likely reduced the grain-to-root ratio.

No significant RSD increase occurred in deeper soil layers from the tillering to the flowering of the crop. Other studies show a decrease in the proportion of C allocated belowground in winter cereals from tillering to flowering due to an increase of the sink activity of the ear (Gregory et al. 1996; Palta and Gregory 1997). For instance, Jensen (1994) observed a reduction in the amount of ^{14}C allocated belowground from the 43% in the tillering stage to a 14% in the early grain filling period of the crop. Gregory and Atwell (1991) studied the fate of C in pulse-labelled wheat and barley during the early tillering and grain-filling stages. During the first stage, 15-25% of the C was respired by the roots and the rhizosphere and between 17-27% was retained in the roots, 45-67% of the C remained in the shoot, and the rest was recovered as water-soluble C. By contrast, during the grain-filling stage, only 2-6% of the C was in the roots, a 2-3% was respired as CO_2 and over 90% was in the shoot. After flowering, decomposition of the finer roots usually occurs (Xu and Juma 1992) and root length, surface and mass get reduced (Xu and Juma 1992; Gregory et al. 1996; Lampurlanés et al. 2001).

Tillage effects on RSD and biomass

No differences between tillage systems were found on RSD in any of the three sites studied in the tillering or the flowering growth stages nor on root biomass in the flowering one. At the LYP and HYP sites, the lack of differences between tillage systems on RSD and root biomass could relate to the fact that all three tillage systems had a similar amount of water in the soil during those growth stages. In the same area of our experiments, Morell et al. (2011a) quantified the effects of tillage on root length density from 2006 to 2009. During dry years they found greater root growth under NT due to higher water content in the soil. However, in 2009, a year with wetter conditions, lower root growth was found under NT. They related reduced root growth to the greater soil penetration resistance measured under NT, when compared to RT or CT. Although statistically not significant (Figures 3 and 4), a trend of greater RSD was observed at the MYP site under CT, which could relate to soil penetration resistance. Although tillage did not affect root growth, a greater number of grains per ear, higher grain weight and higher grain yield were found under NT as an average of the three sites studied. Such results could be related to a better use of water by the crop during the floret development and grain-filling periods. In the same area, Cantero-Martínez et al. (2003) found greater water use efficiency under NT due to better water use in the pre-flowering period of the winter cereals. Thus, the higher water use efficiency under NT could explain the differences between tillage systems that we found. Compared to other geographical areas (Pietola 2005; Munkholm et al. 2008) the use of soil management systems such MT and NT did not limit the grain production due to a reduced root growth in the broad range of conditions of our study.

Root analysis method

To test the validity of our root analysis method a linear analysis was performed between the results obtained with the image analysis software and those obtained with the Newman method (1996) modified by Tennant (1975) based on lineal intersection. The relationship between both methods was highly significant (r^2:0.77; p<0.001) showing the good performance of the new method. Part of the dispersion observed could be explained by the fact that roots with the same length but of different thickness would have different surface area (Kokko et al. 1993).

Another interesting point related with the use of the software is the elimination of operator subjectivity when quantifying root surface area. That fact relies on the use of valid color range thresholds that need to be established depending on the background color used. If the basic color thresholds used to distinguish the roots from debris are maintained during the analysis, the subjectivity inherent in the intersection method is eliminated. The results demonstrate that the RSD index obtained with image processing software is suitable and reliable when studying root dynamics in winter cereals.

Conclusions

In our study in the Mediterranean dryland agroecosystems, winter cereals' above- and belowground biomass ratios increase with yield potential and are strongly regulated by the availability of water in the soil during crop growth. Grain-to-root ratio is more strongly related to the yield potential of the site than is shoot-to-root ratio because drought stress during the grain-filling period can reduce C translocation to the grain but have a small effect on shoot biomass. NT has a greater efficiency in the use of the resources when allocating C to the grain because it is able of obtaining the highest grain yields without having a greater root system. The results obtained with the public domain image processing software ImageJ when analyzing root samples are comparable to those obtained with the most common intersection method.

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Figure captions

Fig. 1 Monthly rainfall during the 2009-2010 cropping season (bars) and historic (30 yr) monthly rainfall (line) at the three sites under study (low, medium and high yield potential).

Fig. 2 Volumetric soil water content of the 0-90 cm soil depth at seeding (S), tillering (T), stem elongation (Se), flowering (F) and harvest (H) in the three locations under study (low, medium and high yield potential). Error bars represent standard errors. For each growing stage and site, different letters indicate significant differences between tillage systems at $P<0.05$.

Fig. 3 Root surface density (RSD) at the tillering stage as affected by tillage system (MT, minimum tillage; NT, no-tillage and CT, conventional tillage) at the three sites studied (low, medium and high yield potential). Horizontal error bars represent standard errors (only positive values of the errors are shown).

Fig. 4 Root surface density (RSD) at the flowering stage as affected by tillage system (MT, minimum tillage; NT, no-tillage and CT, conventional tillage) at the three sites studied (low, medium and high yield potential). Horizontal error bars represent standard errors.

Fig. 5 Linear relationship between the root lenght density (RLD) obtained by the traditional Newman's method and the root surface density (RSD) obtained by the low-cost image analysis method. ***Regression significant at $P<0.05$.

Table 1 Site and general soil characteristics in the 0-30 cm soil depth of the three experimental sites (LYP, MYP, HYP: low, medium and high yield potential).

	Peñalba (LYP)	**Agramunt (MYP)**	**Selvanera (HYP)**
Year of establishment	2005	1990	1987
Latitude	41º25’N	41º48’N	41º 49’N
Longitude	0º04’O	1º07’E	1º17’E
Elevation (m)	352	330	475
Annual precipitation (mm)	336	430	475
Annual ETo (mm)	1250	855	800
Annual water deficit (mm)*	914	425	325
Soil classification**	Typic Haplogypsid	Typic Xerofluvent	Fluventic Xerochrept
pH (H2O, 1:2.5)	8.0	8.5	8.3
Soil organic carbon (g kg^{-1})	3.5	7.56	10.47
EC1:5 (dS m^{-1})	2.59	0.15	0.16
CaCO3 eq. (%)	17	40	35
Water retention (% vol.)			
33 kPa	34.1	29.3	26.9
1500 kPa	15.4	12.6	11.5
Particle size distribution (%)			
Sand (2000-50 µm)	13.0	30.1	36.5
Silt (50-2 µm)	64.9	51.9	46.4
Clay (<2 µm)	22.1	17.9	17.1

* Calculated as the difference between mean annual precipitation and mean annual ETo.

**According to the USDA classification (Soil Survey Staff, 1975).

Table 2 Species and cultivars grown, dates of sowing, harvest and sampling events of the three experimental sites (LYP, MYP, HYP: low, medium and high yield potential).

	Parameter studied	**Peñalba (LYP)**	**Agramunt (MYP)**	**Selvanera (HYP)**
Crop		Barley	Wheat	Wheat
Cultivar		Cierzo	Bokaro	García
Sowing date		11-15-2009	11-12-2009	11-01-2009
Harvest date	Grain-to-root ratio	06-28-2010	07-16-2010	07-14-2010
Below-ground biomass samplings				
Tillering	RSD*	03-29-2010	04-08-2010	03-30-2010
Flowering	RSD; shoot-to-root ratio; root biomass	05-06-2010	05-19-2010	05-12-2010
Above-ground biomass samplings				
Flowering	Above-ground biomass, shoot-to-root ratio	05-03-2010	04-30-2010	05-06-2010
Maturity	Yield components	06-07-2010	07-01-2010	06-27-2010

* RSD: root surface density

Table 3 ANOVA P-values showing significant differences in root surface density (RSD) as affected by site, growth stage, tillage and depth and in root biomass as affected by site, tillage and depth. RSD and root biomass were transformed following the Box-Cox procedure as: $(1/(\text{RSD}-1)^{2})-1$ and $(1/(\text{root biomass}-1)^{0.2})-1$, respectively.

Effects	***P*-values**	
	RSD	**Root Biomass**
Site	<0.0001	<0.0001
Stage	0.0013	
Tillage	0.72	0.83
Depth	<0.0001	<0.0001
Site*Stage	0.68	
Site*Tillage	0.52	0.22
Stage*Tillage	0.25	
Site*Depth	<0.0001	0.0002
Stage*Depth	0.0003	
Tillage*Depth	0.99	0.32
Site*Tillage*Depth	0.99	0.49
Site*Stage*Tillage	0.61	
Site*Stage*Depth	0.64	
Stage*Tillage*Depth	0.46	
Site*Stage*Tillage*Depth	0.99	

Table 4 Root surface density (RSD) at the tillering and flowering growth stages and root biomass at the flowering measured at three experimental sites (low, medium and high yield potential) for three sample depths (0-30, 30-60 and 60-90 cm depth), and the three tillage systems compared (CT, conventional tillage; MT, minimum tillage; NT, no-tillage). Values of RSD are the average of the three tillage systems compared..

Yield potential	**Depth (cm)**	**RSD (cm^2 cm^{-3})**			**Root biomass (g m^{-2})**			
		Growth stage			**Tillage system**			
		Tillering	**Flowering**	**Mean**	**CT**	**MT**	**NT**	**Mean**
Low	0-30	0.027	0.045	0.036 c*	60.42	60.65	75.39	65.48 ab
	30-60	0.007	0.011	0.009 f	23.38	22.76	49.02	31.72 bcd
	60-90	0.006	0.003	0.005 f	1.34	2.19	2.19	1.91 e
	Mean	0.014	0.020	0.017 C†	28.38	28.53	42.20	33.04 B
Medium	0-30	0.046	0.079	0.066 b	100.92	105.06	79.03	95.00 a
	30-60	0.032	0.027	0.030 cd	92.25	65.09	51.97	69.77 abc
	60-90	0.019	0.027	0.023 de	50.45	28.15	35.52	38.04 cd
	Mean	0.032	0.049	0.042 B	84.02	71.66	58.87	71.52 A
High	0-30	0.086	0.110	0.098 a	68.91	136.07	120.33	107.74 a
	30-60	0.032	0.029	0.030 cd	72.14	42.57	44.57	53.10 abcd
	60-90	0.010	0.017	0.013 ef	27.18	23.68	23.82	25.03 d
	Mean	0.043	0.053	0.048 A	56.08	67.44	64.29	62.50 A
Mean all sites		0.030	0.043**	0.037	60.51	58.35	55.28	58.09

* Different lowercase letters indicate significant differences between sites of different yield potential and depths at $P<0.05$. † Different uppercase letters indicate significant differences between sites of different yield potential at $P<0.05$. ** Indicates significant differences between growth stages at $P<0.05$.

Table 5 Shoot-to-root and grain-to-root ratios as affected by tillage (CT: conventional tillage, MT: minimum tillage and NT: no-tillage) in three sites with different yield potential (LYP, low yield potential; MYP, medium yield potential and HYP, high yield potential).

	Yield potential	**CT**	**MT**	**NT**	**Mean**
Shoot-to-root ratio	Low	4.62	5.07	4.90	4.86b*
	Medium	3.32	3.83	6.23	4.12b
	High	7.66	5.87	9.11	7.55a
Grain-to-root ratio	Low	1.43	1.62	1.70	1.58c
	Medium	2.90	3.85	6.11	4.32b
	High	7.98	6.01	7.90	7.30a

* Within each ratio, different letters indicate significant differences between sites with different yield potential at $P<0.05$.

Table 6 Yield components, harvest index and grain yield as affected by tillage (CT: conventional tillage, MT: minimum tillage and NT: no-tillage) in three sites with different yield potential (LYP, low yield potential; MYP, medium yield potential and HYP, high yield potential).

	Yield potential	**CT**	**MT**	**NT**	**Mean**
Ears m^{-2}	Low	362	369	428	386
	Medium	623	597	621	613
	High	675	731	567	658
	Mean	560	569	547	
Grains per ear	Low	9.9 c*	11.3 c	12.7 c	11.3 B†
	Medium	34.4 b	34.4 b	45.1 a	37.9 A
	High	36.6 ab	32.8 b	35.0 b	34.8 A
	Mean	27.7 B	27.0 B	32.3 A	
Grain weight (mg)	Low	21.9	24.5	23.9	23.5 C
	Medium	30.6	34.0	33.0	32.5 B
	High	42.8	42.7	47.5	44.3 A
	Mean	31.6 B	33.8 AB	34.6 A	
Harvest index	Low	0.29	0.32	0.34	0.32 B
	Medium	0.49	0.50	0.48	0.49 A
	High	0.52	0.50	0.53	0.52 A
	Mean	0.44	0.44	0.45	
Grain yield (kg ha^{-1})	Low	218 e	245 e	694 d	386 C
	Medium	4279 b	3240 c	4379 b	3966 B
	High	8134 a	8283 a	8273 a	8230 A
	Mean	4210 B	3923 C	4449 A	

* Different lowercase letters indicate significant differences between sites of different yield potential and tillage treatments at $P<0.05$. † For each variable, different uppercase letters indicate significant differences between sites of different yield potential or between tillage systems at $P<0.05$.

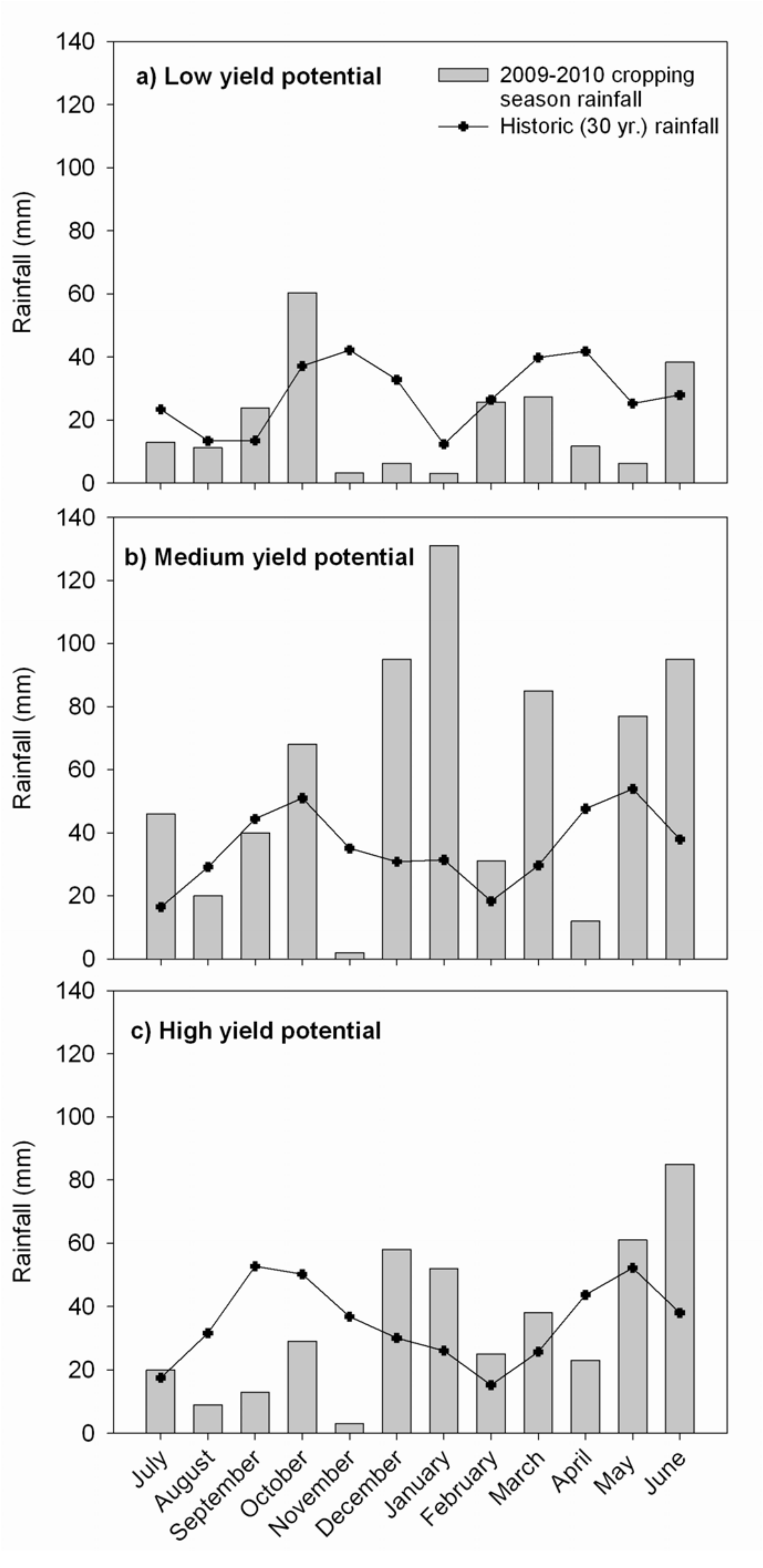
a) Low yield potential
2009-2010 cropping season rainfall
Historic (30 yr.) rainfall
b) Medium yield potential
c) High yield potential
Rainfall (mm)
0
20
40
60
80
100
120
140
July
August
September
October
November
December
January
February
March
April
May
June

Fig. 1

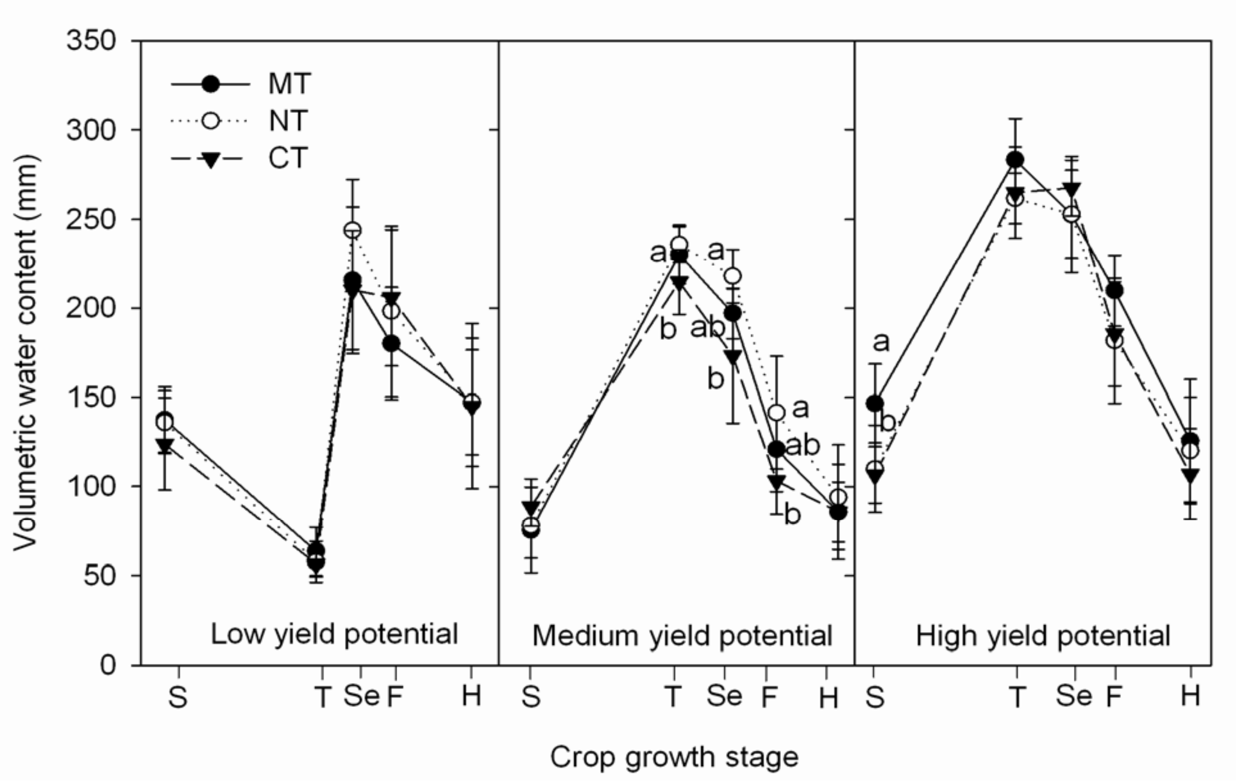
350
300
250
200
150
100
50
0
Volumetric water content (mm)
MT
NT
CT
a
a
b
ab
b
a
ab
b
a
b
Low yield potential
Medium yield potential
High yield potential
S
T
Se
F
H
S
T
Se
F
H
S
T
Se
F
H
Crop growth stage

Fig. 2

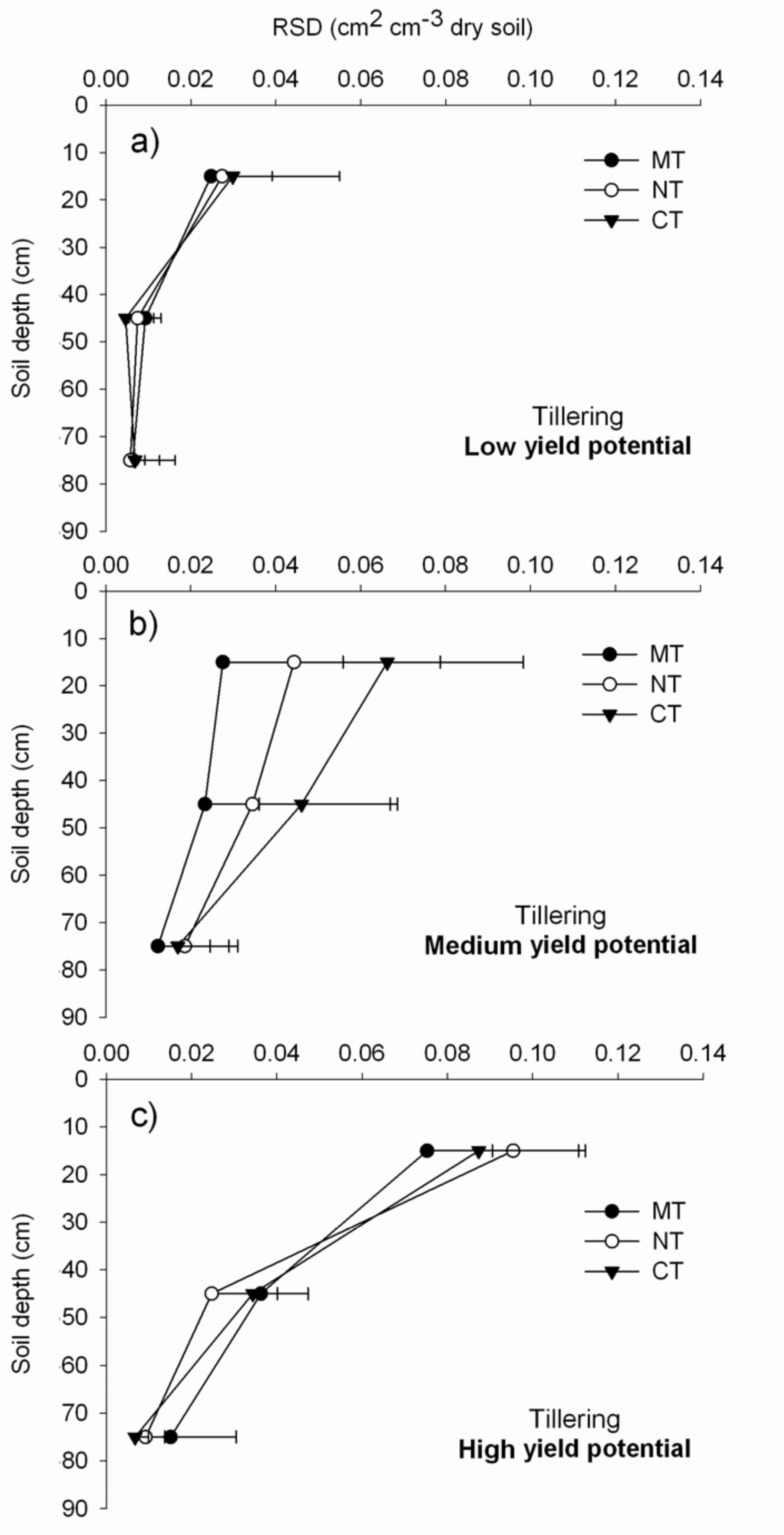
RSD (cm^2 cm^{-3} dry soil)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
Soil depth (cm)
0
10
20
30
40
50
60
70
80
90
a)
b)
c)
MT
NT
CT
Tillering
Low yield potential
Medium yield potential
High yield potential

Fig. 3

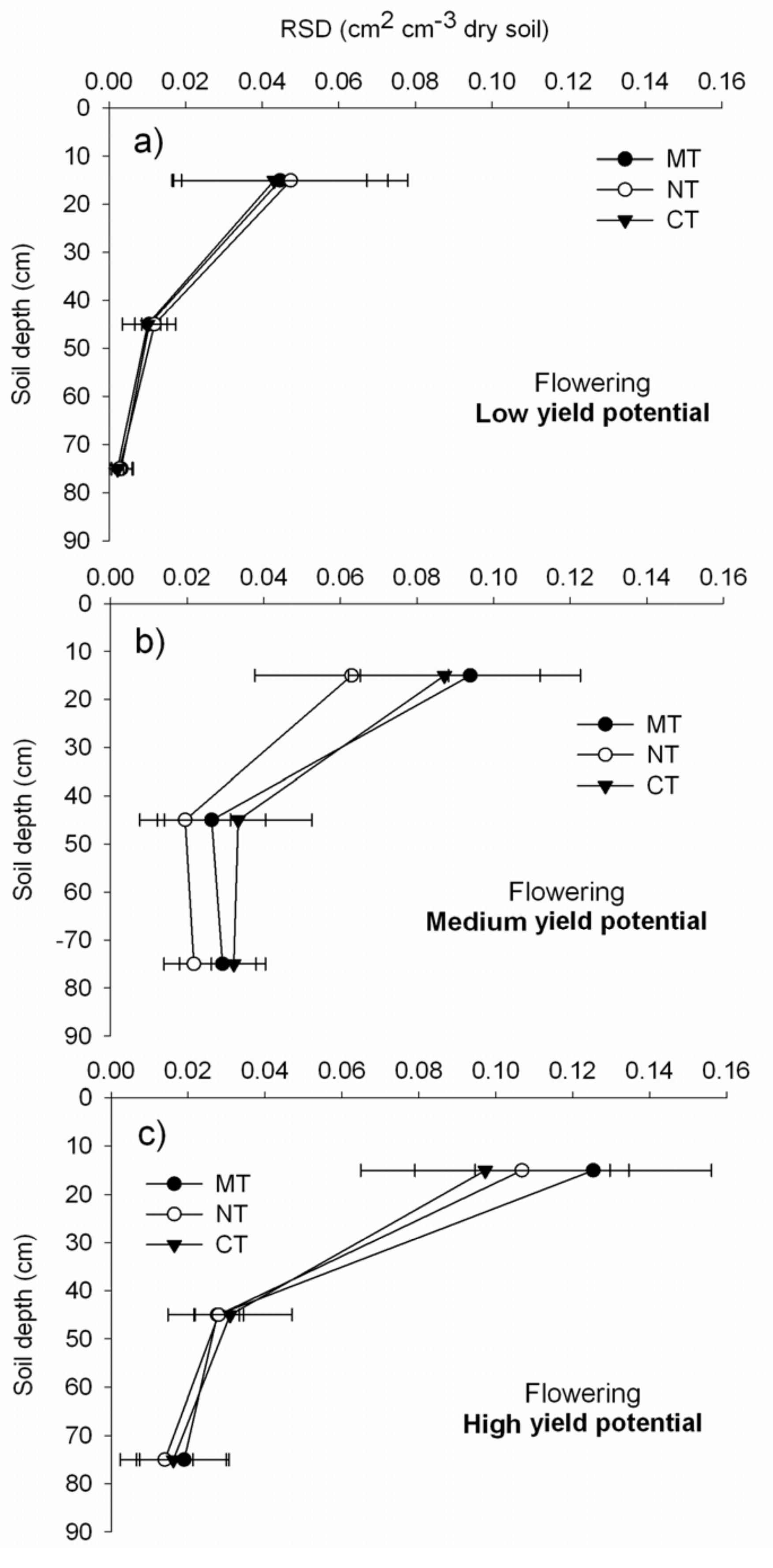
RSD (cm^2 cm^{-3} dry soil)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
Soil depth (cm)
0
10
20
30
40
50
60
70
80
90
a)
MT
NT
CT
Flowering
Low yield potential
b)
-70
Flowering
Medium yield potential
c)
Flowering
High yield potential

Fig. 4

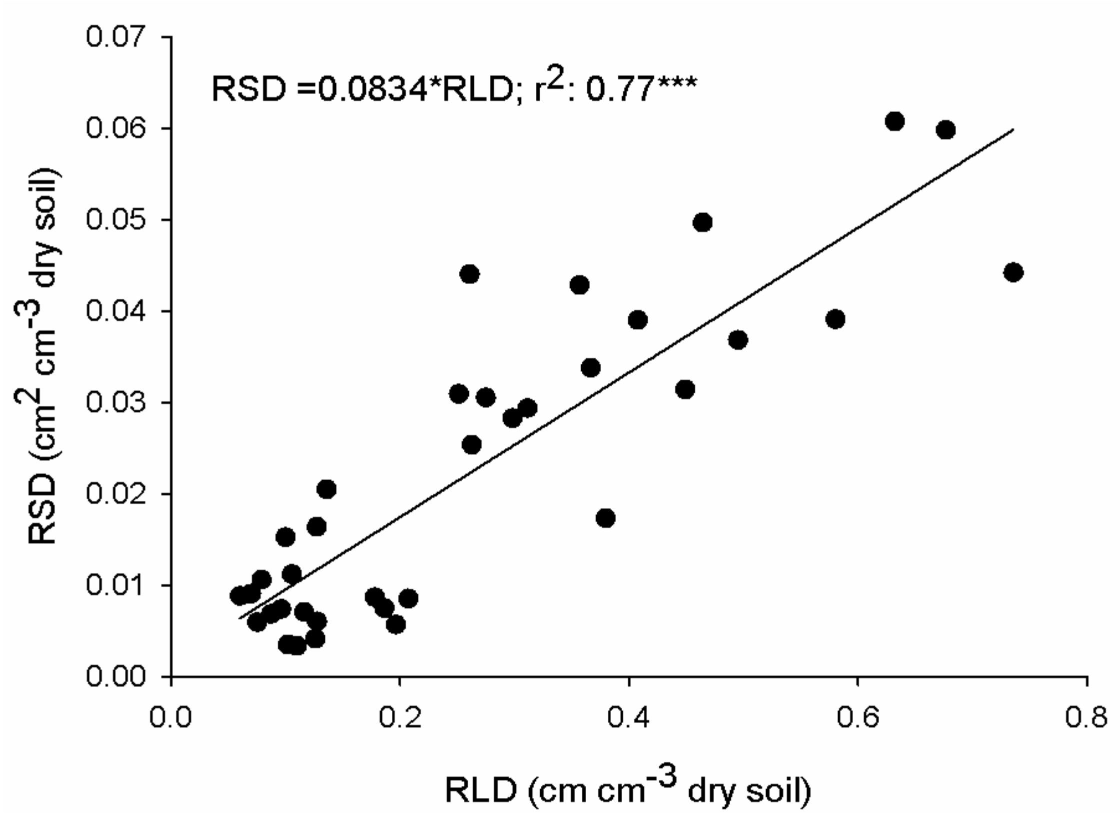
RSD =0.0834*RLD; r2: 0.77***
RSD (cm2 cm-3 dry soil)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.0
0.2
0.4
0.6
0.8
RLD (cm cm-3 dry soil)

Fig. 5