

Root growth dynamics and biomass input of four over-wintering herbaceous crops in boreal conditions

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Root growth of winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.), red clover (*Trifolium pratense* L.), and timothy (*Phleum pratense* L.) was recorded to evaluate the environmental potential of over-wintering crops in a Nordic agroecosystem. In a field experiment on Aquic Haplocryoll soil, root intensities (number of roots/area) were measured to 50 cm depth by minirhizotron microvideo camera technology over a two-year period (21 recording sessions). At anthesis, root biomass and morphological parameters were measured by destructive soil sampling and image analysis of washed roots. Winter cereal roots reached 50 cm depth as soon as the autumn of the seeding year. For both post-seeding years, timothy roots developed the most intensively in spring, while red clover had higher root intensity than timothy in late autumn. At anthesis, the crops were ranked timothy > red clover > winter wheat > winter rye according to root length density, surface area density, and biomass. Based on S:R ratios, red clover appears to offer the most intense carbon sink at 0–60 cm soil depth. Over-wintering crops had living roots in the subsoil both in late autumn and early spring, indicating potential to plant available nutrient uptake outside the growing season of annual crops.

Key words: *Phleum pratense* L., *Secale cereale* L., *Trifolium pratense* L., *Triticum aestivum* L., minirhizotron, root biomass, shoot:root ratio

Introduction

In the Nordic countries, main arable area locates between latitudes 60° and 61° N. By cultivating over-wintering crops, it is possible to prolong the time where crops cover the soil surface, and plants uptake nutrients and water in northern agriculture. This has been found to reduce erosion (Soinne et al. 2016) and the leaching of nitrogen and particulate phosphorus (Jaakkola 1984, Puustinen et al. 2005). Root systems of over-wintering winter cereals (Wicklert 1961, Munkholm et al. 2008, Skovby Rasmussen et al. 2015) and perennial grasses (Steen 1989, Tufekcioglu et al. 1999) have also been found to be active already before the sowing time of spring cereals. Likewise, the recent goal of enhancing soil carbon (C) sequestration has increased the interest in incorporating winter cover and perennial crops into cultivation systems (e.g. Lal 2007, Kätterer et al. 2013).

Knowledge of the root dynamics of over-wintering fodder and grain crops is essential for synchronizing the timing of fertilization in spring. It is also important to evaluate the potential of catch crops in uptaking nutrients outside the growing season of annual crops. In Scandinavian conditions, the root dynamics of winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.) (Wicklert 1961, Munkholm et al. 2008, Thorup-Kristensen et al. 2009, Skovby Rasmussen et al. 2015), grass, and red clover (*Trifolium pratense* L.) leys (Steen 1984, 1989) have been examined. Overall, however, little quantitative information is available on the temporal and spatial dynamics of rooting among different over-wintering plant species under boreal conditions.

Besides root growth dynamics, root biomass allocation and root morphological parameters are important for evaluating a crop's potential for utilizing water and nutrient resources and the input of root-derived C into soil. The latter has been found to contribute more to refractory soil organic matter than aboveground crop residue (Rasse et al. 2005, Kätterer et al. 2011). To estimate the annual C input of arable crops, the need for accurate data on root biomass and shoot:root (S:R) ratios is substantial (e.g. Bolinder et al. 2007, Palosuo et al. 2016). To date, some studies have been published regarding root biomass of over-wintering crops cultivated under long-day conditions (Steen 1984, 1989, Hansson and Andrén 1987, Paustian et al. 1990, Känkänen et al. 1999) but the morphological parameters have seldom been reported.

To obtain quantitative data on root growth to evaluate the timing of agricultural practices and environmental potential of over-wintering crops, we determined crop-specific information regarding the changes in root depth and distribution of two winter cereals (biannual crops) and two perennial grasses in the soil profile. Our main objective was to determine the dynamics of root depth distribution over time in order to assess root growth of over-wintering crops both outside and during the growing season of annual crops and to estimate the duration of the period with potential water and nutrient uptake. A secondary goal was to investigate the biomass and shoot:root ratio of typical over-wintering crops *in situ*, to estimate the C input properties of root systems. Finally, as there is a lack of data on root growth in subsoil, new data on root profiles were gathered to estimate root growth below topsoil.

Material and methods

Field experiment and weather conditions

Data were collected from a field experiment established on a fertile fine sand, Aquic Haplocryoll (Yli-Halla and Mokma 2001, Pietola and Alakukku 2005) at MTT Agrifood Research Finland (at present, Natural Resources Institute Finland, Luke) in Jokioinen (60° 49' N, 23° 28' E) in 1998–2000. Timothy (*Phleum pratense* L. cv. Iki), red clover (*Trifolium pratense* L. cv. Jokioinen), winter wheat (*Triticum aestivum* L. cv. Aura) and winter rye (*Secale cereale* L. cv. Kartano) were grown in a randomized complete-block design with four replicates with a plot size of 2.5 m × 10 m each.

The plots were established in accordance with common farming practices in Finland, i.e. autumn ploughing to a depth of 20–25 cm, harrowing one to two times before sowing, and fertilizing at sowing with a combined drill and then by surface application. The perennials were sown on 28 July 1998 (DOY 209) and winter cereals on 3 September 1998 (DOY 246), which were typical sowing times for the area. The seeding rate of cereals was 600 viable seeds per square meter, i.e. 192 kg ha⁻¹ for winter rye and 270 kg ha⁻¹ for winter wheat. The seeding rates of timothy and red clover were 20 and 12 kg ha⁻¹, respectively. At sowing, red clover was fertilized with NPK at a dose of 310 kg ha⁻¹ (28, 25, 43 kg ha⁻¹ N, P, K, respectively). Timothy and winter cereals were fertilized with NPK at a dose of 300 kg ha⁻¹ (39, 21, 45 kg ha⁻¹). The row spacing was 12.5 cm, and the combined drill placed the fertilizer between every second row, 2–3 cm deeper than the seeds. The following spring (10 May 1999, DOY 130), timothy and winter cereals were surface fertilized with NPK at a dose of 230 kg ha⁻¹ (60, 5, 7 kg ha⁻¹ N, P, K). In 2000, the perennial crops were not fertilized. The grain of winter cereals was harvested on 4 August 1999 (DOY 216) and about 20 cm high stubble remain until August 9. The perennials were not harvested for DM yield during the growing season, but to ensure over-wintering, perennial plots were cut on 21 September 1999 (DOY 264), leaving a high stand height (15 cm). The study was designed to compare root dynamics of two types of over-wintering crops as a biological rather than agronomic study. Therefore, the forage species were let to reach the flowering stage similarly to winter cereals.

After sowing in early autumn 1998, the weather conditions were favourable for the emergence of seedlings (Fig. 1).

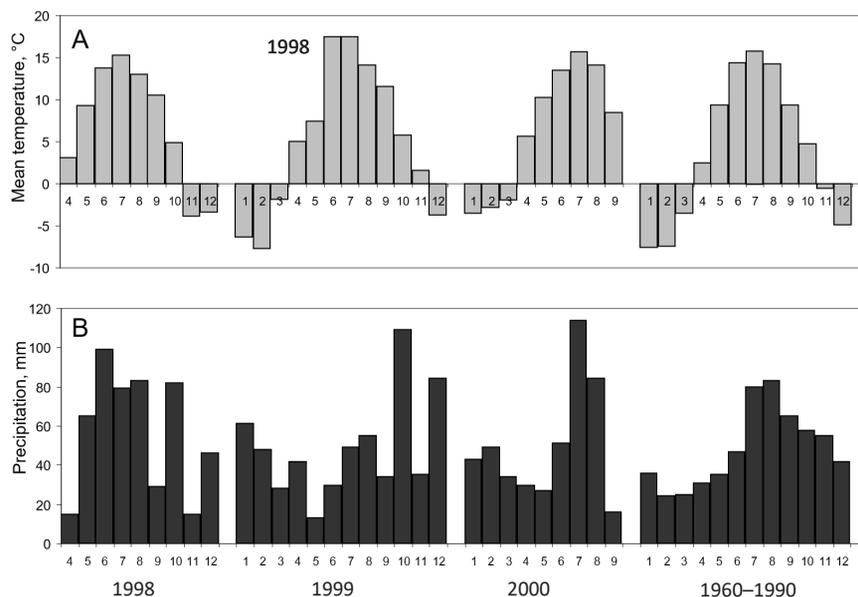


Fig. 1. Monthly mean air temperature (A) and precipitation (B) during the experimental period in 1998–2000 (data from Luke weather database) and the long-term average of years 1960–90 (Finnish Meteorological Institute 1991). Timothy and red clover were sown on 28 July and winter cereals on 3 September 1998.

The stand survived winter 1998–1999 well. Growing season 1999 was dry, as indicated by precipitation (Fig. 1B) and volumetric soil moisture contents in the layers of 0–30 and 0–60 cm (data not shown). Autumn 1999 was warm, and the sum of precipitation for September and October was 16% greater than the long-term average. Precipitation in June 2000 was clearly higher than average (Fig. 1B). Otherwise, precipitation and temperature in the 2000 growing season were within average values.

Root and shoot sampling and carbon input estimation

In 1999, shoot biomass was sampled from an area of 0.2 m² (4 × 0.50 row meter) at anthesis (23 June, DOY 174 for winter rye; 1 July, DOY 182, for winter wheat and timothy; 15 July, DOY 196 for, red clover, Fig. 2). The dry matter of shoots was determined after oven drying at 60 °C for 48 h. After cutting the shoot biomass, four core samples of soil (∅ 5.05 cm, two from the row and two from between rows) were taken at 5-cm increments to a depth of 60 cm to determine root length, diameter, and biomass as described by Pietola (2005). Samples were frozen at –20 °C until the roots were separated from the soil by a hydropneumatic elutriator. Prior to separation, the frozen samples were soaked in a solution of 0.015 M NaOH to disperse soil, and organic debris were manually removed. Roots were dyed with Malachite green oxalate for 2 days before root scanning. Stained roots were placed on a clear glass tray (18 cm × 28 cm) and covered with 3 mm of water; the roots were then scanned, creating image files (600 dpi, brightness 30). The scanned images of roots were analysed with the ROOTEDGE image analysis program which measured the lengths and widths of roots from binary images. Root biomass data were obtained from all soil layers (red clover taproots, defined as roots with a diameter of ≥ 1 mm, were determined separately), but morphological parameters (length and diameter) were only available from soil depths of 10–15, 30–35, and 40–45 cm, representing topsoil, plough pan, and subsoil, respectively.

Annual below-ground C input from roots was estimated based on root biomass at anthesis. A conversion factor of 0.45 (Bolinder et al. 2007) was used to convert dry matter to C. The root biomass near ripening (9 August for winter cereals and 27 August for perennials) was estimated by multiplying the root biomass at anthesis by the ratio of root intensity at ripening versus at anthesis. This ratio was for winter rye 0.57, winter wheat 0.61, timothy 0.51, and red clover 0.78 (Fig. 4). Extra-root production was assumed to be 65% of measurable root C near ripening (Bolinder et al. 2007).

Minirhizotron (MR) determinations

One week after sowing, two transparent polybutyrate tubes (∅ 50 mm, sealed at base) per plot were inserted to a soil depth of 60 cm at an angle of 45° to the soil surface and perpendicular to the plant rows (Pietola 2005). The tubes were video-recorded six times in autumn 1998 and up to 15 (+1 for perennials) times in 1999, to a soil depth of 50 cm, as described by Pietola (2005). In addition, the roots of perennial crops were also recorded twice in 2000 (10 May, DOY 130; 12 September, DOY 255). The mean value of two tubes in a plot was used as the plot's result.

The size of the recording MR-window was 2.43 cm² (1.4 cm × 1.73 cm). The total area of recorded tube surface within the soil profile (0–50 cm) was 121 cm². Root intensities (number of roots/area) were determined as described by Pietola (2005). Following Smit et al. (1994), lateral branches were manually counted as separate roots. Summations of five successive frames were used to calculate the total root intensity for each soil layer at 5-cm intervals. Only living (white) roots were included, and all determinations were carried out by the same person. If the same root with a vertical development continued from one image to subsequent ones, it was included multiple times in the total. Root-number accounting per area from one recording session to the other gave an indication of root growth dynamics. Washed root samples were essential for gaining evidence of real root-length density and biomass. Thus, both methods are needed, and we followed the protocol developed for root studies in the Soil Biophysics Laboratory at Michigan State University (MSU).

Statistical analyses

Data on root biomass, root intensities, and root morphological parameters within each soil layer and determination time (root intensities) were subjected to analysis of variance using a randomized complete-block model with four replicates. The differences were considered significant when the probability level was ≤ 0.05. The means of plant species were compared using Fisher's least significant difference method to determine the least significant differences (LSD, $p \leq 0.05$) for each soil layer (Steel and Torrie 1981).

Results

Root growth dynamics in late autumn and early spring

After sowing in 1998, weather conditions favoured the early growth of plants. In the topsoil layer 0–15 cm, the mean root intensity of perennials was higher than that of winter cereals (Fig. 2), but the difference was statistically significant only in the layer 0–10 cm on 15 September (Fig. 2A). Roots grew deeper from one recording date to the other in the late autumn: the deepest roots reached 35 cm (Fig. 2A), 40 cm (Fig. 2B), and 50 cm depth (Fig. 2C) on 15 September, 1 October, and 3 November, respectively. Root systems of all crops reached the depth of 50 cm (Fig. 2) before soil freezing in November. The first autumn (15 September and 1 October 1998), the mean total root numbers of red clover were higher than that of timothy, winter wheat, and winter rye (68%, 122% and 250 % (1 October), respectively) in the layer 0–50 cm (Table 1). Differences were not statistically significant on 3 November. The next autumn (27 August and 3 November 1999), the mean total root number of red clover was still greater than that of timothy (+111 and +134%) in the 0–50 cm layer (Table 1), especially below the depth of 15–20 cm (data not shown).

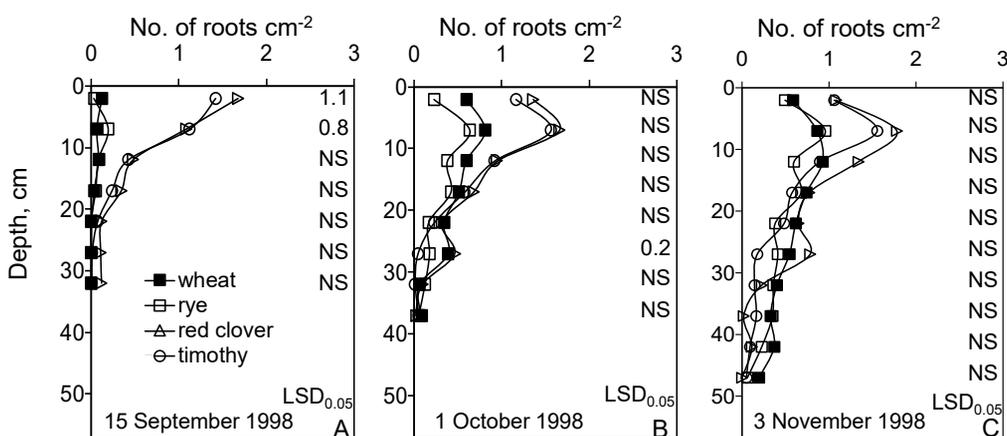


Fig. 2. Root intensity profiles (no. of roots cm⁻²) from the minirhizotron (MR) tube surface before over-wintering on 15 September (A, DOY 258), 1 October (B, DOY 274), and 3 November (C, DOY 307) in 1998. Perennial crops were sown on 28 July 1998 (DOY209) and winter cereals on 3 September 1998 (DOY 253). Least significant difference (LSD_{0.05}) for species effect in each soil layer at 5% risk level. NS = no statistically significant difference.

In springs of both post-seeding years, the number of roots was the highest for timothy (Table 1) especially in the topsoil (Fig. 3A), indicating a quick start of growth after winter. In springs, there were living roots in the whole measurement layer of 0–50 cm already before 10 May (Table 1) both years, clearly before the usual spring sowing time in Finland. The root intensity of timothy early in spring exceeded the intensity late in autumn, but for red clover, the opposite was true (Figs. 2, 3A, Table 1).

Table 1. Total number of roots within 121 cm² in the layer 0–50 cm for winter cereals and perennials before over-wintering in autumns 1998, 1999, and 2000, and just after over-wintering in springs 1999 and 2000 (n = 4).

Day	Winter wheat	Winter rye	Red clover	Timothy	p-value
15 September 1998, DOY 258	4 ^c	4 ^c	67 ^a	32 ^b	< 0.001
1 October 1998, DOY 274	41 ^b	26 ^b	91 ^a	54 ^b	0.004
3 November 1998, DOY 307	67	55	105	71	NS
20 April 1999, DOY 110	52	47	75	66	NS
28 April 1999, DOY 118	69 ^b	57 ^b	96 ^b	132 ^a	0.02
14 May 1999, DOY 134	122 ^b	122 ^b	124 ^b	250 ^a	0.002
27 August 1999, DOY 239	–	–	236 ^a	112 ^b	0.02
3 November 1999, DOY 307	–	–	248 ^a	106 ^b	< 0.001
10 May 2000, DOY 130	–	–	144 ^b	284 ^a	0.05
12 September 2000, DOY 255	–	–	224	141	NS

Comparisons with the different letters differ in significance level of 0.05; NS = no statistically significant difference; DOY = day of year

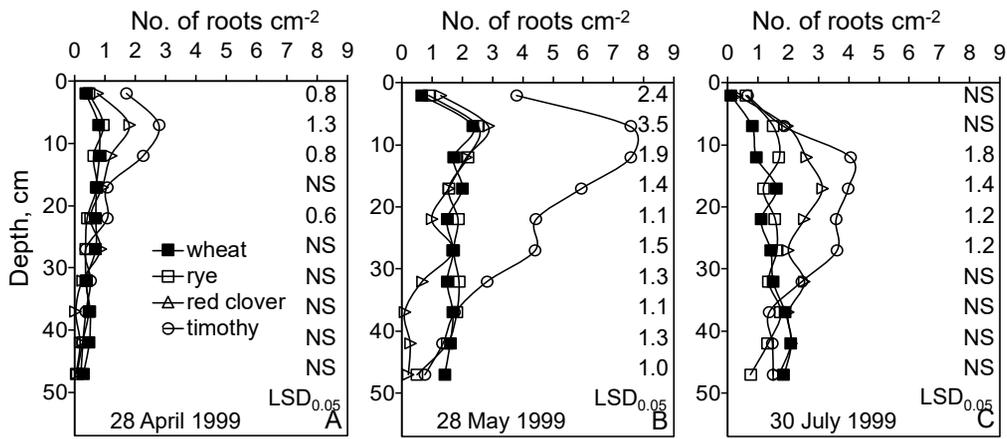


Fig. 3. Root intensity profiles (no. roots cm⁻²) from the MR-tube surface in the first post-seeding spring, on 28 April (A, DOY 118), and later during the growing season of 1999, on 28 May (B, DOY 148) and 30 July (C, DOY 211). Least significant difference (LSD_{0.05}) for species effect in each soil layer at 5% risk level. NS = no statistically significant difference.

Root and crop growth during first growing season after over-wintering

Root growth dynamics

At the beginning of the growing season, the root development of timothy was the fastest (Figs. 3A, B, and 4). Any clear effect of surface fertilization (10 May, DOY 130) was not recognized in the root growth of winter cereals. Timothy was at anthesis in the middle of June, when it had very high root intensities. The root intensities of winter cereals reached their highest values in early July at anthesis (Fig. 4). After the anthesis of winter cereals, the root intensities of red clover were significantly higher than those of winter cereals (Figs. 3C and 4).

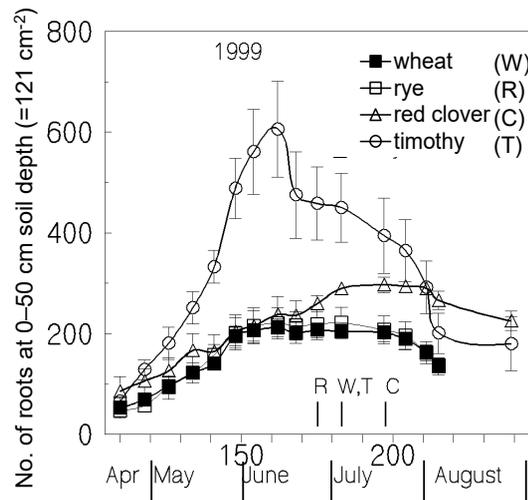


Fig. 4. Total number of roots from MR-tube surface (121 cm²) during the growing season (DOY) of 1999 after the first over-wintering period. R, W, T, and C represent the timing of soil sampling for winter rye, winter wheat, timothy, and red clover, respectively. Bar = ±SE (n = 4).

Above- and below-ground growth and estimation of below-ground carbon input of roots

At anthesis, the mean shoot biomass of winter cereals was not significantly greater than that of perennial crops (Table 2). Although growing season 1999 was mostly dry (Fig. 1B), mean harvested grain yields of 5 200 kg ha⁻¹ for winter wheat and 3 800 kg ha⁻¹ (at moisture content of 15 g 100 g⁻¹) for winter rye represented average local yields.

Table 2. Mean shoot and root biomass at crop anthesis on the first post-seeding year. Root biomass was determined from the soil layer of 0–60 cm (n = 4).

Crop, sampling day	Shoot biomass (g m ⁻²)	Root biomass (g m ⁻²)		Shoot:root ratio	
Winter wheat, DOY182	810	87 ^{ac}	87 ^c	9.3 ^a	9.3 ^a
Winter rye, DOY 174	745	72 ^c	72 ^c	10.3 ^a	10.3 ^a
Red clover, DOY 196	514	218 ^a		2.4 ^b	
- excluding tap roots ¹		125 ^b		4.2 ^b	
Timothy, DOY 182	693	182 ^b	182 ^a	3.8 ^b	3.8 ^b
<i>p</i> -value	NS	0.002	0.002	< 0.000	< 0.000

¹Tap roots were defined as root with a diameter larger than 1 mm. Comparisons with the different letters differ in significance level of 0.05; NS = no statistically significant difference; DOY = day of year

At anthesis, the winter wheat and rye root biomasses (Fig. 5) and total number of roots (Fig. 4) were close to each other. The root biomass of perennial crops was greater than that of winter cereals at soil depths above 20 cm. In the first 20 cm soil layer, the relative proportions of winter wheat, winter rye, timothy, and red clover root biomasses of the total (mass in 0–60 cm) were 60%, 61%, 73% and 83%, respectively, and in the first 30 cm layer, the relative shares were 71%, 79%, 84% and 92% (Fig. 5). In the measured soil profile (0–60 cm), the total root biomasses for red clover and timothy were 2.7 and 2.3 times greater, respectively, than for winter cereals (Fig. 5, Table 2). For red clover, 43% of total root biomass consisted of roots with a diameter of over 1 mm.

The S:R ratios of winter cereals were greater (> 9) than those of timothy or red clover (<4.2) (Table 2). The differences in total biomass (shoot+root biomass in the layer of 0–60 cm) at anthesis between crops were less than 18%, with the total biomass for winter wheat, winter rye, timothy and red clover being 897, 817, 875, and 733 g m⁻², respectively. The high S:R ratio of winter cereals was due to the lower root biomass and greater shoot biomass compared to perennials (Table 2).

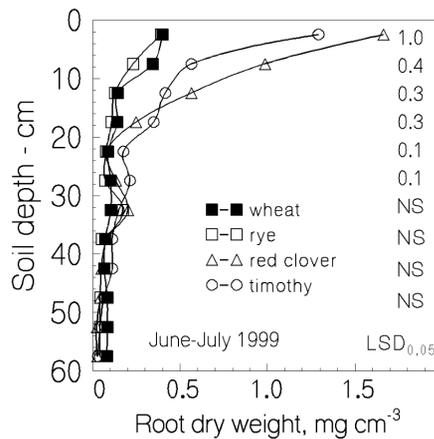


Fig. 5. Root biomass of four over-wintering crops at anthesis at different soil depths. Sampling times are presented in Figure 4. Least significant difference (LSD_{0.05}) for species effect in each soil layer at 5% risk level. NS = no statistically significant difference.

The amount of C in DM of roots at anthesis was 392, 324, 983, and 815 kg C ha⁻¹ for winter wheat, winter rye, red clover, and timothy, respectively. Estimated annual root-derived C allocations were 390, 300, 1 270, and 690 kg C ha⁻¹ for winter wheat, winter rye, red clover, and timothy, respectively.

Root morphological parameters

The root morphological parameters were measured at anthesis. The earliest anthesis was found in winter rye and the latest in red clover (Table 3). The root morphological data revealed that red clover and timothy had 84%–128% larger mean root surface area density (SA) at a soil depth of 10–15 cm than winter cereals (Table 3). Timothy had

a larger RLD than all other crops in the 10–15 and 30–35 cm depth soil layers. The mean fine root diameter of red clover was greater than that of all other crops in the 10–15 cm layer, and greater than that of timothy in the two subsoil layers.

Table 3. Washed root data for fine roots (all recorded roots had a diameter < 1 mm) from soil samples for three soil layers at anthesis (400 cm³ per soil layer). Sampling days: winter wheat 1 July (DOY 182), winter rye 23 June (DOY 174), red clover 15 July (DOY 196), timothy 1 July (DOY 182) (n = 4).

	Soil depth (cm)	Root diameter (μm)	Root length density (cm cm ⁻³)	Surface area density (cm ² cm ⁻³)
Winter wheat	10–15	278 ^b	3.0 ^b	0.25 ^b
Winter rye		264 ^b	3.0 ^b	0.23 ^b
Red Clover		373 ^a	4.1 ^b	0.46 ^a
Timothy		231 ^b	7.9 ^a	0.57 ^a
<i>p</i> -value		0.004	0.005	0.007
Winter wheat	30–35	352 ^a	1.2 ^b	0.14
Winter rye		370 ^a	0.8 ^b	0.09
Red Clover		400 ^a	0.9 ^b	0.11
Timothy		277 ^b	2.2 ^a	0.19
<i>p</i> -value		0.03	0.03	NS
Winter wheat	40–45	354 ^a	1.2	0.14
Winter rye		290 ^{ab}	1.4	0.11
Red Clover		330 ^a	1.1	0.11
Timothy		234 ^b	2.1	0.17
<i>p</i> -value		0.02	NS	NS

Comparisons with the different letters differ in significance level of 0.05. NS = no statistically significant difference

Discussion

Methodological aspects

The study was designated to compare the root dynamics of two bi-annual winter cereals and two perennial forage crops as a biological study. Therefore, the forage species were let to reach the flowering stage similarly to winter cereals. In addition, fertilization levels were adjusted accordingly. This allowed for direct comparisons between the species used. The design and chosen management options must be taken into account when discussing the practical implications of our results.

We used minirhizotrons (MR) data to compare the root intensity and rooting depth of crops over a two-year period (repeated observations from the same place). To reduce inaccuracy due to preferential root growth, the tubes were installed at an angle of 45° to the soil surface as discussed by Johnson et al. (2001). During growing seasons, the observation interval was less than two weeks as recommended by Johnson et al. (2001).

Root growth early in spring and late in autumn

According to MR determinations, winter cereals and perennial grasses had a living root system late in autumn and early in spring as acknowledged by Tufekcioglu et al. (1999) and Houde et al. (2020) for cool-season forage grasses, and by Wikler (1961), Munkholm et al. (2008), and Skovby Rasmussen et al. (2015) for winter wheat and rye. By cultivation of over-wintering crops, the soil's crop-covered period and nutrient uptake by a living root system can be extended outside the common growing season of spring sown annual crops. This has been found to reduce erosion (Soenne et al. 2016) and the leaching of nitrogen and particulate phosphorus (Jaakkola 1984, Puustinen et al. 2005). Likewise, Känkänen et al. (2003) found that undersown grasses reduced soil NO₃⁻-N content late in autumn. They concluded that on soils with a high nitrate leaching risk, overwintering perennial grasses might be more effective in their nitrate uptake than Italian ryegrass (*Lolium multiflorum* Lam. var. *italicum*).

Root growth dynamics of timothy and red clover differed markedly. In autumn, the root intensity of the dicotyledonous red clover was clearly greater than that of the monocotyledonous timothy. In early spring, the result was opposite indicating a rapid beginning of growth for timothy after winter and frost melting, a phenomenon which is typical for the leaf development and growth of timothy as well (Virikajärvi and Järvenranta 2001). This nongrowing

season activity in the roots of perennial grasses was also noted by Houde et al. (2020) with a mixture of timothy and tall fescue (*Lolium arundinaceum subsp. arundinaceum*) as an increase in total root length between the last measurement in autumn and first measurement in spring. In all, the results support the idea of using timothy as an undersown crop, where grass continues to grow after cereal and prevents from erosion and nutrient leaching on high-risk areas during a nongrowing.

One reason for the discovered difference in time \times species response in root growth dynamics was probably that the timothy ripened in July when red clover started to flower. From an agronomical viewpoint, the fast root growth of timothy in spring would imply the early fertilization of swards dominated by timothy. To enhance nutrient uptake in perennial crop covered areas, mixed crops with different growth dynamics are recommended (Nyfeler et al. 2011).

Root intensity, depth, and morphology

For winter cereals, the maximum number of 200 roots per 121 cm² was close to the maximum root intensities of spring sown barley, recorded by using the same method from a soil depth of 0–50 cm (Pietola and Alakukku 2005). In May, the typical sowing time of spring cereals in Finland, the roots of winter cereals had already reached a soil depth of 50 cm, in agreement with the results of Wiklert (1961) and Skovby Rasmussen et al. (2015). This evidently guarantees their intensive nutrient and water uptake in spring which is important in boreal area that often have a rainfall deficiency in the beginning of the growing season (e.g. Peltonen-Sainio et al. 2016). Our yield results for a dry year, especially May to July 1999, support this, since our winter cereal yields were on an average level but the spring cereal yields of surrounding fields were extremely low. In the future, a risk of spring drought is forecasted to increase in Northern Europe where precipitation in early growing season will remain low according to future projections (IPCC 2014).

The studied root intensity profile to the soil depth of 50 cm was inadequate for establishing the maximum rooting depth, which would be important regarding risk reduction in drought years. Under the artificial conditions of the Wageningen Rhizolab, Smit and Groenwold (2005) reported a higher rooting density for winter wheat at 100 cm than in our study at 50 cm. In Scandinavian conditions, maximum rooting depths of 110–160 cm for winter rye (Wiklert 1961, Kristensen and Thorup-Kristensen 2004) and 80–170 cm for winter wheat have been reported (Wiklert 1961, Skovby Rasmussen et al. 2015).

Compared to other examined crops, data on root intensity, root length density (RLD), and surface area revealed the highest values for timothy before and at anthesis. This suggests that timothy had a greater adsorption potential for immobile nutrients, like P, than other crops both in topsoil and subsoil. In subsoil, the mean RLDs of winter cereals and perennials were over 1 cm cm⁻³, which is sufficient for the daily nutrient uptake of crops (de Willigen and van Noordwijk 1987).

Root biomass and allocation in soil profile

At anthesis, the total root biomass of winter cereals was less than that of spring oats but close to that of spring barley, recorded by the same method from the same soil layer (Pietola and Alakukku 2005). Thus, winter cereals may have even less root biomass by anthesis at a soil depth of 0–60 cm than spring-sown cereals. For winter cereals, however, root biomass production lasts longer and occurs deeper in soils than for spring cereals. Compared to our results, Bolinder et al. (1997, in layer 0–30 cm) and Xue et al. (2003, in layer 0–140 cm) reported higher, and McGowan et al. (1984, in layer 0–200 cm) and Wilhelm (1998, in layer 0–120 cm) lower root biomasses for winter wheat at anthesis.

According to the data from our first post-seeding year, red clover and timothy may have twice as much root biomass at 0–60 cm (2 180 and 1 810 kg DM ha⁻², respectively) than winter wheat or winter rye (870 and 720 kg DM ha⁻², respectively). Higher root biomasses for timothy and red clover compared to annual crops have been recorded also in Hansson and Andrén (1987), Hakala et al. (2009), and Bolinder et al. (2012).

In general, in our results the total root biomass of red clover and timothy in the 0–60 cm soil layer was low compared to other studies under a cool climate (e.g. Bolinder et al. 2002, Saarijärvi et al. 2007, Mikola et al. 2009) and to other grass species under a temperate climate (Cougnon et al. 2017). For example, in two Finnish field experiments in the same geographic location, the mean estimates for root biomasses under mixed pastures (in layer 0–10 cm) of timothy and meadow fescue (*Festuca pratensis* Huds.) were 3 800 kg DM ha⁻² according to Mikola et al. (2009) and 7 320 kg DM ha⁻² in layer 0–15 cm according to Saarijärvi et al. (2007). Also, Bolinder et al. (2002)

found a higher root biomass for red clover in layer 0–45 cm (7 400–8 480 kg DM ha⁻²) than in our study. Instead, Känkänen et al. (1999, in layer 0–20 cm) reported considerably varying root biomasses (1 300–3 900 kg DM ha⁻¹) for green manure red clover between experiment treatments and sites. Contrary to our results, Steen (1989), by using long-lasting nylon cloth bags at a depth of 0–30 cm, and Bolinder et al. (2002) in field study, observed a higher root biomass for timothy (1 740 kg DM ha⁻² and 13 510 kg DM ha⁻², respectively) than for red clover (1 160 and 7 940 kg DM ha⁻², respectively).

In all, there are several factors affecting the root dynamics of plants, such as soil properties, prevailing temperature and precipitation as well as management factors such as defoliation, fertilization, irrigation, and drainage (Goss and Watson 2003). In our experiment, the relatively low root biomasses of timothy and red clover may have been affected by a low fertilization level and a low cutting frequency. It is well known that plants alter their C partitioning to favour root growth instead of shoot growth under low nutrient availability, especially that of N and P (Belanger et al. 1994, Hermans et al. 2006, Poeplau et al. 2018). But at the same time, the direct effect of N fertilization on absolute root mass is usually positive (Loiseau and Sousanna 1999, Cougnon et al. 2017). For example, in the study by Cougnon et al. (2017), increasing N fertilization from 190 to 300 kg ha⁻¹ year⁻¹ increased the root mass of all studied species in the 5–45 cm soil layer, but not in the deeper layers (45–90 cm). For meadow fescue, the increase in root mass due to increased N fertilization was 57%. The N rate used in our experiment (60 kg N ha⁻¹) was low for timothy compared to the rate typically used in silage production in Finland (150–240 kg N ha⁻¹ year⁻¹; Virkajärvi et al. (2015) and references therein). It is therefore plausible that it has affected the measured root mass negatively.

In addition, root biomass is affected by defoliation, and the effect depends on the amount, type, and frequency of defoliation, the species, and the plant's growth environment (Richards 1993, Goss and Watson 2003, Mikola et al. 2009). Typically, silage grasses in Finland are fertilized and harvested two or three times per growing season (Virkajärvi et al. 2015), but in this experiment the crops were allowed to grow undefoliated until September, and root mass sampling was done at late anthesis. Therefore, our results for root dynamics and root mass are not directly comparable to studies with frequent defoliations.

Relevant to this, conventional root sampling and washing have been found to clearly underestimate the biomass allocated to roots compared to the ¹³C-labelling method (Subedi et al. 2006, Pausch and Kuzyakov 2018). Bolinder et al. (2007) argued that the uncertainty of root biomass estimates that were used to calculate below-ground net primary production and below-ground C was substantial. They pointed out that there is a certain need for additional field measurements to reduce the uncertainty in root biomass estimates. Differences in sampling procedures (depth, sample size, number of samples) and variation in growing conditions may cause great difficulties in comparing root biomasses from different studies, especially in topsoil layers.

The root intensities (minirhizotron) and root biomasses (soil sampling) of winter cereals were quite similar at different depths in the layer of 0–50 cm, but the intensities and masses of perennial roots were higher in topsoil than in deeper layers. The relative root amount of winter cereals in a topsoil of 20 cm was less than has been found for spring barley (70–75% in topsoil, Hansson and Andrén 1987) and for spring oats (80%, Pietola and Alakukku 2005). Kätterer et al. (1993) reported that 30% of winter cereal root biomass was located below the depth of 25 cm. In agreement with our results, McGowan et al. (1984) found that at anthesis, 60–70% of the root length and biomass of winter wheat occurred within 0–30 cm and 20–25% within the next 30 cm.

Correspondingly, Steen (1989) found in the second ley year that the root biomass in topsoil (0–30 cm) was 76% and 64% of total for red clover and timothy, respectively. Skinner and Comas (2010) found in a pot study that 18% to 25% of root biomass of perennial grasses and legumes was below the depth of 30 cm. Similarly, Hansson et al. (1991) found that the root biomass of lucerne (*Medicago sativa* L.) was greater below the plough layer than that of meadow fescue (*Festuca pratensis*).

There are evident differences between plant species in the depth distributions of root intensity and biomass (Bolinder et al. 2002, Cougnon et al. 2017). Relevant to this, Lorentz and Lal (2005) argued that subsoil horizons may have a high capacity for sequestering organic carbon. Like Ward et al. (2016) recently estimated, 60% of soil C in a one meter layer was below 30 cm in grasslands in the United Kingdom. When the allocation of below-ground biomass and soil C sequestration are evaluated, a soil depth greater than 30 cm needs to be further investigated.

S:R –ratio and estimated rootderived C allocation into soil

First, it must be noted that the determination of S:R can vary between different experiments due to different objectives. In this experiment, the S:R ratio was determined at anthesis for each species. For forage grasses and legumes, however, it is possible to calculate the S:R ratio over the whole growing season (e.g. Bolinder et al. 2002). Obviously, these methods will give different estimates of S:R.

Compared to our S:R ratios, Bolinder et al. (1997, 2002) reported similar ratios for winter wheat but lower ratios for red clover (1.01) and timothy (1.19) for the first production year in Canada (depth 0–45 cm) than we found. In agreement with our results, the S:R ratios of winter cereals (Bolinder et al. 1997) have been reported to be greater than the S:R ratios of red clover and timothy (Känkänen et al. 1999, Huss-Danell and Chaia 2005, Bolinder et al. 2002), even though there were differences in root sampling depths. It must be noted that in the case of perennial forages that are harvested several times per season, the S:R ratio and C allocation vary within a considerable range during the growing season (Bélanger et al. 1994) and among production years (Hansson and Andréén 1986, Bolinder et al. 2002). Especially defoliation radically changes the photosynthetic capacity, S:R ratio, and C allocation of a tiller (Richards 1993). In our study, timothy and red clover were harvested only once during the growing season, and fertilization was low, especially for timothy (60 kg N ha⁻¹), compared to the typical recommendation of 150–240 kg N ha⁻¹ year⁻¹ or the mean average of 155 kg N ha⁻¹ year⁻¹ used on farms (e.g. Virkajärvi et al. 2015). An increase in N supply increases shoot growth more than root growth, and thus the use of a low N fertilization level may have decreased the S:R of timothy (Belanger et al. 1994, Whitehead 1995).

We roughly evaluated the annual below-ground C input of roots based on root biomass at anthesis. The estimated annual root-derived C allocations were 390, 300, 1 270 and 690 kg C ha⁻¹ for winter wheat, winter rye, red clover, and timothy, respectively, when extra-root production was included in the calculations. Kuzyakov and Domanski (2000) examined below-ground C translocation by using a tracer technique. They found that crop translocated C (sum of root biomass and exudates) was 1 500 kg C ha⁻¹ for wheat and barley and 2 200 kg C ha⁻¹ for grasses during the growing season of crops; especially for wheat and barley, these figures are higher than in our study. In Sweden, Bolinder et al. (2012) estimated a mean below-ground C input of forage roots of 1,430 ± 400 kg C ha⁻¹ year⁻¹ in post-seeding years, which is very similar to our estimates for red clover. One reason for the low C-input estimates for red clover and timothy is the above-mentioned management (one cut, low N fertilization). In addition, the factor used for estimating root exudated carbon (0.65 × root biomass carbon; Bolinder et al. 2007) is typically derived from experiments in which forages were cut and fertilized several times in a season.

Increasing soil C storage is a complex process that involves biological activities (plants, micro-organisms, and other soil fauna) and abiotic processes related to soil physical processes (Dignac et al. 2017, Smith et al. 2020, Poeplau 2021). Increasing evidence, however, shows that a key agronomic factor is the C input of crop root systems and root interaction with the soil microbial community: i.e. the amount and location (depth) of C input, as well as the physical structure and chemical composition of DM input (Rasse et al. 2005, Palosuo et al. 2016, Dignac et al. 2017), and the soil organic matter formation in the microbe driven decomposition pathway (Cotrufo et al. 2015). For example, Rasse et al. (2005) and Kätterer et al. (2011) estimated the mean residence time of root-derived C to be 2.3–2.4 times longer than that of shoot-derived C. As discussed by Cotrufo et al. (2013, 2015) and Dignac et al. (2017), the potential of long-term soil C storage increase of arable crops must be evaluated based on soil organic matter formation from root and shoot litter via biochemical and physical pathways.

Conclusions

There were clear differences in root growth dynamics between red clover and timothy, and between biannual winter cereals and perennials. The root growth of timothy started in the topsoil layer quickly after snow melted in spring, which is why timothy-dominated swards would benefit from early fertilization. Compared to timothy, the root system of red clover was dense in the subsoil late in autumn, indicating a better nutrient uptake potential from deeper layers. The mean root intensity of winter cereals decreased less than that of perennials as a function of depth. The roots of winter cereals reached a depth of 50 cm in autumn and continued root growth from that depth early in spring, suggesting good possibilities for water and nitrogen uptake from deep soil layers early in spring and during the growing season compared to spring cereals.

At anthesis, the four investigated species ranked in descending order according to root length density, surface area, and biomass: timothy>red clover>winter wheat> winter rye. Based on the shoot:root ratios, red clover appeared

to offer the most intense C sink at a soil depth of 0–60 cm. Our data indicate the potential of over-wintering crops as root-driven C sinks in early and late seasons. Knowledge concerning root systems of different species under different managements and pedoclimatic conditions is important for both enhancing C storage in agricultural soils as well as for developing more accurate soil C models.

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