Electrostatic method to separate roots from soil

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Summary – Zusammenfassung

Complete removal of roots from soil samples is a prerequisite for most of the chemical and biological analyses. A simple electrostatic method of separating roots from sieved, largely mineral soil substrates was optimized and examined by the addition of ^{14}C labeled fine roots to sandy, silt loamy and clay loamy samples. Depending on soil texture, between 40% and 50% of fine roots can be removed from 100 g of sieved soil in less than 10 minutes. The root-free soil substrate and the extracted roots can be used for analyzes or experiments immediately after the separation. The proportion of the mineral particles remaining in the root fraction depends on duration of separation, distance between the charged plate and the sample, and soil texture. The proportion of separated mineral particles is about 90%–95% (w/w) in sandy and 70%–85% in silt loamy and clay loamy substrates. The electrostatic method of root separation may take place before the analysis of C_t and N_t contents, and is suitable for soil samples preparation for incubation experiments.

Key words: methods / roots separation / sample preparation / ^{14}C / C_t and N_t analysis

Eletrostatische Methoden zur Abtrennung der Wurzeln vom Boden

Eine möglichst saubere Abtrennung der Wurzel vom Boden ist Voraussetzung für viele bodenchemische und -biologische Analysen. Die Wurzelabtrennung bleibt ein Problem, sowohl von der Sauberkeit als auch vom Zeitaufwand her. Eine einfache Methode zur elektrostatischen Wurzelabtrennung vom gesiebten Boden wurde optimiert und durch Zugabe von ^{14}C-markierten Wurzelresten zum sandigen, schluffig-lehmigen und tonig-lehmigen Böden überprüft. Im Laufe von 5 bis 10 Minuten kann man 100 g eines trockenen und gesiebten Mineralbodens von den Wurzeln abtrennen. In dieser Zeit werden je nach der Bodenart ca. 40% bis 50% aller nach dem Sieben verbliebenen Feinwurzeln entfernt. Der Bodenrest und auch die Wurzeln sind nach der Auftrennung sofort für Analysen oder andere Experimente verwendbar. Der Anteil der beigemischten mineralischen Bodenpartikel hängt sowohl von der Zeit der Auftrennung und der Höhe der aufgeladenen Platte über den ausgebreiteten Boden als auch von der Bodenart ab. Im sandigen Boden liegt dieser Anteil bei ca. 90–95% (Masse-%), im schluffig-lehmigen und tonig-lehmigen Boden ist er bei 70–85%. Die elektrostatische Methode zur Wurzelabtrennung kann sowohl vor den Analysen der C_t- und N_t-Gehalte im Boden erfolgen als auch für die Vorbereitung der Bodenproben für die Inkubationsversuche empfohlen werden.

1 Introduction

Most of the chemical analyses of C_t or N_t content in the soil as well as laboratory experiments on C and N turnover (e.g. incubation experiments, soil respiration, mineralization, etc.) require a neat removal of root and leaf residues from the soil (*Black*, 1965, pp. 572–574; *Schinner* et al., 1993, p. 9; *Schlichting* et al., 1995, p. 93). However, a review of the known methods for soil analyses (e.g. C_t, N_t content) shows that none of them recommends the procedure of separation of roots from soil samples before the analysis (*Black*, 1965; *Hoffman*, 1997; *Carter*, 1993; *Gerthsen* et al., 1986; *Schlichting* et al., 1995; *Blume* et al., 2000). Only sieving < 2 mm is recommended for sample preparation of some biological and enzymatic analyses (*Schinner* et al., 1993, p. 9; *Schlichting* et al., 1995, pp. 158–159). The description of most of the soil chemical methods begins with the grinding procedure and is followed by sieving with 0.5 mm mesh size (*Black*, 1965; *Carter*, 1993). However, the separation of organic residues from the soil after the grinding is not possible at all.

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The removal of roots from soil samples larger than 100 g is based on sieving with different mesh sizes. However, a considerable part of medium and fine roots passes through the sieve if the mesh size > 1 mm is used. Smaller mesh sizes are seldom used. For samples smaller than 100 g manual picking (with a pair of tweezers) is recommended, although it is very subjective and laborious. By use of root washing machines (*Smucker* et al., 1982), only the large and medium size roots will be collected and the whole soil material with fine roots is lost together with the wash water. In addition, leaching effects of some nutrients from the roots can occur. For some special aims, heavy liquids can be used to remove roots and other specific light particles from soil samples (*Magid* et al., 1997; *Gaiser* et al., 1998). Thereafter, the specific heavy soil substrate and light organic particles, including roots, must be washed and it is impossible to use the sample materials for some analyses at all (C and N analysis, DOC, microbial biomass etc.).

Insufficient separation of root can lead to many artifacts such as high variation in the contents of C_t in soil samples during vegetation period. Thus, a cleaner separation of soil samples from roots remains a problem, for purposes of neatness as well as due to tidiness and time consumption.

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Table 1: Properties of soils used in the experiment
Tabelle 1: Die Eigenschaften der getesteten Böden.

Short description	Full description	Origin and field trial	Clay %	Clay + fine silt %	Silt %	C_t %
Sandy	Sandy Gleyic Cambisol	Großbeeren, Long-term field trial „Trasse 2“	4.6	6.0	9.9	0.75
Silt loamy	Silt loamy Haplic Luvisol from loess	Karlshof, field trial of Hohenheim Univeristy	20	22	8.0	1.2
Clay loamy	Clay loamy Gleyic Cambisol	Allgäu, pasture, previous field trail of Hohenheim University by Siggen	28.4	44.1	47.1	4.7

The aim of this contribution is to optimize and examine the electrostatic method of separating root and other organic remains from the soil. The duration of procedure for root removal from soils, neatness of separation and admixture of mineral particles to the extracted roots were tested.

2 Material and methods

2.1 Principle of the electrostatic root separation procedure

The principle of the electrostatic separation of roots from mineral soils has been known for a long time, although it was not described in the literature and not tested for the separation efficiency. The electrostatic root separation procedure is based on the attraction of organic or mineral particles, which cannot conduct electricity to the electrostatically charged stick (Fig. 1a). The electrostatically charged stick moved above the spread-out soil layer (1–2 mm) attracts the plant residues as well as mineral particles. The specific weight of roots is much lower than that of mineral soil particles. So, it is possible to choose a distance between the stick and the soil layer such that preferably organic particles will be absorbed.

The shortcomings of this known method are that an electrostatic field from a stick is not regular and not uniform for the particles situated at different distances from the stick (Fig. 1a). According to the Coulomb rule, the attracting force (F) decreases with the distance square (r^2):

$$F = \frac{1}{4\pi\varepsilon_r\varepsilon_o}\frac{Q \cdot Q'}{r^2} \cdot v_0 \qquad (1)$$

Where Q and Q' are the electrostatic charge of the stick and the induced charge of the particle; v_0 is the vector in the connection direction, ε_r and ε_o are the permittivities of the medium between the stick and the particle and of vacuum, respectively (*Gerthsen* et al., 1986). So, the force of the particles on the plate situated 45° to the stick is only 50% of the force that affects the particles directly under the stick. This difference leads to the fact that from the place under the stick not only the root residues but also the mineral particles will be attracted. On the other hand, only a small part of the roots will be attracted from the places situated farther away from the stick and that the whole area of the spread soil will not be treated equally. Additionally, the small effective impact field of the stick requires repeated recharging.

Our modifications of the method include (Fig. 1b):

1. The exchange of the stick for a plate because:
   a) The electrostatic field of the plate is much more regular than that of a stick. Therefore, the particles with similar electrostatic features and similar specific gravity will be attracted.
   b) The impact area of a plate is much larger than that of a stick. Therefore, the separation can be carried out much faster.
2. The padding with soil material (and not the plate) will be shaken. This leads to the mixing of soil on the padding during separation and the root particles initially situated on the bottom are moved upwards.

2.2 Soil properties and sample preparation

Three soils with different texture were used to test the method of electrostatic separation of roots from the soil (Tab. 1). The soil samples were taken from Ap or Ah horizon (0–10 cm) and were sieved on a 5 mm screen to remove stones and large roots. After air-drying, the samples were sieved < 2 mm to homogenize the sample and to remove middle and long (> 5–7 mm) roots.

2.3 Examination of the method

The method of separation of roots from soil was tested in two experiments:

Table 2: Time (minutes) taken for the separation of roots from 100 g soil and amount of roots (mg DW 100^{-1} g^{-1} ± SD, in parentheses the percentage of the total organic matter content before separation is presented) separated from the three sieved soils using the electrostatic method.
Tabelle 2: Zeit (Minuten) für die Abtrennung der Wurzeln von 100 g Boden und die Wurzelmengen (mg TS 100^{-1} g^{-1} ± Standardabweichung, in Klammern Prozent der gesamten organischen Substanz des Bodens vor der Abtrennung), die nach dem Sieben aus den drei Böden mittels elektrostatischer Methode abgetrennt wurden.

Separation time (Minutes)				Root amounts separated (mg 100^{-1} g^{-1}, %)		
	Sandy	Silt loamy	Clay loamy	Sandy	Silt loamy	Clay loamy
1st step	2	3.5	5	27.8 ± 5.6 (1.5)	63.7 ± 9.1 (2.1)	114.3 ± 9.1 (1.4)
2nd step	3	5	5	20.2 ± 4.8 (1.1)	40.9 ± 8.0 (1.3)	95.7 ± 13.3 (1.1)
Total	5	8.5	10	48.0 ± (2.6)	104.6 ± (3.4)	210.0 ± (2.5)

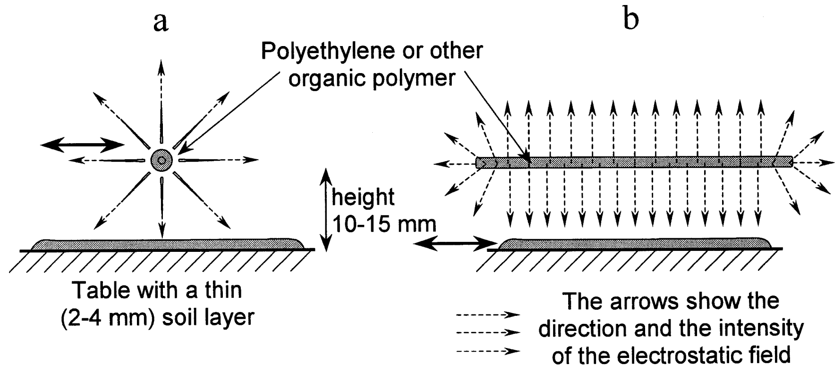

Figure 1: Sketch of the conventional method of electrostatic root separation (left) and our modification (right).
Abbildung 1: Schema der üblichen Methode zur elektrostatischen Wurzelabtrennung (links) und unsere Modifikation (rechts).

The experiment 1 aimed at the selection of the optimal separation time for differently textured soils and the assessment of admixture of mineral particles to the separated roots. The soil samples were spread out on a DIN A3 paper. A polyethylene plate (diameter 300 mm and height 4 mm) was charged by means of intensive rubbing (4–5 seconds) on a stretched wool fabric. The plate was set over the padding with spread-out soil and the padding was shaken horizontally. So, the roots with admixture of mineral particles were attracted by the charged plate and collected manually. The separation times were chosen for each soil according to the amount of mineral particles mixed with the separated roots. After separation, the roots and soil remains were weighed and the C_t contents were determined. Nine replicates per soil were carried out in this experiment.

The experiment 2 was aimed at examining the recovery rates of roots mixed with the soil and the estimation of roots remaining in the soil after the separation as well as the proportions of mineral particles removed together with the roots. The ^{14}C labeled roots of *Lolium perenne* L. were cut to a length of 0.2 to 3 mm. Roots of such length usually pass through a 1 mm screen sieve. About 0.555 g of ^{14}C labeled root residues (14.9 kBq g^{-1}) were carefully mixed with 100 g soil. The labeling of roots with ^{14}C was as described by *Kuzyakov* et al. (2001). After the mixing, the roots were separated from the soil as described above and analyzed for ^{14}C activity. Three replicates were carried out in this experiment.

The two experiments were carried out with 100 g soil per replicate. All separations were done in two steps: (1) The charged plate was held 1.5 cm above the spread soil. Afterwards the soil material was collected, mixed, and spread out for the second time. (2) The height of the charged plate over the spread soil was reduced to 1 cm, and the separation was repeated. For treatment 100 g soil about 10 charging procedures were necessary.

2.4 C_t and ^{14}C analysis

The C_t contents of soil (from 0.9 g) and of root samples (from 0.1 g) were analyzed on the C_t analyzer (Carlo Erba). The root amount was calculated according to the assumption that roots free of mineral particles contain 40% carbon. The ^{14}C activity of roots and soil samples were analyzed on a β-spectrometer Tri-Carb 2000CA after the combustion of the sample in the oxidation unit and collection of $^{14}CO_2$ in the scintillation cocktail Permafluor E^+ (Canberra Packard Co. Ltd). All results are presented with standard deviation (SD).

3 Results and discussion

3.1 Separation time and root amounts extracted

For the sandy, silt loamy, and clay loamy top-soils examined, different time was needed to separate the roots depending on the amounts of roots extracted in each step (Tab. 2). About 5 minutes were necessary for 100 g of the sandy soil. The same procedure takes twice as long for the clay loamy soil. However, in this soil the root amount was about two times larger than that in the silt loamy and four times larger than that in the sandy soil (Tab. 2). These root amounts are presented as organic matter of roots, so that the mineral particles are subtracted. The root amounts extracted in the second separating step are 1.5 to 2 times smaller than in the first step, although the duration of separation was 1.5 times longer for the sandy and silt loamy soil. All at all between 2.5 and 3.4% of all organic substances remaining in

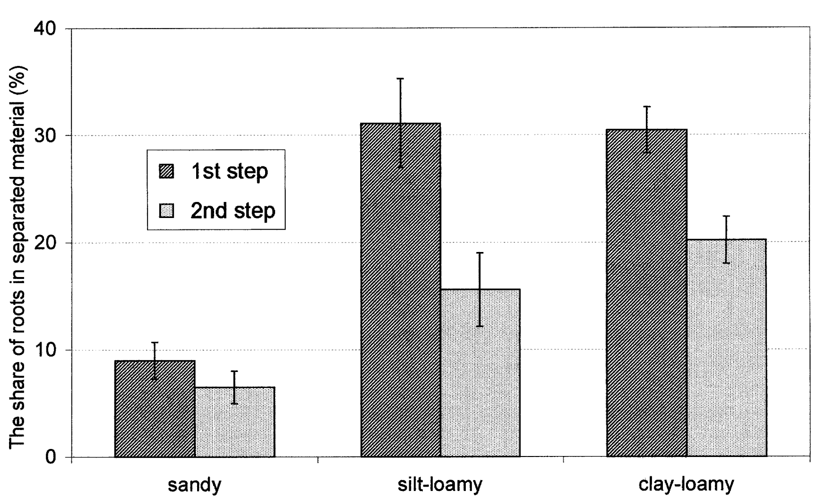

Figure 2: Amount of roots in the separated material (± standard deviation). The difference to 100% corresponds to the mineral admixtures.
Abbildung 2: Anteil der Wurzelmasse im abgetrennten Material (% ± Standardabweichung). Die Differenz zu 100% wird durch mineralische Beimischungen verursacht.

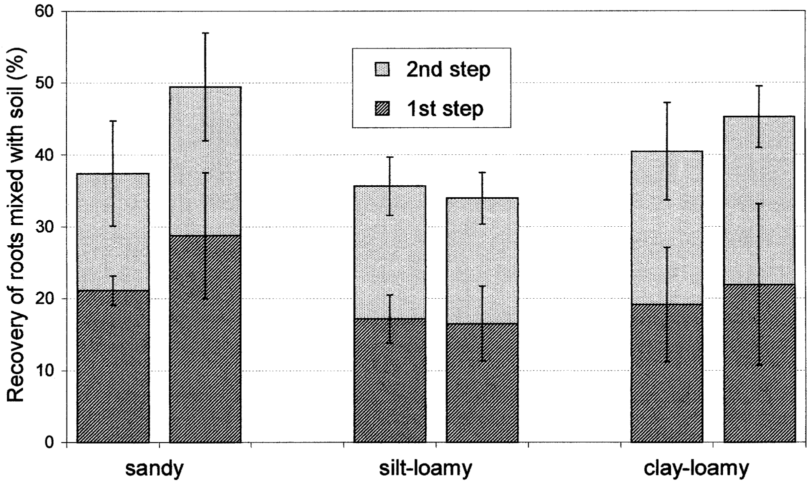

Figure 3: Percentage of roots removed using the electrostatic method. (Left bars correspond to the percentage of root-^{14}C mixed into soil; right bars correspond to the percentage of ^{14}C recovery).
Abbildung 3: Anteil der in den Boden eingebrachten Wurzeln, die mit der elektrostatischen Methode entfernt wurden (linke Säulen: in % der eingebrachten ^{14}C-Menge; rechte Säulen: in % des wiedergefundenen ^{14}C).

the soil after sieving were extracted with the suggested separation method (Tab. 2).

3.2 Admixture of mineral particles

The suggested electrostatic separation method also leads to the absorption of fine mineral particles together with roots. Based on the C_t content of the extracted material, the proportion of roots was calculated (Fig. 2). The proportion of organic particles is minimal in the sandy soil. The sandy soil is less aggregated than silt loamy and clay loamy soils. As a result, the non-aggregated mineral fine particles are possibly stronger attracted to the charged plate in spite of their smaller number compared to silt and clay loamy soils. Obviously the real root content of the material extracted in the 2nd separation step is 1.5 to 2 times less than in the first separation (Fig. 2). Depending on the study and accepted cleanness, different numbers of separating steps can be recommended to reach different purity grades of soil or the roots.

3.3 Purity of separates obtained

The recovery rate is one of the important criteria of the method quality. Referring to root separation, the completeness of the extraction of roots admixed to soil can be considered as such a criterion. It is impossible to extract all roots from the soil sample so cleanly that the sample can be reused for the estimation of recovery rates. Therefore, we mixed the ^{14}C labeled roots of *Lolium perenne* with the soil to differentiate between the roots that were previously in the soil (unlabeled) and newly added roots (labeled). The results of the separation procedure show clearly the necessity of labeling: despite the fact that only 0.555 g of roots were added to 100 g of each soil, 2.1 (sandy), 1.5 (silt loamy) and 0.8 g of roots (clay loamy) were extracted from the differently textured samples. These weights include mineral particles. The ^{14}C labeling shows a different situation and allows for the estimation of which part of the roots added to the soil was really removed (Fig. 3). The difference between the proportions of ^{14}C input (left) and ^{14}C recovery (right) can result from (1) root losses during the manual collection of roots from the charged plate and (2) high variability of ^{14}C in the roots. In two separation steps about 43% (sandy and clay loamy) and 35% (silt loamy soil) of the roots mixed with the soil substrates could be removed.

In contrast to the experiment 1, the root amounts separated here in the second step were approximately the same as in the first step (Fig. 3). This indicates that a third separation step possibly would lead to an increase in the total root amount extracted from soil. A third separation step was not carried out because the results of ^{14}C analysis, necessary for deciding on the third step, were available only after the separation itself.

4 Conclusions

An electrostatic method separating the roots from sieved soil was optimized and is recommended as pre-treatment for many biochemical analysis of soil samples: C_t, N_t, incubation experiments, extraction - fumigation/incubation, DOC etc. The root-free soil and the extracted roots can be used immediately after the separation. The method can be further optimized to enable a higher extraction of roots and minimize the admixture of mineral particles. The method is much more effective for small soil samples (5 to 20 g).

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