The use of the ingrowth core method for measuring root production of arable crops – influence of soil conditions inside the ingrowth core on root growth

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Summary – Zusammenfassung

The ingrowth core method can be used to measure root gross growth (i.e. root production). A mesh bag filled with root free soil is buried into the root zone. After about 14 days, the bag is pulled out and root length inside the core can be determined. An objection against this method is the inability to obtain the same soil conditions inside the bag as outside, which can result in different root growth pattern in the ingrowth core compared to the bulk soil. To study this, mesh bags were buried in a stand of oilseed rape and were filled with soil at different nitrate, phosphate, moisture, and bulk density levels. Results showed that root growth was only influenced by a high nitrate content and a high soil density in the cores, which resulted in higher and lower root length densities (RLD), respectively. In a long-term ingrowth experiment similar root length densities in the cores and in the bulk soil were measured, indicating that there were no root growth enhancing or impeding conditions inside the ingrowth cores. The conclusion is drawn, that the ingrowth core method gives reliable results, provided the N content and the soil density inside the bags are comparable to the bulk soil.

Key words: Ingrowth core / nitrate / phosphate / root growth / soil density / soil moisture / soil texture

Die „ingrowth core"-Methode zur Messung der Wurzelproduktion von Nutzpflanzen – Der Einfluss der Bodenbeschaffenheit innerhalb des „ingrowth core" auf das Wurzelwachstum

Die „ingrowth core"-Methode wird genutzt, um das Bruttowachstum von Wurzeln zu bestimmen. Hierfür werden Schläuche aus Netzgewebe (Strümpfe) mit wurzelfreiem Boden gefüllt und im Bestand vergraben. Nach ca. 14 Tagen werden die Strümpfe herausgezogen und die Wurzellänge darin bestimmt. Ein möglicher Einwand gegen diese Methode ist, dass innerhalb der Strümpfe nicht dieselben Bodeneigenschaften vorliegen wie im Restboden. Dies könnte das Wurzelwachstum verändern. Um dies zu prüfen, wurden Strümpfe in einen Rapsbestand eingebracht. Dabei wurden der N-, P- und Wassergehalt, sowie die Bodendichte variiert. Ein Einfluss auf das Wurzelsystem konnte nur bei einem hohen N-Gehalt und einer hohen Bodendichte gefunden werden, die das Wurzelwachstum anregten bzw. behinderten. In einem weiteren Versuch, in dem der Boden innerhalb der Strümpfe über einen langen Zeitraum durchwurzelt werden konnte, stellten sich innerhalb und außerhalb der Strümpfe vergleichbare Wurzellängendichten ein. Auch dies zeigt, dass innerhalb der „ingrowth cores" keine generelle Förderung oder Hemmung des Wurzelwachstums gegeben war. Es wird der Schluss gezogen, dass die „ingrowth core"-Methode geeignet ist, das Bruttowachstum von Wurzeln zu messen, wenn sich der N-Gehalt und die Bodendichte innerhalb der Strümpfe nicht zu stark vom Restboden unterscheiden.

1 Introduction

Plant roots die during the vegetation period and are replaced by new roots (*Sauerbeck* and *Johnen*, 1976; *Cheng* et al., 1990; *van Noordwijk* et al., 1994). Therefore, we have to distinguish between actual root growth, i.e. root gross growth, sometimes called root production, and net growth of a root system, which results from the difference between gross growth and root mortality. The net development of a root system can easily be measured by subsequent auger sampling, which gives a good indication of the size of the standing root system (*Böhm*, 1979). Determination of root gross growth is more difficult. Attempts were made using minirhizotrons or radioactive isotopes (*van Noordwijk* et al., 1994; *Sauerbeck* and *Johnen*, 1976; *Swinnen* et al., 1995a/b). However, the suitability of minirhizotron data to quantify root growth on a field scale is still doubtful (*Box*, 1996) and the use of isotopes is restricted to single plants or very small plots.

A promising approach for measuring root gross growth on a field scale is the ingrowth core or mesh bag method. This method is frequently used in forestry or grassland research to determine the yearly fine root production (*Steen*, 1984; *Steen* 1985; *Hansson* and *Andrén*, 1986; *Vogt* et al., 1998; *Makkonen* and *Helmisaari*, 1999). Mesh bags are inserted into the root zone and filled with root free soil. After a period of time (usually 2-3 weeks) during which roots can grow into the soil inside the bags, the cores are pulled out and root length can be determined. If the time period the bags are accessable to root ingrowth is shorter than the life span of a

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single root, no root mortality inside the mesh bags occurs and root growth into the ingrowth cores can be assumed as root gross growth. But this assumption is only valid if root growth inside the bags is neither enhanced nor reduced compared to the bulk soil. Root injury, which happens during inserting the mesh bags into the ground, was one of the objections against the ingrowth core method. However, *Steingrobe* et al. (submitted) showed that root injury caused no differential growth pattern inside or in the direct vicinity of the mesh bags. Another major objection against the ingrowth core method is the difficulty in obtaining exactly the same soil conditions inside the mesh bag as outside (*Vogt* et al., 1998). The soil can be taken immediately before filling the bags from the same site. In this case roots and root debris must be sieved out, which changes soil moisture, soil density and perhaps the content of mineral N by fast mineralization. To avoid this intense sieving, the soil can be collected before the growing season starts and stored in a cool room. In this case nutrient contents, especially mineral nitrogen, soil moisture, and soil density are also different to field conditions.

Before the ingrowth core method can be widely used for measuring root gross growth, it has to be clarified that the differences in soil conditions inside and outside the mesh bags have no influence on root ingrowth. Soil factors that are known to influence root growth are nitrate and phosphate content, soil moisture and soil density. In this study these soil factors were changed during the filling of the bags and root ingrowth was compared to control bags that were filled with soil as similar to the bulk soil as possible.

In another experiment with spring wheat the cores were kept open for root ingrowth over a period longer than usual. If there would be favorable growth conditions inside the mesh bags, this should result in higher root length densities inside the cores compared to the bulk soil.

2 Materials and methods

2.1 Variations of soil conditions

Oilseed rape was grown in 1998 on a sandy loam in southern Lower Saxony, Germany. Phosphorus content of the soil was 103 mg P kg^{-1} (0–30 cm soil layer) as determind by the Ca-acetate-lactate extraction method (CAL). Mineral N content at the beginning of March was 12 kg ha^{-1} (0–30 cm soil layer) and the plants received a N fertilization of 80 kg N ha^{-1} as calcium ammonium nitrate at mid March. It was not possible to apply a second dose of N fertilizer as intended, accordingly plants suffered slightly from N deficiency from May, when the experiment was carried out. Soil bulk density was 1.48 g cm^{-3} and soil moisture at the time of opening the ingrowth cores was 0.32 (v/v).

The mesh bags were inserted into the ground at the end of April. Holes were drilled into the soil at an angle of 45° with a 4 cm diameter hand auger. The mesh bags (diameter 4 cm, length 42 cm, mesh size 3 mm) were pulled onto a PVC-tube and pushed into the holes. The tubes remained closed by end caps over 17 days and at 11th of May the mesh bags were "opened" for root ingrowth. At this time, the plants were flowering which appeared as the period of most vigorous root growth (*Steingrobe*, submitted). At "opening" the bags the soil was filled in. The tube was pulled out of the mesh bag for a few cm and some soil was filled in through the tube and compressed with a wooden stick to a density comparable to the bulk soil. The tube was pulled out step by step and the procedure was repeated until the mesh bag was completely filled with soil. To meet the correct soil density the necessary amount of soil was weighed before filling the bags according to the bag volume (527 cm^3). The weight balance left after filling the bags indicated whether the desired average density was reached or not. After a training exercise with a few bags, a density corresponding to the bulk soil could be adjusted in the bags quite well. After 15 days the mesh bags were pulled out of the soil, the roots were washed out carefully over a 200 µm sieve and root length was determined with a line intersection method according to *Tennant* (1975). Root length density (RLD, cm cm^{-3}) was calculated by relating root length to soil core volume.

The soil for filling the mesh bags was taken from the experimental site at the beginning of march before nitrogen fertilizer had been applied. It was sieved under cold air conditions (about 6°C) to remove old roots and debris and was stored at 4°C until it was used. There were different treatments as follows:

- The untreated soil served as the control.
- The nitrate content was changed by fertilizing the soil with 42 mg N per bag given as $Ca(NO_3)_2$ which corresponds to 240 kg N ha^{-1} in the soil layer 0–30 cm. The mineral N content of the unfertilized soil was equivalent to 25 kg ha^{-1}.
- A fertilization of 88 mg P per bag was given as K_2HPO_4 which corresponds to 500 kg P ha^{-1} (0–30 cm). With the phosphate fertilization a large amount of potassium was also given (about 1200 kg K ha^{-1}). However, potassium is not known to influence root growth, therefore this treatment will be announced as P treatment.
- The volumetric water content was adjusted to 0.25, 0.32 and 0.41 at the filling of the bags. The water content of 0.32 was equivalent to the soil moisture content two days before the filling took place.
- Soil bulk density was varied by greater or less compaction inside the mesh bags than in the control. From the weight of the soil used the average soil density could be calculated. The soil density of the bulk soil and of the control treatment in the mesh bags was 1.48 g cm^{-3}, the higher soil density was 1.64 g cm^{-3} with a range from 1.57 to 1.72, and the lower soil density was 1.2 g cm^{-3} with a range from 1.16 to 1.27.

2.2 Long-term ingrowth cores

Spring wheat was grown 1995 on a sandy loam in Bavaria, Germany. Mesh bags were buried as described above at April 26th, May 22nd, and June 16th and were opened immediately after insertion for ingrowth. Every 2–4 weeks some of the bags were "harvested" for measuring root development inside the ingrowth cores. The longest time period a bag was open for root ingrowth was 14 weeks between April and the final harvest at 6th of August. At each time mesh bags were "harvested" soil samples were taken with an 8 cm hand auger to determine root length density in the bulk soil. Roots were washed out and root length was determined as described above.

2.3 Statistics

Each oilseed rape treatment was replicated 4 times. Anovas were performed and at significant differences ($p = 0.05$) the treatment means were compared to the untreated soil as a control by the Dunnet-test. Replications in the long-term experiment with spring wheat varied between 4 and 10. The comparison of means was done by the Scheffé test.

3 Results and discussion

3.1 Nutrient content

The preparation of the soil for filling the mesh bags, i.e. sieving, transport, and storage, may cause differences in the

nitrate concentration compared to the field conditions. To simulate this, the soil filled into the mesh bags was fertilized with nitrate equivalent to 240 kg N ha^{-1}. This fertilization increased root ingrowth into the cores in tendency ($p = 0.10$) compared to the control (Fig. 1) which had a mineral N content of 5.6 mg kg^{-1} dry soil equivalent to 25 kg N ha^{-1} in the 0–30 cm soil layer. The mineral N content of the bulk soil was not measured at the time the bags were opened for ingrowth. But the plants had been fertilized with only 80 kg N ha^{-1} 7 weeks before the bags were opened and, therefore, the mineral N content of the bulk soil can be assumed to have been low. This could also be deduced by a reduced shoot growth in comparison to a well fertilized treatment of another oilseed rape experiment located nearby on the same site. Despite this slight N deficiency root growth response to the high N content in the fertilized bags was rather small. This is in contrast to findings that a localized supply with nitrogen enhances root growth and root branching in the zone of increased supply (*Gliemeroth*, 1953; *Drew* and *Saker*, 1975). Especially, spots of ammonium in high concentration are reported to increase root growth. However, it is unlikely under ordinary circumstances that the soil prepared for filling the cores has a higher ammonium concentration than the surrounding soil because the sieving will result in a good aeration and, hence, a high nitrification rate. However, the root growth enhancing effect of a localized nitrate fertilization in the field seems less pronounced compared to pot or solution experiments (*Skinner* et al., 1998).

The nitrogen mineralization caused by the usual preparation of the soil before filling the bags is probably less than the 240 kg ha^{-1} used in our experiment. Root growth enhancement inside the bags due to this nitrogen mineralization can, therefore, be assumed to be less than shown in Figure 1. Nevertheless, these results demonstrate the importance to avoid much higher mineral N levels inside the mesh bags compared to the bulk soil. This can be achieved by collecting the soil in early spring before fertilization, when the mineral N content should be low, followed by a subsequent cool storage of the soil. The mineral N content can be measured and adjusted to the bulk soil before filling the bags. Collecting the soil immediately before filling the bags is disadvantageous because it requires an intensive sieving to remove the fine roots. This sieving is usually done at high air temperatures during spring or summer and bears the risk of inducing a rapid mineralization (*Craswell* and *Waring*, 1972; *Cabrera* and *Kissel*, 1988), thus, leading to higher mineral N contents inside the ingrowth cores compared to the bulk soil.

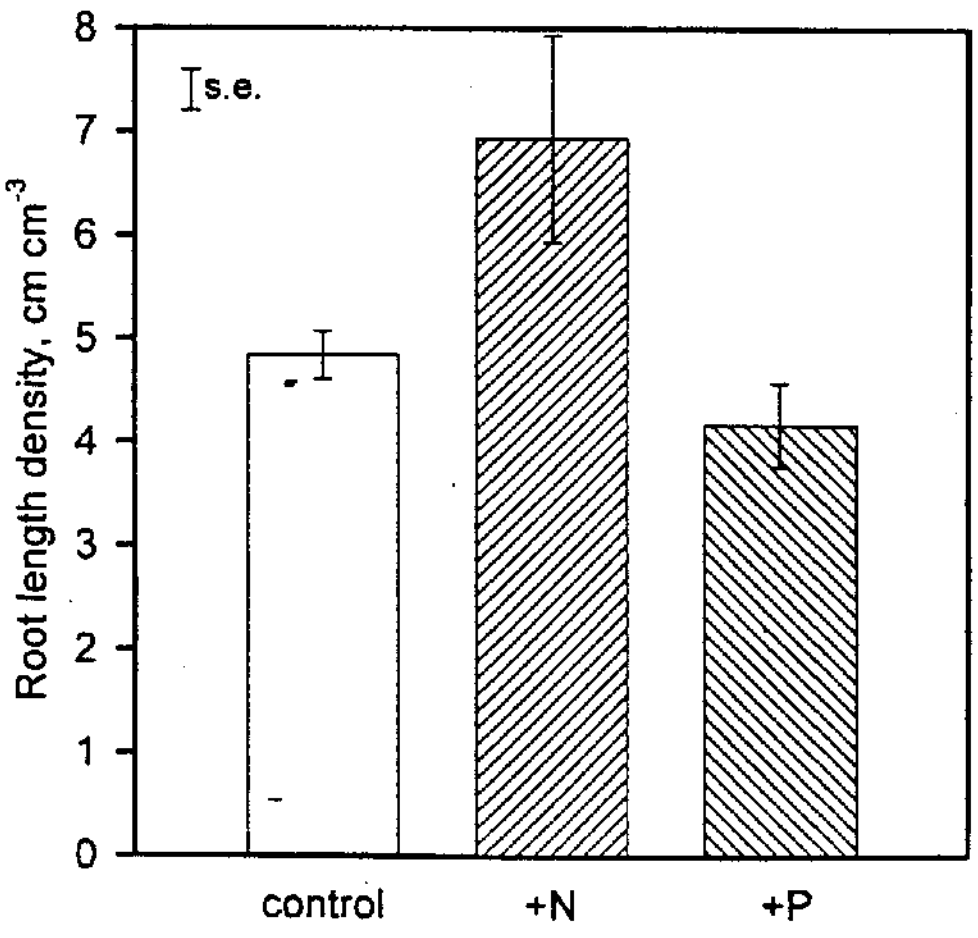

Figure 1: Influence of nitrate N (240 kg ha^{-1}) and P (500 kg ha^{-1}) fertilization of the ingrowth cores soil on root length density of oilseed rape inside the cores. (Differences to control are not significant at $p \leq 0.05$).
Abbildung 1: Einfluss einer Düngung des Bodens in den „ingrowth cores" mit Nitrat (240 kg N ha^{-1}) und P (500 kg ha^{-1}) auf die Wurzellängendichte von Raps innerhalb der „cores". (Die Werte unterscheiden sich nicht signifikant bei $p \leq 0.05$).

The P fertilization of the soil inside the mesh bags equivalent to 500 kg P ha^{-1} had no effect on root growth into the ingrowth cores (Fig. 1). This might be due to the P level in the bulk soil of 103 mg P(CAL) kg^{-1} being a high P content according to the German recommendation scheme and sufficient for plant supply. Together with the P fertilization the potassium supply was also increased. However, an influence of potassium on root growth is not known. This is confirmed by the results presented here. In contrast to potassium a localized P supply may enhance root growth (*Drew* and *Saker*, 1978) and, therefore, root development inside the cores might be increased if the bulk soil is low in available P and the P content in the cores would be much higher. However, the content of available P is unlikely to change much by collecting, sieving, and storing the soil before filling it into the bags. *Römer* and *Claassen* (2000) demonstrated that desorbing P from different dried soils with several water:soil ratios resulted in comparable inorganic P concentrations in the desorption solutions and in the soil solution of the field-fresh soils. This might be due to the high P buffer capacity of most soils. Therefore, special care in handling the soil before filling it into the mesh bags seems not to be necessary with regard to phosphate.

3.2 Soil moisture

After a long period of storage, the soil moisture may be different to field conditions. Different volumetric water contents of the soil at the time of filling it into the bags, however, had no influence on root ingrowth (Fig. 2). The soil water content inside and outside the bags seemed to adjust quickly and there were no noticeable differences in soil moisture between the cores after 15 days of ingrowth.

3.3 Soil density

Root ingrowth into the cores was not altered by the lower soil density compared to the control but the higher soil density reduced root ingrowth significantly (Fig. 3). An even greater reduction in root growth of spring rape at high soil

compaction in ingrowth cores was also reported by *Steen* and *Håkansson* (1987) for a sandy soil. They found in compacted soil cores (1.40 g cm^{-3}) less than 20% of the root mass than in loose soil cores (1.09 g cm^{-3}). These greater differences in root growth compared to our results might be due to the longer period the soil cores were open for root ingrowth (several months). Root growth reduction of wheat, however, was less pronounced by an increase of soil density inside the cores from 1.09 to 1.40 g cm^{-3} (*Steen* and *Håkansson*, 1987).

To avoid root growth reduction at high soil densities, special care should be taken to reach a density in the cores equivalent to the bulk soil. This seems to be possible by weighing the soil before filling it into the mesh bags as described above. But by this procedure only the average density of the ingrowth cores will correspond to the average bulk soil density. It is rather difficult to reach a uniform distribution of soil density inside the cores or even a distribution as in the bulk soil in the direct vicinity to the cores.

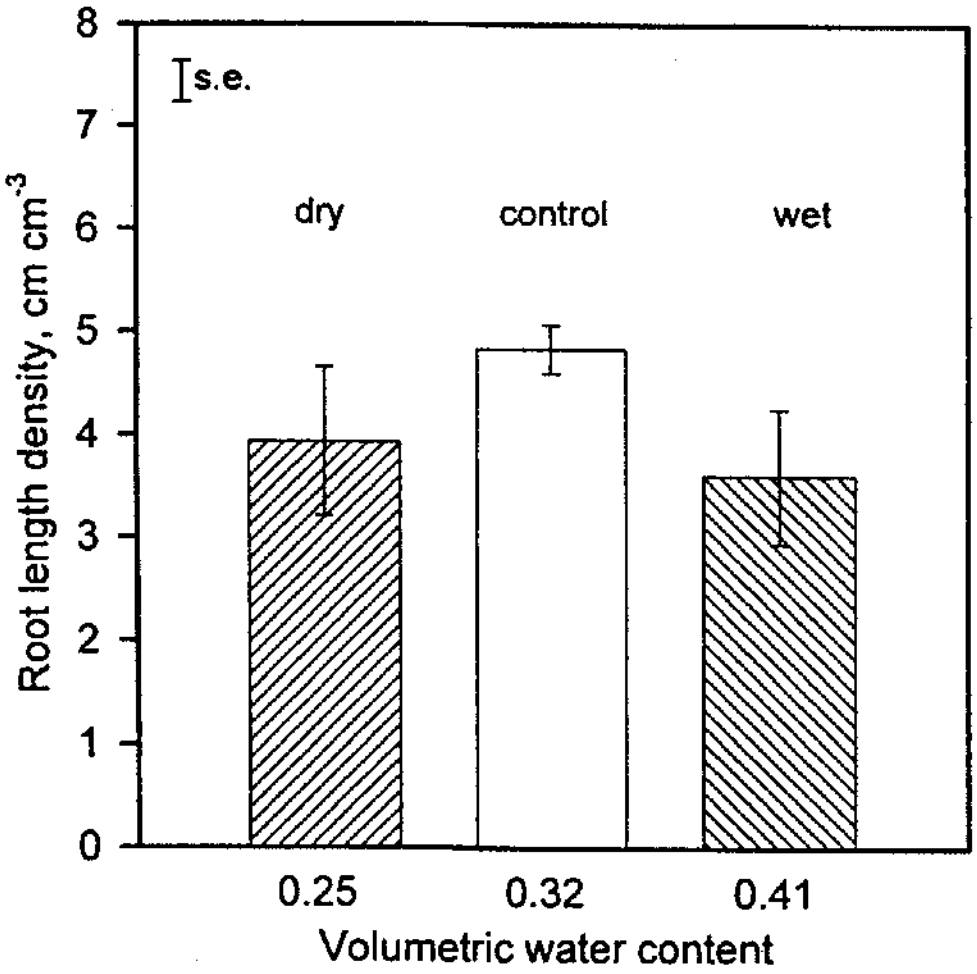

Figure 2: Influence of the initial volumetric soil water content of the ingrowth cores on root length density of oilseed rape inside the cores. (Differences to control are not significant at $p \leq 0.05$).
Abbildung 2: Einfluss des volumetrischen Bodenwassergehaltes in den „ingrowth cores" zum Zeitpunkt der Befüllung auf die Wurzellängendichte von Raps innerhalb der „cores". (Die Werte unterscheiden sich nicht signifikant bei $p \leq 0.05$).

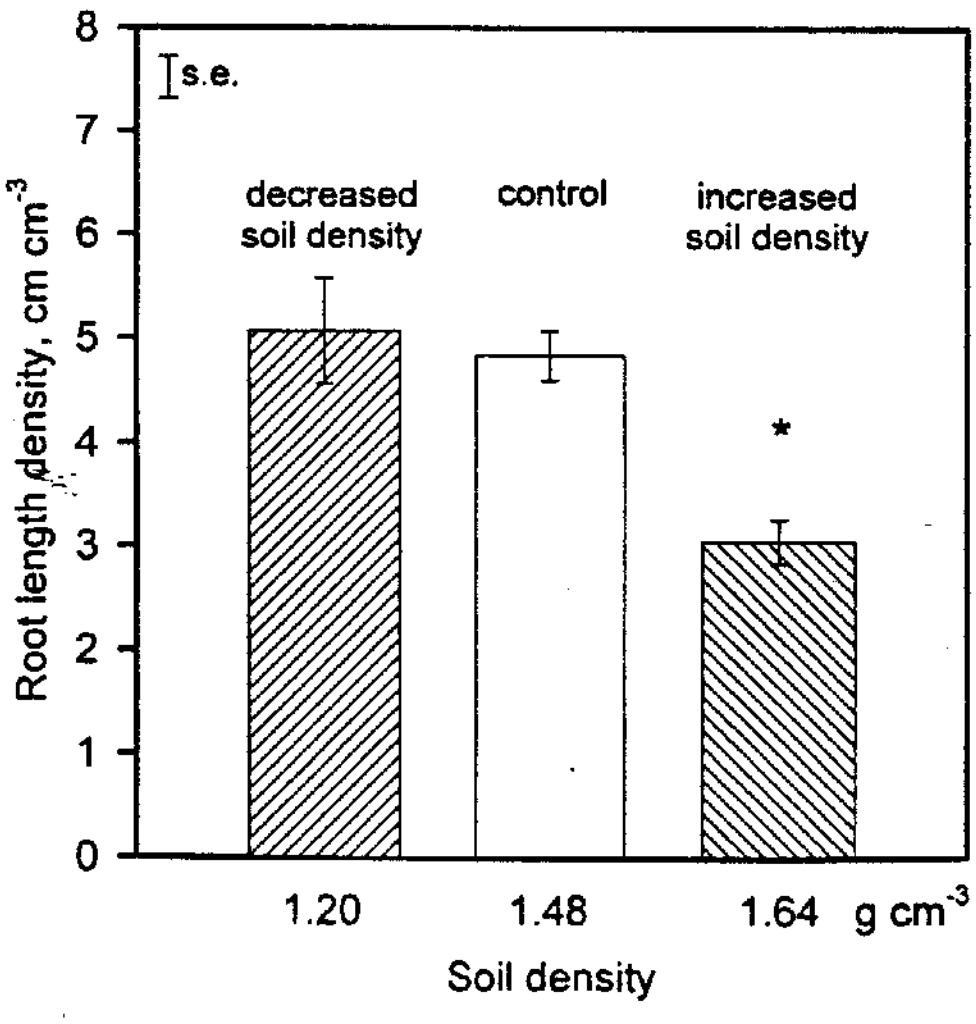

Figure 3: Influence of soil density in the ingrowth cores on root length density of oilseed rape inside the cores. (* denotes significant difference to the control at $p \leq 0.05$).
Abbildung 3: Einfluss der Bodendichte in den „ingrowth cores" auf die Wurzellängendichte von Raps innerhalb der „cores". (* signifikante Unterschiede bei $p \leq 0.05$).

3.4 Long-term ingrowth cores

The preparation of the soil before filling the bags may also change soil properties like pore volume or aggregate size. The size of soil aggregates, for example, was found to influence root growth of maize in a pot experiment (*Donald* et al., 1987). Keeping the bags open over a long period for root ingrowth until a constant root length density inside the bag is reached should give information about enhancing or impeding influences on root growth inside the bags compared to outside. For this, mesh bags were inserted in a spring wheat stand and were opened for ingrowth at 3 dates. Every 2 to 4 weeks some of the bags were "harvested" over a period of 14 weeks in order to determine root development over a long period.

Root length density in the bulk soil increased from 4 cm cm^{-3} at end of April up to 12 cm cm^{-3} during July (Fig. 4). The further increase in August was not significantly different to the RLD in July. Root length density in the ingrowth cores increased even faster after opening the cores than in the bulk soil and remained constant after reaching the RLD of the surrounding soil. It can be assumed that the root growth in the ingrowth cores immediately after opening the mesh bags reflects root gross growth, whereas the development of the root system in the bulk soil is a result of root gross growth and root death. After a while, which appears to be about 1.5 months in this experiment, the growth curve of the roots inside the bags began to level out. This indicates the beginning of root mortality inside the bags because root growth continued as the root development in cores opened later indicates. At the end of the growth period root development slowed down reflected in a lower RLD in the ingrowth cores opened at 16th June.

Similar root length densities inside and outside of long-term ingrowth cores have also been reported by *Claassen* (1990) and *Steen* (1991). These results do not necessarily show that the root growth dynamics in the cores were always the same as outside. But these findings indicate that the preparation of soil for filling the mesh bags did not create favorable growth conditions which led to a different root system with a higher RLD.

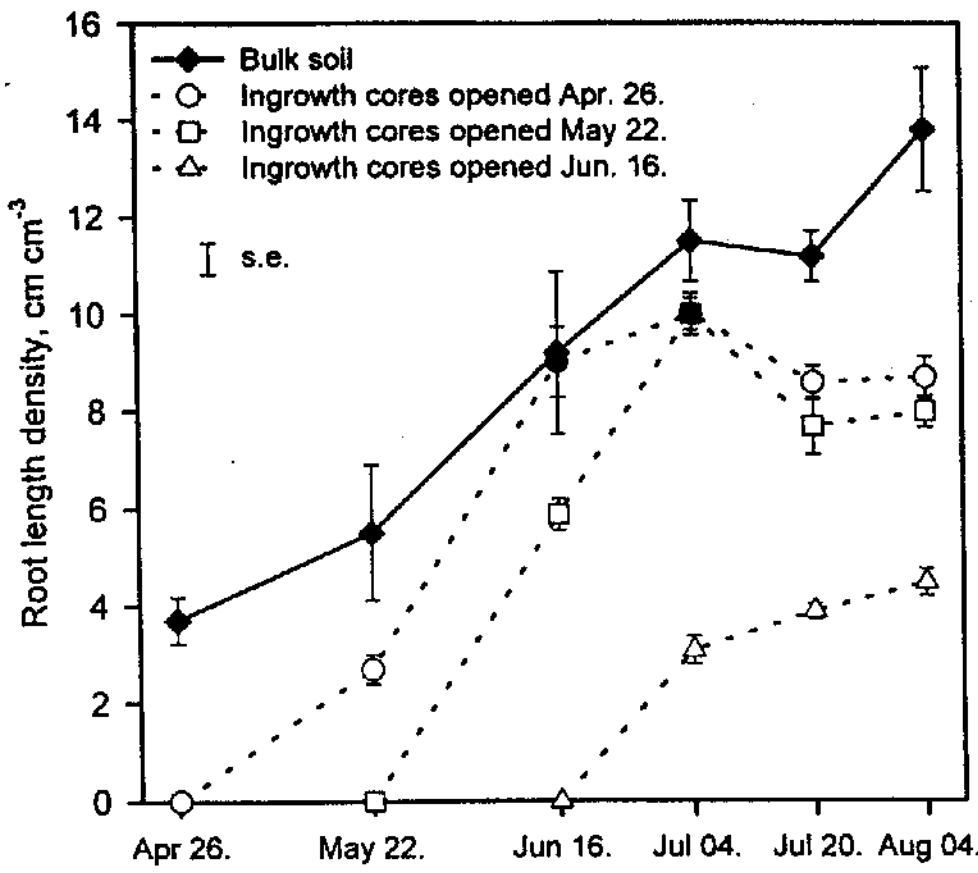

Figure 4: Development of root length density (RLD) of spring wheat in the bulk soil and in long-term ingrowth cores. (Open symbols of ingrowth cores denote significant differences ($p \leq 0.05$) to bulk soil RLD, closed symbols denote no significant differences to bulk soil RLD).
Abbildung 4: Langfristige Entwicklung der Wurzellängendichte (RLD) von Sommerweizen in den „ingrowth cores" und im Restboden. (Offene Symbole für die „ingrowth cores" zeigen signifikante ($p \leq 0.05$) Unterschiede in der RLD zum Restboden an, gefüllte Symbole bedeuten keine signifikanten Unterschiede zum Restboden).

Sometimes a kind of "allelopathic mechanism" that favors root growth into the root-free zone of the ingrowth core is also assumed as a possible objection against this method. This experiment was not designed to answer this question, but it is obvious that root growth inside the cores during the second measurement period (22nd May to 16th June and 16th June to 4th July for the cores opened at 26th April and 22nd May, respectively) was in the same order than root growth in freshly opened cores. The already existing roots in the cores seemed not to impede further root development.

4 Conclusions

The investigation of the effect of different soil conditions inside the ingrowth core on root growth showed that there were no influences of soil moisture, P fertilization (at a already high P background), and low soil density on root ingrowth. A higher N concentration in the ingrowth core compared to the bulk soil, however, increased root ingrowth. A higher soil density in the cores resulted in less root growth. These problems, however, can be avoided by taking special care during filling the mesh bags to meet the N content and soil density of the bulk soil. A long-term opening of the ingrowth cores did not reveal favorable root growth conditions inside the cores. *Steingrobe* et al. (submitted) could show that injuring the roots by drilling the holes for inserting the mesh bags did not influence root ingrowth either and that root production of cereals measured with the ingrowth core method was in a similar order than derived from minirhizotrons and isotope techniques by other authors.

Therefore, the ingrowth core method seems suitable as an easy to handle field method for measuring root gross growth. An advantage of this method is that there is no need for special equipment and that no radioactive isotopes are required. It allows to run experiments with many replications to get reliable quantifications of root growth under field conditions. A disadvantage is the labour-intensity of this method.

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