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The use of the ingrowth core method for measuring root production of arable crops – influence of soil and root disturbance during installation of the bags on root ingrowth into the cores

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Abstract

To measure root production by the ingrowth core method mesh bags filled with root-free soil are buried into the root zone of plants. After a time period which should be shorter than the lifespan of the roots the mesh bags are pulled out and root length inside the cores is determined. A major objection against this method is a possible alteration of root growth pattern induced by root injuries or soil disturbances that occur during the insertion of the mesh bags into the soil. Root length density (RLD) in the mesh bags was between 0.7 and 5.0 cm cm^{-3} after 14 days of ingrowth of wheat or barley roots depending on soil type or plant age. RLD in the bulk soil next to the mesh bags was between 7.7 and 14.8 cm cm^{-3} in the 0–30 cm soil depth. Different time periods between inserting the mesh bags and opening them for root ingrowth in which the installation disturbance could settle, had no effect on RLD in the ingrowth cores. There were also no differences in RLD in the direct vicinity around the ingrowth cores and the bulk soil, which was tested by counting roots at profile walls adjacent to the mesh bags and by taking soil samples around the cores with an auger. The conclusion of these results was that no alteration of root growth pattern occurred in or around the ingrowth cores due to the installation of the mesh bags. However, further investigations are necessary to validate this method as a reliable and easy field method for measuring root growth. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The investigation of root systems of arable crops is often limited to one or only a few sampling dates because this work is labour-intensive. To quantify root biomass, length, or surface area

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soil samples are usually taken with an auger, the roots are washed out carefully and length or surface area is measured (Böhm, 1979; Kücke et al., 1995). This method reveals the size of a standing root system at a specific date or the development of a root system during a time interval. However, it is known that parts of root systems senesce and die during plant development and are replaced by growth of new roots (Steen, 1985; Cheng et al., 1990; Swinnen et al., 1995b; Goins and Russelle, 1996). The root development which is measured by sequential auger samplings is, therefore, only the net result of root birth and death. Sometimes knowledge about the actual growth of roots, i.e. the gross growth, is of more importance than the net growth, e.g. for investigations of carbon or nutrient flows in the plant and rhizosphere or for modelling root growth. However, gross root growth and/or root death and decay cannot be reliably determined by the conventional auger sampling methods.

Attempts to measure gross root growth and root decay of arable crops are scarce, probably because there is no existing reliable and easy field method. Sauerbeck et al. (1975) and Sauerbeck and Johnen (1976) determined the carbon distribution between shoot, root growth, root respiration and root decay of wheat and mustard plants by continuously labelling with ^{14}C in pot experiments. But the transferability of root data obtained in pot experiments to field conditions is doubtful. Under field conditions a ^{14}C pulse labelling is more appropriate like it was used by Swinnen and co-workers for wheat and barley grown in conventional and integrated farming systems (Swinnen, 1994; Swinnen et al., 1995a,b). However, this technique is not suitable for widespread field experiments. The release of radioactive isotopes in the field is often restricted by authorizing procedures and only single plants or small plots can be labelled, which causes statistical implications.

Another method to measure gross root growth and root decay that is suitable for field experiments is the use of minirhizotrons (Cheng et al., 1991). Minirhizotrons allow a non-destructive view of roots and their growth patterns, but the quantification on a field scale is still a problem (McMichael and Taylor, 1987; Upchurch, 1987). A comparison between root counts using minirhizotrons and root length density (RLD) obtained by auger sampling resulted in a correlation coefficient of only 0.72 for a wheat experiment (Box and Ramseur, 1993). Problems that may occur from an improper installation of minirhizotrons are reviewed by Box (1996). Van Noordwijk et al. (1994) tried to avoid improper installations by using inflatable minirhizotron tubes and by calibrating the minirhizotron data with data obtained by the conventional auger sampling method. The amount of carbon translocated to the roots they measured was similar to that obtained by Swinnen (1994).

In forestry or grassland research, yearly fine root production is sometimes measured by the mesh bag or ingrowth core method (Persson, 1980, 1983; Steen, 1984; Ahlström et al., 1988; Vogt et al., 1998). Bags made of nylon nets are filled with root-free sieved soil and buried in the rooting zone. After a period of time (from a few weeks up to more than a year), these bags are taken out and parameters of the ingrown roots can be determined. Steen and co-workers used this method also to investigate the influence of herbicides on sugar beet root growth (Steen and Al-Windi, 1984; Steen et al., 1984) or the influence of soil compaction on rape and wheat root growth (Steen and Håkansson, 1987).

Under the assumption that root growth into the ingrowth cores is the same as in the bulk soil, this method would be an easy to handle field method to measure gross growth. However, for measuring gross root growth, the death or decomposition of roots in the ingrowth cores must be avoided. This can be done by reducing the time of ingrowth. Data on the root lifespans of annual crops are scarce (see review of Eissenstat and Yanai (1997)). Krauss and Deacon (1994) measured a half-life of individual groundnut roots of about 4 weeks. A similar half-life can be calculated from the data, van Noordwijk et al. (1994) presented for sugar beet. Therefore, the time of ingrowth into the mesh bags should not exceed 4 weeks with 10–20 days preferred.

There are still several objections against the ingrowth core method for measuring gross root growth (Steen, 1991; Vogt et al., 1998). It is not possible to adjust the same soil conditions inside the mesh bags as outside. However, by altering soil density, N, P, K, and soil moisture content of the soil filled into the bags Steingrobe et al. (2000) showed that only a high soil density and a very high N content compared to the bulk soil decreased and increased root ingrowth of rape, respectively, compared to control bags. Another problem may occur by inserting the mesh bags. Drilling the hole may lead to a smearing and/or compaction of the hole walls which may reduce root ingrowth. Furthermore, roots are injured by inserting the bags and little is known about the reaction of annual plants on root injury. Joslin and Wolfe (1999) measured an increased root elongation in a forest ecosystem during the first year after inserting minirhizotrons compared to 2 years later. They assigned this to a plant reaction on root injury and/or to N rich microsites near the minirhizotron due to an increased mineralisation. An enhanced root ingrowth into the mesh bags may be avoided by burying the bags and protect them against root ingrowth by an inserted tube as long as roots need to show normal growth patterns after injury and as soil disturbances around the bags need to settle. But by doing this an enhanced root growth may have lead to a greater RLD around the mesh bags compared to the average density, which also may lead to a faster ingrowth into the cores compared to the growth pattern in the undisturbed soil.

The objective of this work was to investigate the influence of soil and root disturbances due to installation of the mesh bags on root growth in the ingrowth cores and in the direct vicinity of the core. This was part of a series of experiments to prove the ingrowth core method as an easy to handle field method for measuring root gross growth.

The influence of the installation disturbance on ingrowth into the cores was tested by protecting the mesh bags against ingrowth with a tube inside the bags. By doing this, different periods of settlement could be adjusted, assuming that the growth enhancing effect of the disturbance is more pronounced when it is fresh. The root development around the mesh bags was tested by digging profile walls along the ingrowth cores and by taking soil samples around the cores with an auger to compare RLDs in the direct vicinity of the cores and the bulk soil.

2. Materials and methods

The experiments were conducted in Scheyern and Freising (Bavaria, Germany) as a part of the Agroecosystem Research Network Munich (FAM, Forschungsverbund Agrarökosysteme München). The experimental site is hilly with a variation of different soil types. Spring wheat was grown in 1995 on a sandy loam. In 1996, three different stands of winter wheat on loamy sand, silty loam and sandy loam and a stand of potatoes on a loamy sand were used for the investigations. In 1997, winter barley was grown on a sandy loam. Nutrient supply was sufficient according to the German fertilization recommendations for all crops.

2.1. Ingrowth core method

Mesh bags were sewn using a synthetic fibre net with a mesh size of about 3 mm. The bags had a diameter of 4 cm and were about 45 cm long. For inserting them into the soil, they were pulled onto a PVC-tube also 4 cm in diameter and about 50 cm long. A hole was drilled into the soil with a 4 cm hand auger at an angle of 45° to soil surface and the tube covered by the mesh bag was pushed in. In this way the mesh bag covered a soil depth of 0–30 cm. The bags were placed between the rows of cereals in parallel to the rows to avoid too much harm to the young plants by drilling the holes. The bags in the potato experiments were placed in the ridges where most of the roots are located. There was a distance of at least 1 m between each bag. Depending on the experiment, the mesh bags were filled immediately or the tubes were closed by end-caps and left undisturbed in the soil for different time periods. For filling the mesh bags, soil was not taken directly out of the drilled hole but from the experimental site (0–30 cm soil depth) and sieved to get out stones, roots and organic debris. The tube was pulled out a few centimetres and a handful of soil was filled in. This soil was compacted to an average density comparable to the bulk soil density by a wooden stick. This procedure was repeated until the mesh bag was completely filled. The bulk soil densities of the different sites were determined before by weighing soil cores of a known volume. The desired soil density in the bags was reached by weighing the soil filled into the bags according to the bag volume and the soil water content.

In 1995, mesh bags were inserted into the soil 0 (EC 65), 19 (EC 47), 40 (EC 32) and 69 days (EC 23) before opening them for root ingrowth on 4th of July. In 1996, the opening of the mesh bags was on 3rd and 12th of June with an insertion of the bags 0 (EC 51), 12 (EC 32), 26 (EC 28) and 41 days (EC 22) and 0 (EC 55), 12 (EC 41) and 36 days (EC 22) before, respectively. In 1997, opening was on 30th of May and the bags were inserted 0 (EC 67), 18 (EC 33), 46 (EC 30) and 60 days (EC 27) before.

After 14 (winter wheat, 1996), 16 (spring wheat, 1995) and 19 (winter barley, 1997) days of ingrowth the mesh bags were pulled out and the roots were washed out carefully from the soil over a 200 μm sieve. Afterwards, root length was determined by a line intersection method according to Newman (1966). By relating the measured root length to the volume of the mesh bag the average RLD (RLD, cm root cm^{-3} soil) was calculated.

2.2. Profile walls

For measuring RLD around the mesh bags, three profile walls were dug in a stand of potatoes and four profile walls in the winter barley. Two of the 'barley walls' were dug along mesh bags that were opened immediately after inserting them, so that at the time the profiles were set the installation disturbances were about 14 days old. The other two profiles were at mesh bags opened 68 days after insertion. For potato, ingrowth cores opened several weeks after insertion were chosen. The walls were dug around the bags so that half of the bags were still stuck into the soil. After smoothing the profile wall, about 3 mm of soil was sprayed off to make the roots visible. The correct thickness of this 3 mm layer was controlled by nails that were pressed into the profile wall. A grid with 2 cm line distance was fixed at the wall and root length in each grid square was determined by counting root length units of 5 mm. This method is described in detail by Böhm (1979) and Kücke et al. (1995). RLD was calculated from the root length per surface area of the profile wall and a soil depth of 3 mm which had been sprayed off. RLDs in a distance of 2 cm to the mesh bags were compared to the average densities of the rest of the profile wall in increments of 10 cm soil depths.

In addition, soil samples directly around the mesh bags were taken with a soil core auger. This auger had a diameter of 8 cm and was placed directly over the mesh bag. In this way, the core contained the mesh bag in the middle and 2 cm of the surrounding soil. To quantify the root density in the undisturbed soil, samples were also taken with an auger at a distance of 0.5–1 m from the ingrowth cores. Roots were washed out and root length was determined as described above.

At each installation date in 1995, 10 mesh bags were inserted and each bag was taken as a replicate. For the cereals in 1996 and 1997 there were three different plots as replicates and for each plot and 'installation date' four mesh bags were inserted. These four bags per plot were joined together for root length determination. ANOVAs were performed for each year and soil type separately with the installation date as single influence factor. Profile walls were replicated two or three times for barley and potato, respectively. The Tukey test was used to compare the treatment means. Soil cores directly around the bags were taken with four replicates for each crop and installation date. ANOVAs were performed for each crop, soil depth, and installation date separately to compare RLDs in 2 cm distance around the bags and in the bulk soil.

3. Results and discussion

A major objection to the ingrowth core method is the assumption that the soil and root disturbance while inserting the mesh bags could alter root growth pattern. Joslin and Wolfe (1999)

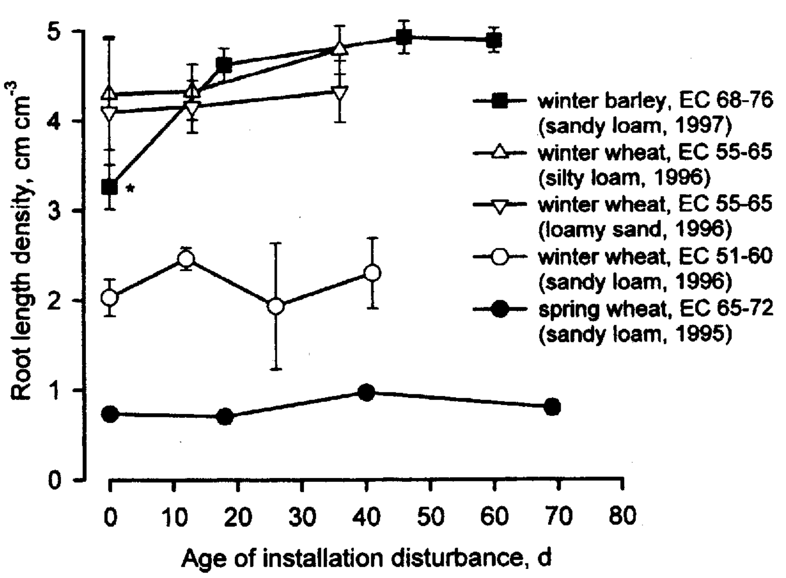

Fig. 1. Influence of the age of the installation disturbance on RLD in the ingrowth cores. The different ages were obtained by different time intervals between inserting the bags and opening them for root ingrowth. All bags of each treatment were opened at the same time. Growth stages during which roots grew into the bags are given in the legend. Bars reflect standard error, which is not shown if smaller than symbol size; spring wheat in 1995 had 10 replicates instead of 3–4. (*) Significant different from other means of the treatment ($P = 0.05$).

measured an enhanced root growth in a forest ecosystem due to the installation of minirhizotrons. Such a growth stimulation would lead to an overestimation of root gross growth using the ingrowth cores method especially when the period the cores are open for root ingrowth is rather short. However, it can be assumed that this growth stimulation is more pronounced immediately after disturbing the soil–root system and calms down with time. Fig. 1 shows the RLDs inside the ingrowth cores determined for different cereal crops and years. All mesh bags of each crop were filled with soil and opened for ingrowth at the same time (day 0), but the mesh bags were inserted into the soil at different dates before and were protected against ingrowth by a PVC-tube. By this, the installation disturbances had different ages at the opening of the bags.

The RLD measured in the ingrowth cores varied between 0.7 and 5.0 cm cm^{-3} depending on the crop and growing conditions. The rather low root growth of the spring wheat grown in 1995 is caused by the late opening time of the ingrowth cores in the vegetation period (4th July). At this time the vegetative growth of the plant was completed (EC 65) and, thus, root growth was reduced. The lower root growth of winter wheat grown 1996 on sandy loam compared to the other winter wheat crops of this year corresponds with the smaller standing root system of this treatment (Table 1). This might be due to different growing conditions and microclimates of the specific experimental site, which is located 40 km apart from the other sites.

However, of more relevance for this study is the influence of the time period between inserting and opening the bags on the RLD inside the ingrowth cores. With the exception of winter barley in 1997, there was no influence of the age of the installation disturbance on root ingrowth at all. For winter barley, ingrowth in the recently installed bags was even lower than at older installations. This indicates that neither root injury nor soil disturbance due to the bag insertion had an influence on root growth into the bags. It seems also unlikely, that root growth in the direct vicinity of

Table 1
RLD in the bulk soil of several cereals at the beginning and at the end of the time period the ingrowth cores were opened for root growth

Crop	Soil	Year	Root length density	
			Beginning cm cm^{-3}	End cm cm^{-3}
Winter barley	Sandy loam	1997	6.4 (0.5)[a]	7.7 (0.2)
Winter wheat	Silty loam	1996	14.3 (0.4)	12.3 (0.8)
Winter wheat	Loamy sand	1996	13.4 (0.7)	14.8 (1.3)
Winter wheat	Sandy loam	1996	8.4 (1.7)	8.9 (0.3)
Spring wheat	Sandy loam	1995	11.5 (0.8)	11.1 (0.5)

[a] Standard error, $n = 4$.

the ingrowth cores was enhanced as long as the bags were protected against ingrowth by a plastic tube. Such an enhancement of root growth around the bags should have resulted in higher RLDs inside the ingrowth cores, which was not determined.

However, to confirm this deduction profile walls around ingrowth cores of potato and barley were dug and root length in 2×2 cm^2 of a grid closer to and further away from the cores was counted. Fig. 2 shows three examples, one for potato with an installation disturbance that happened several weeks ago and two for winter barley with a recent (14 days) and an old bag installation (82 days). In no case differences in root length between the direct vicinity of the cores and locations further away were visible.

Taking into account a depth of the profile of 3 mm, RLDs could be calculated. This was done for soil within a 2 cm distance of the cores and averaged for the rest of the profile wall in steps of 10 cm soil depth (Fig. 3). With the exception of a single soil depth (10–20 cm) in case of recently installed bags for barley there were no significant differences in RLD between soil around the cores and the bulk soil, which confirms the findings given in Fig. 2. There seemed to be a slight trend ($P = 0.19$) for potato towards higher RLDs in the vicinity of the cores. But for barley, this trend was in the opposite direction. The greatest differences in RLD were for recently installed barley bags. Consistent with the results shown in Fig. 1, the RLD was reduced near the cores. This might be the reason for the low ingrowth into the recently installed bags shown in Fig. 1 for barley grown in 1997.

To confirm these results another attempt was made by measuring the RLD in soil samples taken within a distance of 2 cm around the ingrowth bags with an auger. This was compared with the RLD of undisturbed soil nearby (Fig. 4). The results were comparable to those obtained with the profile wall method. There was no significant difference between RLDs near the cores or further away in case of potato and the older bag installation of barley. Only in the case of recently installed bags, the RLD of winter barley around the ingrowth cores was significantly lower than in the bulk soil. Thus, an increased root development around the tube-protected mesh bags like it was reported by Joslin and Wolfe (1999) for minirhizotrons in a forest stand could not be determined for potato and barley. In contrary, a recent disturbance of the soil and/or fresh root

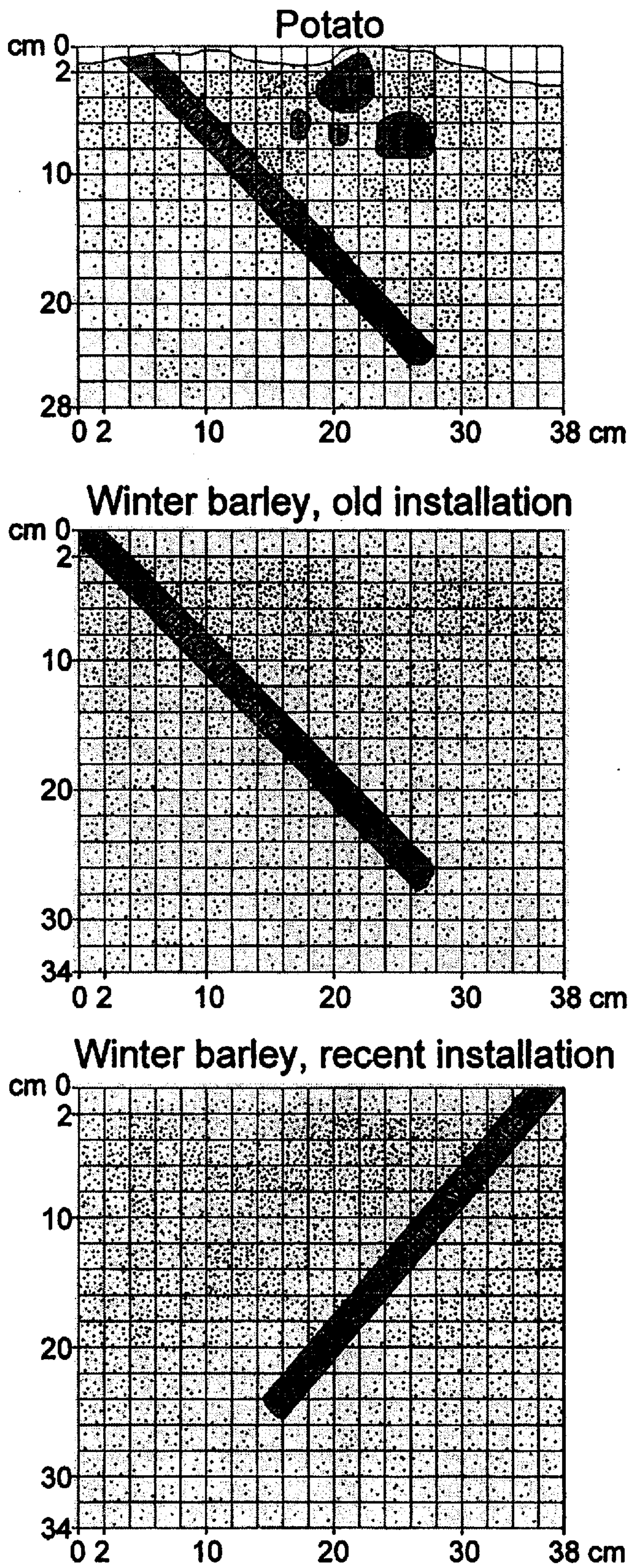

Fig. 2. Root counts at profile walls along ingrowth cores of potato and winter barley (EC 76) with old and recent disturbance due to installation of the ingrowth cores. The walls were placed in a way that one half of the cores still stuck in the soil. Each dot represents a root counting unit of 5 mm (t = potato tuber).

injury seems to reduce root length of barley around the bags and the root ingrowth into the bags.

For potato, the absolute RLD obtained with the auger (Fig. 4) was comparable to the average RLD of the profile wall (Fig. 3). This should not necessarily be the case as Böhm (1979), Kücke et al. (1995) pointed out. In most cases RLD, is underestimated by counting roots at the profile wall, as was the case for barley in our findings. Böhm (1979) assumed that this might be due to an adherence of fine roots to thicker ones, when they were still wet after the preparation of the profile wall.

4. Conclusions

The results presented showed that the soil and root disturbance during the installation of the mesh bags does not enhance root growth, neither in the vicinity of ingrowth cores nor in the cores itself. In the case of winter barley a trend in all experiments was visible that the ingrowth core method leads to an underestimation of gross root growth rather than to an overestimation. Such a trend was not given for potato, winter and spring wheat. Therefore, root injury and soil alteration associated with installing the bags should not preclude this method from being adapted.

The advantage of the ingrowth core method for measuring gross root growth would be its simplicity. There is no need for special equipment and the use of isotopes can be avoided. This method allows experiments with several treatments in the field and enough replications to run statistical analysis. It has already been shown that this method is not sensitive against an altered soil condition inside the bags compared to outside with the exception of a much higher N content and higher soil density (Steingrobe et al., 2000). Both can be avoided. However, there is still no

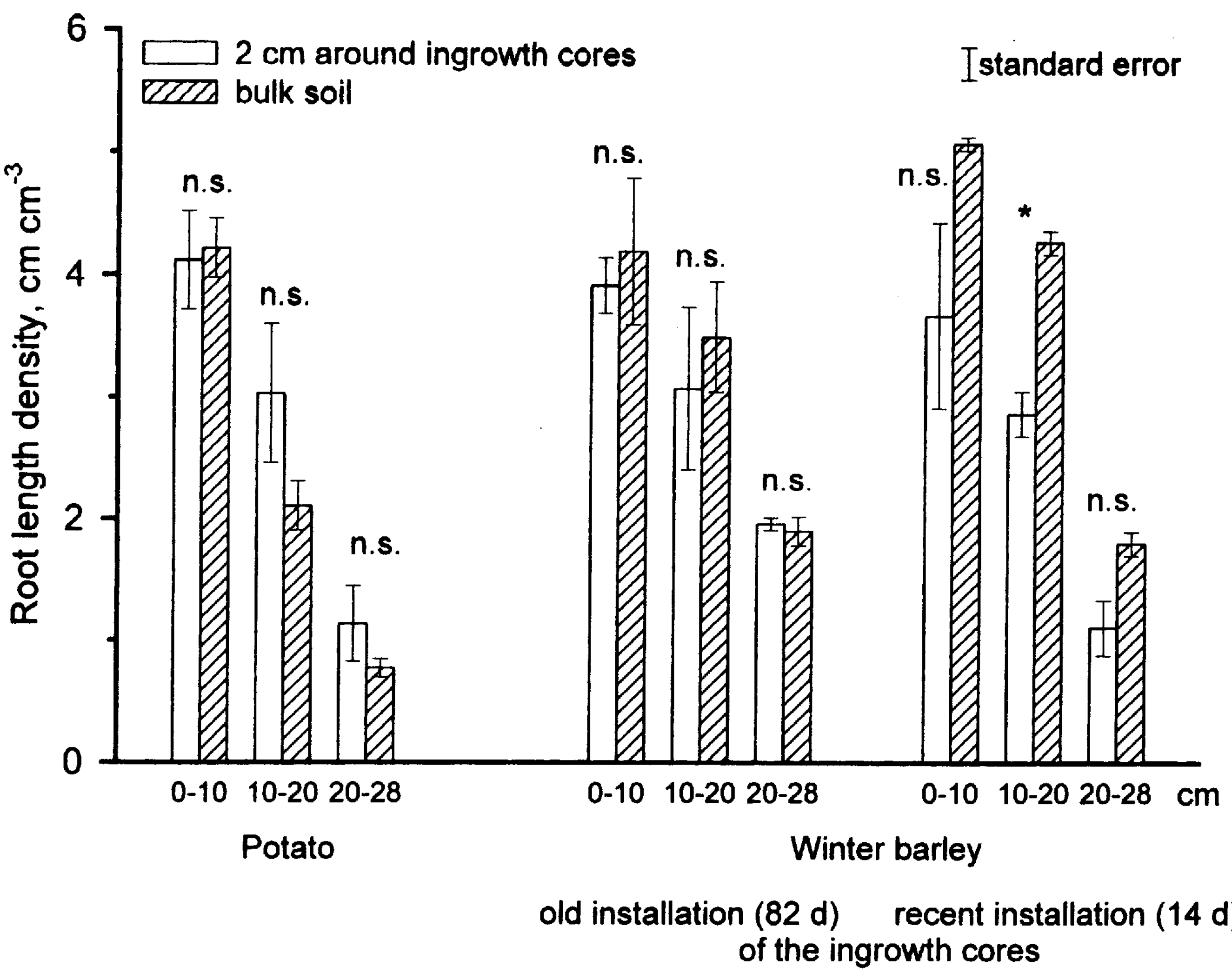

Fig. 3. RLD of potato and winter barley (EC 76) in different soil layers (0–10, 10–20, and 20–28 cm) measured at profile walls within 2 cm distance from ingrowth cores and in the bulk soil (n.s., not significant; (*) significant at $P \leqslant 0.05$; $n = 3$ for potato, $n = 2$ for barley).

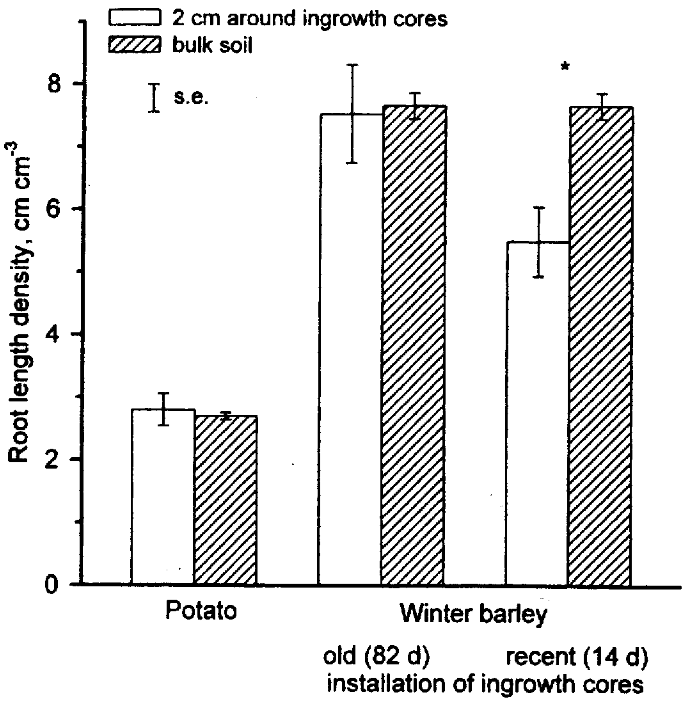

Fig. 4. RLD of potato and winter barley (EC 76) in soil samples taken with an auger around ingrowth cores and in the bulk soil (soil layer 0–30 cm; (*) significant at $P \leq 0.05$; $n = 4$).

clear evidence that root growth into the ingrowth cores is the same as gross root growth of the whole root system.

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