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Modified ingrowth core method plus infrared calibration models for estimating fine root production in peatlands

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Abstract

Aims: Fine root production is a major carbon flux in many peatland ecosystems and least understood because of methodological difficulties. We aimed to develop a robust method for inventorying fine root production in peatlands.

Methods: The ingrowth core method was modified to reduce disturbance and facilitate faster colonization by developing a novel type of corer-installer that reduces cutting of roots during installation, and using smaller volume of initially root-free substrate. The number of cores needed for a reliable site-level mean was estimated based on the variation observed in the test sites. Infrared calibration models were constructed for estimating the mass proportion of roots in the ingrowth cores without manual separation.

Results: We observed first-year production of 118, 216, 170 and 125 g m⁻² yr⁻¹ in the 0-50 cm soil layer for boreal drained bog forest, drained fen forest, wet sedge fen and dry sedge fen, respectively. Between 10 and 30 cores per site was estimated to be enough for obtaining reliable mean estimates of production. The infrared calibration models yielded high coefficient of determination (0.72-0.83) and low standard error of prediction (5-8%).

Conclusions: We suggest that the methodology presented here saves time and manual labour and may be applied with a high number of replicates for inventorying fine root production in peatlands. Practical guidelines are presented.

Introduction

Knowing the structure of an ecosystem is a prerequisite for understanding and modeling its functioning. Concerning peatlands, the carbon hotspots of our planet (e.g., Page et al. 2011), the information gap regarding roots and rhizomes of vascular plants contrasts with our detailed view of above-ground vegetation structure and dynamics. Open bogs dominated by *Sphagnum* mosses, the nutrient-poor, acid end-points of peatland succession, may spring to one's mind first when peatlands are mentioned. However, peatlands where the carbon cycle is dominated by fluxes mediated by vascular plants, sedges, shrubs, and/or trees, are at least as common in many regions (Sjörs 1991; Saarinen 1996; Laiho et al. 2003; Jauhiainen et al. 2005). Consequently, root-related C fluxes, such as the production rates of fine roots in peatlands are of global importance but are poorly known.

The shortage of information largely stems from methodological difficulties. Separating roots from soil and live roots from dead roots is arduous; especially so when it comes to peat soils that solely consist of plant remains, including roots, at various stages of decay (Sjörs 1991). The same holds for visual species identification, which is time consuming and requires a high level of expertise. Root production cannot be measured without disturbing the system in one way or the other, which may affect the outcome (Milchunas 2009). Further, the extreme spatial variation (e.g., Finér and Laine 1998) adds to the uncertainty of the estimates.

The methods that have been used for estimating root production include sequential coring, minirhizotrons, ^{14}C -labelling, and ingrowth cores or nets; all with their specific drawbacks (Finér and Laine 1998; Finér and Laine 2000; Lukac and Godbold 2001; Lukac and Godbold 2010; Iversen et al. 2012; Rytter and Rytter 2012). The ingrowth methods have been commonly applied, and their results are relatively easy to interpret (Milchunas 2009). Thus, we chose them as a starting-point in our search for methods that would allow inventorying root production in a wide range of peatland types and environmental conditions that is needed for efficient parameterizing of peatland ecosystem models. The ingrowth core method involves installing mesh cores filled with root-free soil to holes cored in the ground, lifting these up at certain intervals, and separating and weighing the roots that have grown in and represent root production during the incubation period. In the ingrowth net method, cores are replaced by simple nets, and roots that have grown through the net during a certain period are used for estimating production (Lukac and Godbold 2010). Even these methods involve manual separation of roots from the peat substrate, which is the most time-consuming and error-prone phase of such studies (Sjörs 1991; Milchunas 2009). Further, they involve uncertainties associated with cutting the existing root systems at installation (Milchunas 2009), and, in the case of cores, inserting a rather large amount of root-free and thus atypical substrate in the soil. To capture both the production of new roots and the diameter growth of older roots, several years' observation period is needed. This brings forth the issue of root mortality, and separating live roots from the dead.

Infrared spectroscopy (IRS) has been found to enable identification of species composition in simple root mixtures (Roumet et al. 2006; Picon-Cochard et al. 2009; Lei and Bauhus 2010). The same holds for separating live roots from the dead (Picon-Cochard et al. 2009). Each

chemical bond absorbs IR radiation in a specific manner that depends on the nature of the bond. Thus, an IR absorbance spectrum, showing for each wave-length or wave-number the proportion of radiation absorbed by the sample, shows the relative abundance of different chemical bonds in the sample, i.e., a summary of the chemical composition of the sample. This information may be used for constructing calibration models, regression models based on a calibration data set that consists of samples with known composition plus their IR spectra. These models are then used to predict the composition of unknown samples based on their IR spectra only. Following the same approach, it should be possible to estimate the mass proportion of roots in a sample consisting of roots and peat, that is, from an unseparated ingrowth core, where peat is used as the substrate. Such samples may be pulverized and homogenized to a sufficient extent that the small samples used for spectral analyses represent the root-peat ratios in the whole cores. For success, the minimum requirement is that live roots share some common chemical patterns that are absent in the peat substrate, and that these chemical differences are quantifiable.

The substrate used as filling for the ingrowth cores should match the soil of the study sites as closely as possible. Higher-than-ambient level of nutrients limiting plant growth may lead to overestimation of root production (Steingrobe et al. 2000). In mineral soil sites, it has been common to use soil cored from the study sites, with roots removed and the original sequence of soil horizons reproduced in the packing of the cores. This is generally not feasible in peatland sites, since root removal would be far too laborious. It is, however, possible to find peat without live roots to generally match different peat soils: commercial non-fertilized horticultural moss peat for bogs, and sedge or woody peat harvested for energy use for fens and swamps. In minerogenic wet, open peatlands, where different sedges are the dominant vascular plants (e.g., Anderson et al. 1996; Saarinen 1996), water-borne nutrients may actually play a significant role in plant nutrition. In such sites, it might be possible to use an inert, inorganic material, such as crushed glass or plastic granules, as the substrate in the ingrowth cores. Such material would facilitate easy separation of the ingrown roots. Further, it would be a more homogeneous material than peat, and could be re-used several times. However, whether the root production patterns are similar in such materials to those in peat soil needs to be tested.

Our overall aim is to develop a robust method, operating at a yearly time step, that could be used in inventorying fine root production in peatlands. In this paper, we introduce a modification of the ingrowth core method that is both less time-consuming and causes less disturbance in the soil system than those previously applied. Combined with Infrared spectroscopy (IRS), it could yield production estimates without manual separation of the samples. In case IRS would not prove successful, we tried to find a way for easy manual separation of roots. The specific aims of this paper were

i) to estimate production of new roots (one-year ingrowth results obtained with manual separation of roots from the cores) in different types of peatland ecosystems, regarding which we tested the hypotheses that 1) root production would be higher in a nutrient-rich than nutrient-poor peatland forest, and 2) drying would lead to lower root production in an originally wet sedge fen,

ii) to test whether an inorganic, inert filling could be used instead of peat to facilitate fast separation of roots from the cores, regarding which we tested the hypotheses that 3) peat and inorganic substrate (glass or plastic) yield similar results in a wet site, but 4) the inorganic substrate yield lower results in a dry site, where plants need to rely more on the actual peat nutrient stores, and

iii) to test the potential of IRS calibration models for estimating root production without manual separation of the cores.

Materials and methods

Approach to the modification of the ingrowth core method

The idea has been developed based on our earlier experience on using the method in a more traditional form (Murphy et al. 2009) and testing the ingrowth net method (Lukac and Godbold 2010; data unpublished). We abandoned the net method for two reasons: difficulties in removing the nets with the necessary amount of undisturbed peat caused by the strong suction of the wet peat soils, and difficulties in obtaining estimates including radial growth and branching of older roots in addition to the production of new roots that the method was designed for.

The idea behind the modification has been to decrease the diameter of the cores and to increase the number of replicates. A smaller diameter reduces the disturbance in the soil environment in two ways: the cores may be installed without massive cutting of the root systems, and the volume of the “unnatural” competition-free rootless substrate inside the cores is smaller, and may have a smaller overall influence in the root growth patterns. Moreover, a small diameter may also minimize delays in the colonization of the cores (Hertel and Leuschner 2002) and thus, reaching the stage where all components of root production are possible. With a large number of replicates, the high spatial variation may be captured and reliable mean values estimated. A high number of replicates may be processed by applying IRS for estimating the root mass instead of manual separation and weighing. Cutting of the root systems is avoided by pushing the holes for the cores in the peat instead of cutting. This is possible since peat is predominantly organic matter, and as such, flexible (e.g., Price and Schlotzhauer 1999). Peat volume, and even the position of the peatland surface, depends on and changes with the peat water and gas contents (Päivänen 1973; Price 2003; Kellner et al. 2005; Waddington et al. 2010; Kettridge et al. 2013). Thus, the root systems in peat soils need to be flexible as well.

We have developed a two-piece corer for installing the cores (Fig. 1). The inner, closed and sharp-end tube pushes the hole to the ground. When the desired depth has been reached, the lock linking the two tubes is released, and the inner tube pulled out (Fig. 2). The hollow outer tube then allows us to drop the core into the hole. The outer tube has an outer diameter of 4.7 cm, and an inner diameter of 4.2 cm. To ensure that the cores fall freely, we set the theoretical core diameter to 3.18 cm (perimeter 10 cm), when preparing the cores. When the tube is pulled out, the displaced soil closes in around the core. The proper closure and the surface

position of the core must be checked. If the closure is not satisfactory, which is possible if the surface soil is dry, the surface soil around the core should be gently compressed around the core. Ideally, the cores are installed in late autumn, after root growth has ceased, to be well settled before the onset of root growth the next year. Study sites

The test sites included two drained peatland forests and one treeless sedge-dominated fen. In the sedge fen site, we chose two site conditions: wet fen site, and slightly drained fen site, where slight drainage was done by a shallow ditch to mimic the effects of climate change (Table 1). The drained sites were Lettosuo in Tammela and Kalevansuo in Loppi, while the sedge fen was Lakkasuo in Orivesi. These are all intensive study sites, where the root production C flux may eventually be compared with the ecosystem NEE estimated with either the eddy covariance method (the forested sites; Lohila et al. 2011) or closed chambers (the treeless site; Silvan et al. unpublished).

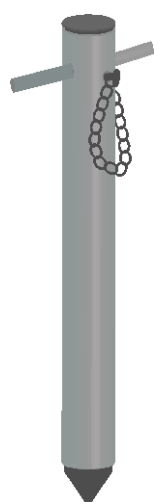


Fig. 1 The corer-installer. Inner tube in darker grey, outer tube in lighter grey, lock in black.

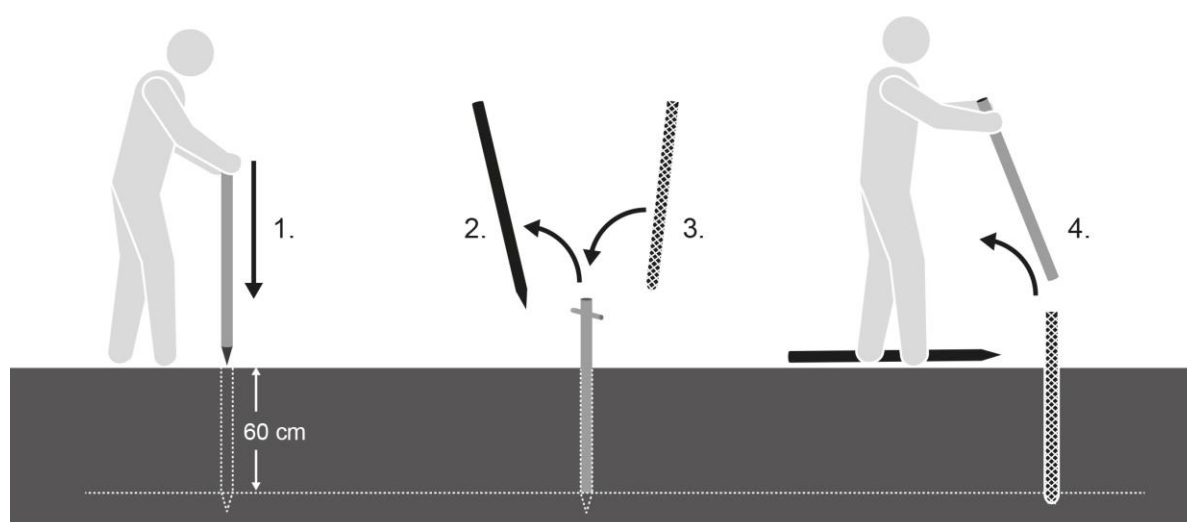


Fig. 2 Installation of ingrowth cores using the novel corer-installer.

Table 1 Some basic characteristics of the study sites. Soil bulk density (BD; g cm⁻³), N, P, and K concentrations (mg g⁻¹) and C:N ratio are average for 0-20 cm layer. Stand basal area = sum of tree stem areas measured at 1.3 m, in m² ha⁻¹, separately for Scots pine (*Pinus sylvestris*), downy birch (*Betula pubescens*) and Norway spruce (*Picea abies*).

Site	Type	BD	N	P	K	C:N	Stand Basal Area		
							Pine	Birch	Spruce
Kalevansuo	drained bog forest	0.093	14	0.56	0.31	36.0	17.6	0.7	<0.1
Lettosuo	drained fen forest	0.162	23	0.79	0.23	24.5	17.4	6.1	4.5
Lakkasuo	wet sedge fen	0.072	24	0.82	0.39	20.4	<0.1		
Lakkasuo/slightly drained	dry sedge fen	0.087	25	0.84	0.36	19.7	<0.1		

Kalevansuo (60°39'N, 24°22'E) was originally an ombrotrophic dwarf-shrub pine bog. It shall henceforward be called “drained bog forest”. It was drained for forestry in 1969 and was classified as dwarf shrub type drained peatland forest (cf. e.g., Westman and Laiho 2003) at the time of our study. The tree stand was dominated by Scots pine (*Pinus sylvestris*), with a minor understorey of stunted downy birch (*Betula pubescens*) and Norway spruce (*Picea abies*). The forest floor vegetation mainly consisted of dwarf shrubs (*Betula nana*, *Empetrum nigrum*, *Ledum palustre*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idea*) with some cottongrass (*Eriophorum vaginatum*) and cloudberry (*Rubus chamaemorus*).

Lettosuo (60°39'N, 23°57'E), our “drained fen forest” is located about 22 km west from Kalevansuo. The site was originally a mesotrophic fen classified as herb-rich tall-sedge birch-pine fen, and at the time of the study it was *Vaccinium myrtillus* type 2 drained peatland forest (Westman and Laiho 2003). The site was drained in the early 1970's. The tree stand mainly consisted of Scots pine with some co-dominant downy birch, and Norway spruce formed a vigorous understorey. The forest floor vegetation was more diverse than in Kalevansuo. In addition to species found in Kalevansuo, also herbs (*Dryopteris carthusiana*, *Trientalis europaea*) and some *Carex* species occurred in Lettosuo.

The sedge fen in Lakkasuo (61°47'N, 24°18'E) was a mesotrophic (moderately nutrient-rich) fen classified as herb-rich tall sedge fen. The vegetation was characterized by sedges (*Carex lasiocarpa*, *C. chordorrhiza*, *C. limosa*) along with bog bean (*Menyanthes trifoliata*). Also some dwarf shrubs (*Andromeda polifolia*, *Betula nana*, *Vaccinium oxycoccos*) occurred. A part of the wet sedge fen area had been surrounded by circa 40-cm deep ditch in 2008 to moderately lower the water-level. This slight drainage did not affect the coverage of sedge vegetation; however, the coverage of shrubs slightly increased and that of bog bean diminished.

Soil temperatures (Fig. 3) were monitored at different depths using Maxim iButtons. Water-levels (Fig.4) were monitored with Intech WT-HR dataloggers installed in perforated tubes.

Preparation of the ingrowth cores

The cores were made of elastic polyester fabric with a mesh size approximately 1 mm x 1 mm. The cores were sown to have a perimeter of 10 cm and, consequently, theoretical diameter of 3.18 cm. The effective length was planned to be 50 cm (drained peatland forests) or 60 cm (sedge fen). Additionally, a “tail” was included that would remain unfilled and aboveground to facilitate locating the cores after incubation, as well as some margin at the lower end (Fig. 2).

Four substrates were used as fillings: two types of peat and two types of inorganic materials. The peats were chosen to mimic the soil quality of the recipient site as well as possible, while the choice of the inorganic materials was based on finding a material that would facilitate easy separation of the ingrown roots. The materials were also expected to be as homogenous as possible, easily accessible and affordable. Non-fertilized horticultural moss peat was used for the drained bog forest, where the peat was mostly of *Sphagnum*-origin. Deep-horizon sedge (*Carex*) peat harvested for energy use was used for all the fen sites that were characterized by sedge peat. Deep-horizon peat was chosen to exclude the possibility of having fresh live root remains in the substrate. Two different inorganic materials were tested; crushed recycled glass (diameter of the glass pieces in average 0.8 mm), and polyethylene granules (diameter of the granules 0.3 mm) commonly used in plastic industry. The inorganic materials were used in the sedge fen only.

For the drained peatland forests, the bulk density of the site was checked, and the filling was planned to mimic that in 10-cm sections. For this purpose, 10-cm sections were marked in the effective part of the cores before filling. Subsamples of the peat substrates were dried to determine their average moisture contents, and the amount of fresh peat needed to achieve the desired dry mass in each 10-cm layer was calculated. The cores were then filled with the weighed batches of peat using a funnel. With the fresh *Sphagnum* peat, filling to the desired mass was well feasible. However, with the semi-dry sedge peat this was problematic, since the dry particles were escaping through the mesh, small-sized though it was. In the end, we decided to abandon the idea of a target mass for the sedge peat, and to fill them “safely” with rather too much than too little peat.

The diameters of the peat-filled cores were not measured accurately, since even the fresh *Sphagnum* peat was drier than peat in field conditions; it could be expected that the cores would expand to some extent after installation. Consequently, the effective diameter should be measured after recovery. It was obvious, however, that the diameters of the peat-filled cores were less than the theoretical 3.18 cm. The crushed glass and plastic granule fillings resulted in diameters at the theoretical value, or in the case of glass-filled cores, even higher.

The filled cores were closed with rubber bands tightly above the substrate surface. There was a short (2-3 cm) section of the filled core that was not fully circular in both the top and the bottom of each core; these were planned not to be included in the effective length of the core.

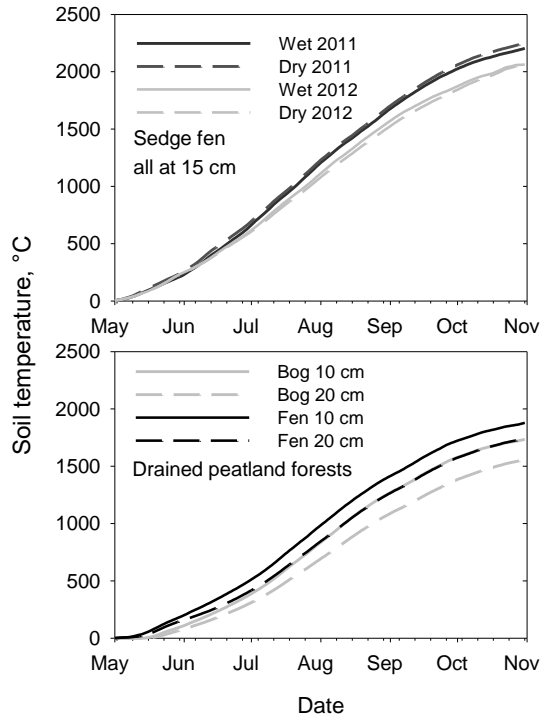


Fig. 3 Cumulative soil temperature sums, at 10 cm and 20 cm depths in the drained peatland forests, and at 15 cm depth in the wet and slightly drained site conditions in the sedge fen, during the growing seasons studied in each site: 2010 for the drained peatland forests, 2011-2012 for the sedge fen.

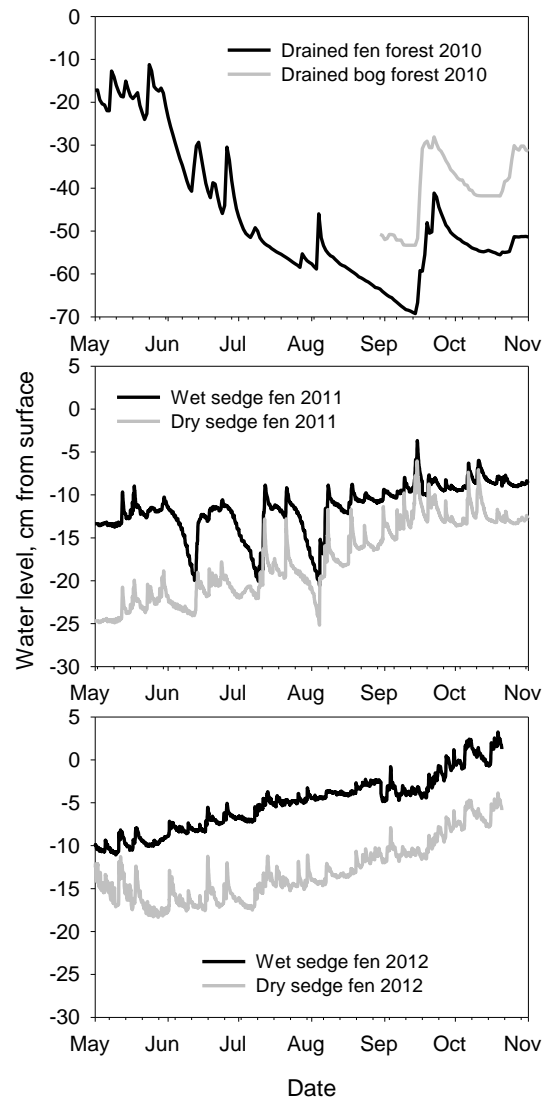


Fig. 4 Water levels of the study sites during the growing season studied

Installation and recovery

At the drained peatland forest sites, twenty in-growth cores were installed at each site in two different transects; ten cores in each transect. Each transect stretched across a strip between two ditches, with the first core installed at few meters away from the ditch margin to avoid the difficulties in inserting the core due to unevenness in the ditch bank, and the rest at about 4 m intervals. The cores of the drained bog forest were installed in late October 2009, and those of the drained fen forest in the beginning of May, 2010, because early soil frost prevented

installation in the autumn before. The cores were recovered after one growing season in November, 2010.

In the sedge fen, 36 cores filled with peat were installed in pairs in irregular order in both the wet and slightly drained sites in October 2010. In October 2011, 12 cores per site were collected for the analysis and rest of the cores were left on the site to monitor the root growth patterns in longer term. Twenty-four cores filled with plastic granules were installed in pairs next to the remaining peat cores in October 2011, along with 14 cores filled with glass installed close to gas exchange measurement points in an irregular pattern. The cores filled with glass and plastic were removed after one growing season in October 2012.

Vigorous moss growth in some locations made it obvious that the tails of the cores are not enough to facilitate finding the cores in longer term, but more extensive markings are needed. After one growing season, the new moss growth did not contain any roots, but in longer term, it must be secured that any root growth above the cores is accounted for with specific sampling if needed.

During removal of the cores, a long sharp knife was used to cut around the cores to detach any aboveground plant parts attached to or growing through the cores, and to cut the root systems, especially rhizomes and any hard lateral expansion, to avoid risk of pulling out roots from the cores. Next, the cores were simply gently pulled out of the soil, wrapped in plastic foil with their incubation location marked, and laid straight in plastic containers. The cores were then kept in freezer (-20 °C) until further treatment.

Post-recovery treatment of the cores

All ingrowth cores were first hand-separated to get the actual amount of root ingrowth, which represented the rate of fine root production over one growing season (production of new roots into the rootless material), and to gain test data for the IRS calibration.

Each core was cut into 10-cm segments, five segments down to the 40-50 cm depth for peat cores, and four cores down to the 30-40 cm depth for the cores filled with either glass or plastic. For the peat cores, the diameters of the segments were measured (two measurements at right angle of both the top and the bottom of each segment). For the glass and plastic cores, this was not feasible since the contents fall loose when the segments are cut, so the perimeters were measured instead. All roots found outward from the core segments were cut and discarded, while the roots inside the cores were recovered and gently washed clean with water. Estimation of whether the roots were living or dead was based on colour and friability. The roots were oven-dried to constant mass at 30°C and then weighed. Similarly, the peat material of each core was collected and oven-dried at 30°C and weighed.

Infrared spectroscopy and the calibration models

We used ten ingrowth cores from both the drained bog and the drained fen forest in this part of the study. The roots separated from these cores were powdered with an oscillating ball-mill MM400 (Retsch, Germany). Similarly, the rootless peat substrate (0-10 cm layer) of each core was powdered after oven-drying at 40° C for six days. Then, we prepared ten peat-root

mixtures from each site in the same root/peat mass concentration as was determined by hand-separation to mimic the original, unseparated ingrowth cores. In addition, we prepared ten artificial peat-root mixtures for each site. We mixed the roots in 0-5 % concentration with incremental addition of 0.5 % root. The 5% upper limit was used because roots formed 5% or less of the total mass of the bulk samples. Altogether, 40 mixtures were thus created to form the calibration data.

The infrared spectrum of each root, peat, and peat-root mixture sample was measured using a Bruker VERTEX 70 series FTIR (Fourier Transform InfraRed) spectrometer (Bruker Optics, Germany) equipped with a horizontal ATR (Attenuated Total Reflectance) sampling accessory. Powdered samples were inserted directly on the ATR crystal and the MIRacle high-pressure digital clamp was used to achieve even distribution and contact of the sample and crystal. Each spectrum consisted of 65 averaged absorbance measurements between 4000 and 650 cm^{-1} , with 4 cm^{-1} resolution. For correction of differences in the amplitude and baseline between different runs (samples) we used two different transformation methods: 1) standard normal variate transformation and de-trending and 2) first derivative, the Savitzky-Golay method. The standard normal variate transformation and de-trending gave better results in the analysis of the variation in chemical composition of the samples, while the first derivative performed somewhat better in the calibration models.

Variation in the chemical composition between the in-grown roots, peat substrates and the total ingrowth core mass (peat substrate plus the in-grown roots) was explored by principal component analysis (PCA). The transformed absorbance measurements were used as response variables.

For building calibration models for estimating the mass proportion of roots in the ingrowth cores, the data were divided into two subsets. Every fourth sample was systematically removed from the full dataset. The larger of the subsets thus created was used for building the calibration models (calibration samples; 75% of the dataset). The smaller subset was used for testing the performance of the calibration models (testing samples; 25% of the dataset).

Calibration models were built using partial least squares (PLS) regression. PLS regression reduced large numbers of correlated spectral data into a limited number of principal components (orthogonal components, loading vectors), each representing one independent gradient of variation in the chemical composition of the ingrowth core mass samples. The principal components were then used as independent variables in a multivariate regression with the measured proportion (%) of in-grown roots in the total ingrowth core mass (peat substrate plus the in-grown roots) used as the dependent variable.

The r^2 , root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used for estimating the accuracy of the models. RMSEC tells how well the reference values are fit by the calculated regression line in the calibration model while RMSEP shows the accuracy of prediction on independent (testing) samples. RMSEC/P is expressed in the same units as the dependent variable (% of in-grown roots in the total ingrowth core mass).

The spectra transformations and calibration models were done using the Unscrambler software (CAMO, Norway), the analysis of the variation in chemical composition of the samples were done using Canoco 5 (ter Braak and Šmilauer 2012).

Statistical analyses

First, we evaluated the distributions of the core-level root production values concerning skewness (symmetry) and kurtosis (peaked or flat relative to a normal distribution). Next, we evaluated potential of the number of our samples for producing reliable sample means. The relation between sample size (within a site and the maximum deviation of the sample mean from the population mean was analyzed making use of the central limit theorem (e.g., Ranta et al. 1989) according to which sample means follow asymptotically the normal distribution, with μ as expected value, and σ^2/n as variance. The theoretical maximum deviation of the sample mean from population mean with a probability $1 - \alpha$ can be expressed as

$$d = z_{1-\alpha/2} \sigma / \sqrt{n} \quad (1)$$

where $z_{1-\alpha/2}$ is the $1 - \alpha/2$ fractile of the normal distribution. We chose $\alpha = 0.05$. The standard deviations σ of root production were estimated for each site from the samples as the average s.d. of 1000 bootstrapped replications of the samples. The maximum deviations were transformed to percentages using the average mean of the bootstrapped samples as the estimate of μ .

Differences between i) the two drained peatland forests, ii) the wet and dry site conditions of the sedge fen, and iii) substrate performance were evaluated using Repeated Measures Analysis of Variance. Site or substrate was the between-subjects (grouping) factor, and depth (layer within a core) was the within-subjects (repeated) factor. Since there was considerable departure from sphericity in all cases (variances differed among depths), and values of the epsilon test statistic were always smaller than 0.75, the Greenhouse-Geisser correction was applied in the interpretation of the F-ratios. Since the sample distributions deviated from normal in some cases, and the number of samples was not always likely to lead to accurate sample means (see Results section), we applied resampling by bootstrapping to confirm the analyses. All analyses were repeated with 50 bootstrapped data sets. All analyses were done with SYSTAT11.

Results

General

After incubation, the average substrate diameters in the peat cores, which were measured for each 10-cm core section, varied between 2.3 – 2.9 cm (Fig. 5). Overall, the variation was not clearly correlated with the bulk densities of the ambient soils. This indicates that the diameters were not determined by the pressure of the (partially displaced) surrounding soil: in such a case, there would have been a negative correlation. No changes in the vertical dimensions of the cores were observed. The measured post-incubation diameters were used for transforming root production into area-based values.

While the top section perimeters of the glass-filled cores were at the theoretical maximum of 10 cm, the lower parts of these cores had yielded and the perimeters were typically 11-11.5 cm. Consequently, the area-based values were calculated using a diameter of 3.18 cm for the topmost 10-cm section, 3.5 cm for the 10-20 cm section, and 3.66 cm for all following lower sections. For the plastic-filled cores, the diameter of 3.18 cm was used all over, since no deviations were observed in their dimensions.

The bulk density of the peat inside the cores was close to that of the surrounding peat soil in the drained bog (Fig. 5). This was the only site with *Sphagnum*-dominated peat, where ambient bulk density was carefully targeted. In all cases with *Carex*-dominated peat, the bulk density inside the cores was clearly higher than that of the surrounding soil.

No dead roots were observed in any of the cores after one growing season.

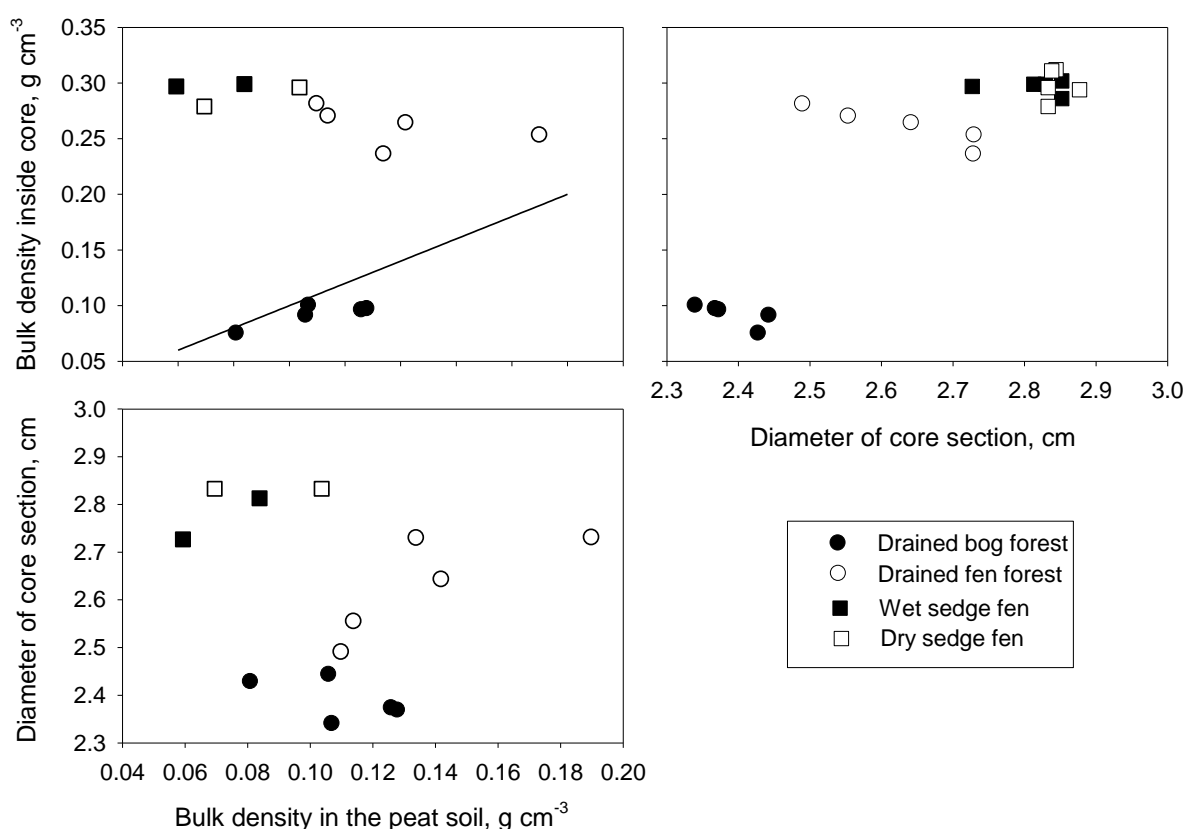


Fig. 5 Relations between peat bulk density of the recipient site, post-incubation bulk density of the peat substrate in the ingrowth cores, and post-incubation average diameter of the ingrowth core. All parameters were measured for 10-cm sections. The line corresponds to 1:1. For the sedge fen, bulk density in the peat soil was available only for the topmost 20 cm.

Production of new roots in different peatland sites

In the drained sites, production of new roots in the 0-50 cm peat layer totalled 118 g m⁻²yr⁻¹ in the drained bog and 216 g m⁻²yr⁻¹ in the drained fen (Table 2), and supported our hypothesis that root production was higher in the more nutrient-rich and floristically diverse site (Table 3). Also, the depth distribution of root production differed between the two sites: production peaked in the top layers more clearly in the drained fen (Fig. 6). Both results were confirmed by analyses of bootstrapped samples: 46 samples out of 50 showed a significant site effect, while all 50 samples showed a significant site x depth interaction. The within-site variation was considerable (Table 2), and showed no clear trend relative to the distance to ditch (not shown).

In the sedge fen, root production in the 0-50 cm peat core totalled 170 g m⁻²yr⁻¹ in the wet site, while in the slightly drained site it totalled 125 g m⁻²yr⁻¹ (Table 4). In the 50-60 cm layer studied in these sites, average root production between 1.1 g m⁻²yr⁻¹ (slightly drained site; SE 0.8 g m⁻²yr⁻¹) and 3.2 g m⁻²yr⁻¹ (wet site; SE 1.2 g m⁻²yr⁻¹) was additionally observed (Fig. 7). The difference between the two sites was not statistically significant, however, so our hypothesis that drying would lead to lower root production in an originally wet sedge fen was not supported (Table 5). Neither were the depth-related patterns of production significantly different between the two site conditions. Both results were again confirmed by analyses of bootstrapped samples, although somewhat less convincingly: 17 samples out of 50 showed a significant drying effect, and 12 samples out of 50 showed a significant site x depth interaction.

Table 2 Fine root production (g m⁻²yr⁻¹) with standard deviation (SD), standard error (SE), minima and maxima by depth in the drained peatland forests. N = 20 for both sites.

Depth, cm	Drained bog forest					Drained fen forest				
	Mean	SD	SE	Min	Max	Mean	SD	SE	Min	Max
0 to 10	47.4	42.3	9.4	0.0	141.0	142.7	75.6	16.9	36.3	312.4
10 to 20	34.8	33.9	7.6	0.0	145.0	38.2	30.2	6.7	0.0	101.9
20 to 30	14.4	17.8	4.0	0.0	49.4	18.1	20.1	4.6	0.0	78.5
30 to 40	10.6	22.6	5.1	0.0	96.3	10.5	11.8	2.6	0.0	45.9
40 to 50	10.6	18.6	4.2	0.0	66.6	6.0	10.0	2.2	0.0	37.4
Total 0 to 50	117.8	86.2	19.3	0.0	371.6	215.5	110.7	24.8	46.6	439.8

Table 3 Repeated Measures Analysis of Variance on A) the effect of Site (drained bog, drained fen) on fine root production ($\text{g m}^{-2}\text{yr}^{-1}$) in drained peatland forests, and B) the effect of Drying (wet, slightly drained; see text) on fine root production in a sedge fen. Data are from ingrowth cores filled with peat and were divided into five (A) or six (B) 10-cm layers per core (within-factor Depth). P-values for within-subjects effects involve Greenhouse-Geisser correction (epsilon 0.47 in A, and 0.46 in B).

Effect	df	MS	F	P
A) Drained peatland forests				
<i>Between subjects</i>				
Site	1	19070	9.68	0.004
Error	38	1969		
<i>Within subjects</i>				
Depth	4	52549	56.58	<0.001
Depth x Site	4	18036	19.42	<0.001
Error	152	929		
B) Sedge fen				
<i>Between subjects</i>				
Drying	1	1977	2.23	0.149
Error	22	886		
<i>Within subjects</i>				
Depth	5	18812	20.92	<0.001
Depth x Drying	5	799	0.89	0.430
Error	110	899		

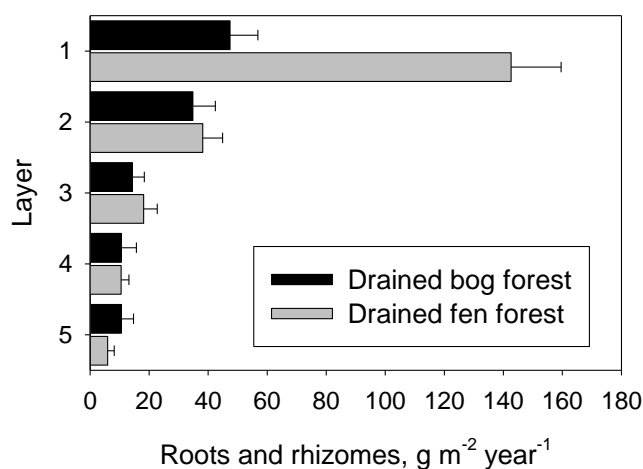


Fig. 6 Root production in the drained peatland forests. Error bars are standard errors of mean. Layer 1: 0-10 cm depth from moss surface; layer 2: 10-20 cm; layer 3: 20-30 cm; layer 4: 30-40 cm; layer 5: 40-50 cm.

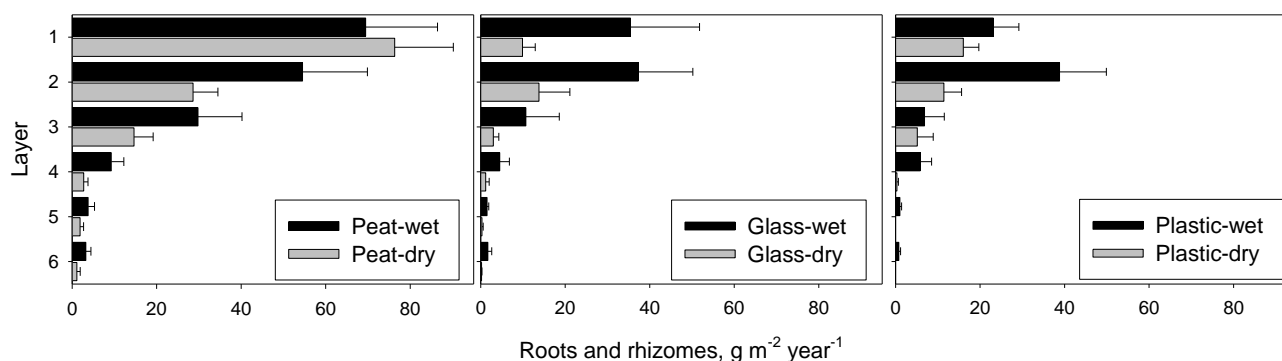


Fig. 7 Root production in the wet and slightly drained site conditions in the sedge fen, measured with ingrowth cores filled with: A: peat; B: crushed glass, C: plastic granules. Layer 1: 0-10 cm depth from moss surface; layer 2: 10-20 cm; layer 3: 20-30 cm; layer 4: 30-40 cm; layer 5: 40-50 cm; layer 6: 50-60 cm.

Table 4 Fine root production ($\text{g m}^{-2}\text{yr}^{-1}$) with standard deviation (SD) and standard error (SE) by depth in the sedge fen (wet and slightly drained site conditions), obtained with A) peat substrate ($n = 12$ for both sites), B) crushed glass ($n = 7$ for both sites), and C) plastic granules ($n = 11$ for both sites).

Depth, cm	Wet sedge fen			Min	Max	Dry sedge fen			Min	Max
	Mean	SD	SE			Mean	SD	SE		
A) Peat										
0 to 10	69.4	59.0	17.0		217.6	76.3	48.0	13.9		151.9
10 to 20	54.5	53.2	15.4		197.4	28.6	20.3	5.9		68.1
20 to 30	29.8	36.0	10.4		119.1	14.7	15.7	4.5		52.1
30 to 40	9.3	10.2	3.0		29.8	2.7	3.5	1.0		12.5
40 to 50	3.8	5.3	1.5		14.1	1.9	2.9	0.8		8.6
Total 0 to 50	170.0	76.9	22.2	41.3	292.8	125.3	69.1	20.0	9.9	228.2
B) Crushed glass										
0 to 10	35.4	43.3	16.4	2.5	98.2	9.9	8.0	3.0	0	23.9
10 to 20	37.3	34.2	12.9	7.3	85.2	13.8	19.3	7.3	1.0	54.0
20 to 30	10.7	21.0	7.9	0	58.0	3.0	3.3	1.3	0	7.6
30 to 40	4.5	6.0	2.3	0	17.1	1.2	2.2	0.8	0	5.7
40 to 50	1.5	1.1	0.4	0	2.9	0.3	0.7	0.3	0	1.9
Total 0 to 50	89.4	77.6	29.3	13.8	204.3	28.2	25.1	9.5	3.1	71.7
C) Plastic granules										
0 to 10	23.1	20.1	6.1		66.7	16.0	12.2	3.7		32.7
10 to 20	38.8	36.8	11.1		129.7	11.4	13.9	4.2		39.0
20 to 30	6.9	15.5	4.7		52.9	5.1	12.5	3.8		41.5
30 to 40	5.8	8.9	2.7		30.2	0.3	1.1	0.3		3.8
40 to 50	1.0	1.4	0.4		3.8	0.0	0.0	0.0		0.0
Total 0 to 50	76.4	55.6	16.8	26.4	207.7	32.9	26.0	7.8	2.5	66.7

Table 5 Repeated Measures Analysis of Variance on the effects of Drying (wet, slightly drained; see text) and Substrate (peat, crushed glass, plastic granules) on production of new roots ($\text{g m}^{-2}\text{yr}^{-1}$) in a sedge fen. Data were from ingrowth cores and were divided into six 10-cm layers per core (within-factor Depth). P-values for within-subjects effects involve Greenhouse-Geisser correction (epsilon 0.48).

Effect	df	MS	F	P
<i>Between subjects</i>				
Drying	1	5993	10.04	0.003
Substrate	2	10089	16.91	<0.001
Drying x Substrate	2	78	0.13	0.877
Error	54	597		
<i>Within subjects</i>				
Depth	5	14697	29.21	<0.001
Depth x Drying	5	1141	2.27	0.097
Depth x Substrate	10	2675	5.32	<0.001
Depth x Drying x Substrate	10	276	0.55	0.734
Error	270	503		

Substrate performance

Production values obtained with peat substrate were generally the highest, and they peaked in the topmost, 0-10 cm layer (Fig. 7, Table 4, Table 5). In the data set covering all substrate types, also the drying effect was significant, and there was no significant interaction between drying and substrate, which contrasted the result obtained for peat-filled cores alone. In the analyses of resampled data sets, six samples out of 50 yielded a significant interaction between drying and substrate. Further, 11 samples yielded a significant interaction between depth, drying and substrate. A significant interaction between depth and drying was supported with 36 samples out of 50. (All samples yielded significant drying and substrate effects, as well as interactions between depth and substrate.)

Since different substrates clearly resulted in different depth distribution patterns (Fig. 7, Table 5), we analyzed separately if they would reproduce total production in the 0-50 cm layer similarly, when the (dominant) depth effect was not involved. The main effects of substrate and drying remained significant (not shown). Post hoc tests showed that in the wet sedge fen, the production values obtained with peat and glass were not significantly different, while those obtained with plastic were significantly lower than those with peat. This lent partial support to our hypothesis that peat and inorganic substrate yielded similar results in a wet site. In the slightly drained sedge fen, the production values obtained with glass and plastic were similar, and significantly lower than those obtained with peat. Thus, the hypothesis that inorganic substrates yielded lower results than peat in a dry site was confirmed.

Number of cores needed for reliable mean estimates

The accuracy of the mean production values depends strongly on the number of cores they are based on (Fig. 8). Between 10 and 20 cores were needed for the theoretical maximum deviation of the sample mean to drop below 10%; except for the drained bog where as much as 30 cores were needed.

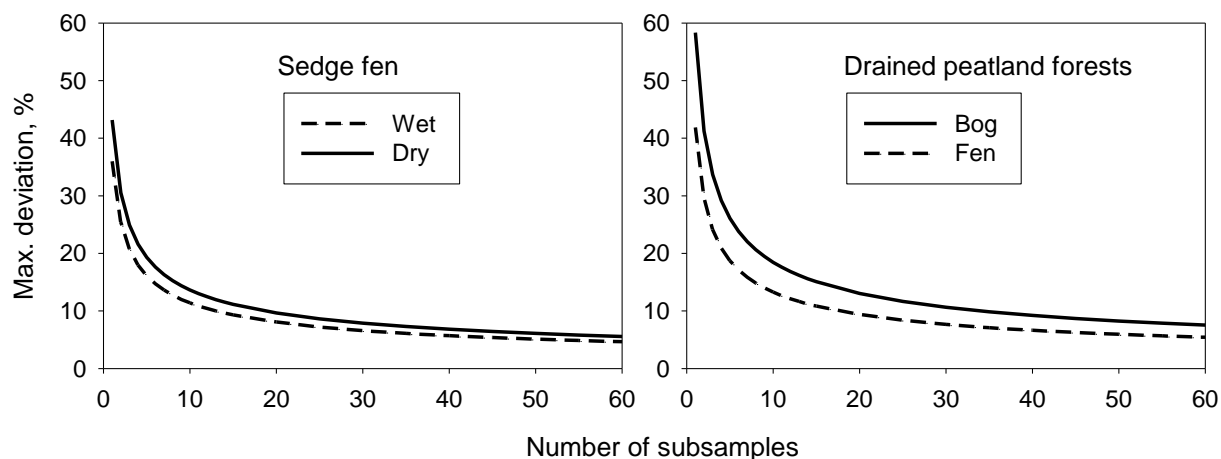


Fig. 8 Theoretical maximum deviation of sample mean from population mean, as percent of population mean, relative to the number of ingrowth cores constituting the sample. Estimation was based on the central limit theorem, and one thousand bootstrapped samples were used for estimating population mean and standard deviation used in the estimation.

Estimation with IRS

The *Sphagnum*-dominated peat used at the drained bog site, and the *Carex*-dominated peat used at the drained fen site are chemically rather different, and the overall contribution of the peat substrate to the spectra of the total ingrowth core mass (peat substrate plus the in-grown roots) is essential (Fig. 9).

The mass proportion of roots in the total ingrowth core mass (peat substrate plus the in-grown roots) was generally less than 5%. The calibration models based on small data sets, 20 spectral samples for each site, yielded very promising results (Fig. 10). For all the models, the coefficients of determination for prediction were high; r^2 ranged between 0.72 and 0.83, and the prediction error (RMSEP) did not exceed 8.2%. Site-specific models performed better, with RMSEP as low as 5.2 % and 6.7 % for drained bog and fen, respectively. When just one calibration model was built using data from both sites, the result was somewhat worse because a slight systematic error was introduced in the estimation (Fig. 10).

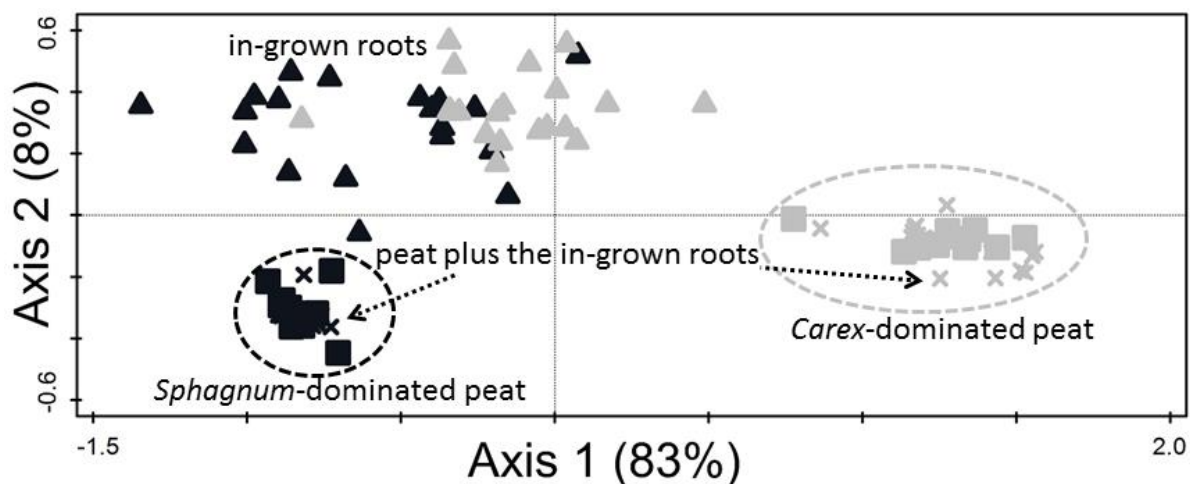


Fig. 9 Ordination diagram from principal component analysis (PCA) showing relations between infrared spectra of fine roots (triangles), peat substrates (squares) and peat-root mixtures mimicking the total ingrowth core mass (X marks). Samples from the drained bog forest are shown with black symbols and those from the drained fen forest with gray symbols, respectively.

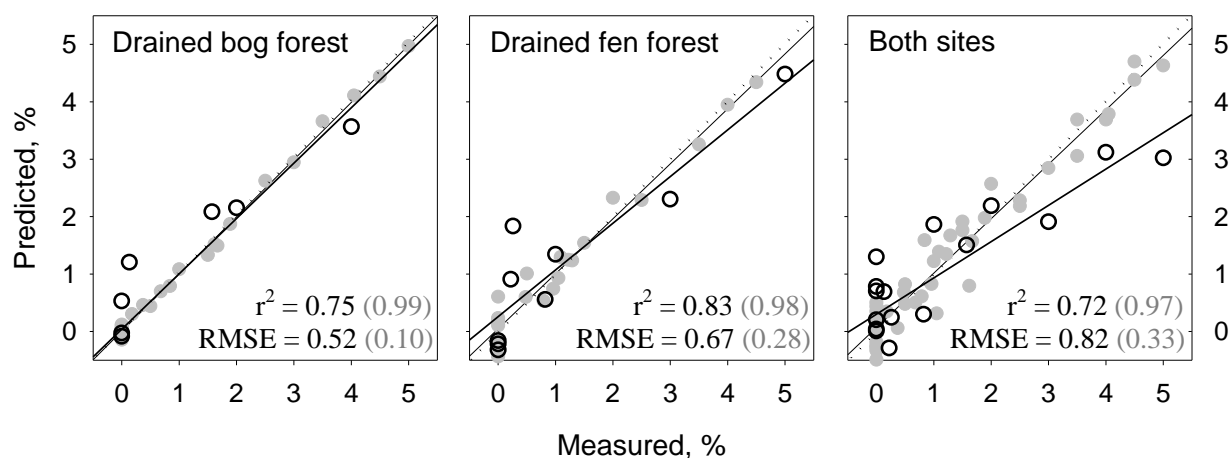


Fig. 10 Relationship between the actual mass proportion of roots in the ingrowth cores and the proportion predicted by infrared reflectance spectroscopy using partial least squares regression (PLS) calibration. The dotted line represents the 1:1 line. Altogether forty test samples (twenty from each site) were used, of those $\frac{3}{4}$ for calibration (grey circles) and $\frac{1}{4}$ for prediction (open circles). The coefficient of variation (r^2) and root mean square error (RMSE) of prediction are shown in black numbers and those for calibration in grey numbers.

Discussion

Patterns in the production of new fine roots

Our first-year fine root production estimates of the drained sites were clearly higher than those obtained earlier for drained bogs and fens (6.6-77.6 g m⁻²yr⁻¹; Finér & Laine 2000; Murphy et al. 2009), while those of the sedge fen were of the same magnitude as those observed earlier (Bernard et al. 1988; Finér & Laine 2000). Overall, our first-year results were already of the same magnitude as annual fine root production rates estimated for three drained peatland forests by Finér and Laine (1998, 2000) using sequential coring and ingrowth cores over a three-year period. Thus it seems that our modified method is successful in causing less disturbance and facilitating faster colonization in drained sites dominated by trees and shrubs. In wet sites dominated by sedges the advantage seems to be minor. Finér and Laine (2000) observed that root biomass in the ingrowth cores of drained sites started to increase clearly only during the second growing season, whereas on the sedge fen the ingrowth of roots began already during the first growing season. This is in line with the observation of, e.g., Lukac and Godbold (2001) that cutting resulted in a lag in tree root growth, while cut avoidance resulted in faster colonization of ingrowth cores. In contrast, Steingrobe et al. (2001) observed no alteration of root growth pattern in herbaceous plants. Unfortunately, in field studies there is no absolute root production value available to compare the estimates with (Milchunas 2009). We will follow the production patterns with the ingrowth cores over two more years to find out the optimal incubation period (Finér and Laine 2000).

The first-year results reflect the regenerative potential of the root systems rather than actual production rates of established root systems (Cudlín and Chmelíková 1999). Thus, the values presented in this paper must be considered indicative of this potential only, and cannot be used to ascribe actual root production to any of the sites. First-year results cover only the production of new roots, while branching of older roots and radial increment of perennial arboreal roots are not fully represented. On the other hand, production of new roots may be overestimated if they grow preferably to the root-free substrate, as might be expected. The estimates for both total production and depth-related patterns may change in the two- and three-year recoveries to follow. Also the variability and the deviation of the distributions from normal may be relieved to some extent; however, the spatial variation is most likely to remain high (Finér and Laine 1998; Finér and Laine 2000). In any case, it seems obvious that fine root production is a major C flux in drained peatland ecosystems, as well as in sedge-dominated fens, and should receive more attention in the future.

Whether root production is higher in nutrient-poor sites, where new resources must be explored continually, or in nutrient-rich sites, where resources may be “wasted” for root regeneration has long been debated (Nadelhoffer et al. 1985; Helmisaari et al. 2007). Our results suggest that at least the regenerative potential is higher in a more nutrient-rich and floristically diverse forest than a nutrient-poor forest. Somewhat unexpectedly, production was higher only in the topmost layer; a system with a wider range of species and plant functional types would be expected to show complementary resource use mediated by belowground niche differentiation (Lei et al. 2012; Brassard et al. 2013; Lang'at et al. 2013),

especially since in the richer site, the water table level was lower thus presumably offering more oxic rooting space. However, final conclusions should not be drawn from the one-year data only.

Unexpectedly, drying did not lead to significantly lowered root production in the sedge fen. Water-level drawdown by 5-10 cm seems not have been enough to impede sedge performance in a situation where competition by shrubs and trees has not yet increased considerably (also Hultgren 1988, 1989). Increased shrub root production may have counteracted any decreases in root production of sedges and/or herbs; at least the latter may be expected to have occurred because of lowered above-ground coverage of *Menyanthes trifoliata*. With certain caution, the drained fen forest may be seen as an end-point to a persistent drying succession in the sedge fen, and suggests that in long-term, drying and forest succession (Laiho et al. 2003) maintains high root production in such sites (root production in 0-50 cm peat layer, wet fen – drained fen forest comparison: LSD $p=0.186$). In wet sedge fens, carbon sequestration is largely facilitated by root inputs (Saarinen 1996); however, root production in drained fens is generally not enough to compensate for increased decomposition losses induced by water-level drawdown (Ojanen et al. 2010).

Methodological considerations

The diameter of ingrowth cores used earlier in peat soils has ranged from 4 cm to 10 cm (Finér and Laine 2000; Steingrobe et al. 2001; Murphy et al. 2009; Murphy and Moore 2010), while in our version it was less than 3 cm. A smaller diameter leads to a smaller area: perimeter ratio. In a 10-cm-diameter core this ratio is 2.5, while in a 3-cm core it is only 0.75. This, in addition to reduced disturbance, may facilitate faster colonization of smaller-diameter cores. However, when the diameter is reduced, also the relative error in measuring the core area increases in practice. It is difficult to suggest what would be an optimal diameter; the one that we chose was based on intuition, not thorough testing with different diameters that could in principle be done.

Peat bulk density inside the cores matched rather well the density of the surrounding peat soil in the drained bog forest, where ambient density was specifically targeted. Some substrate decomposition seems to have taken place, since the post-incubation diameters were lower than the theoretical 3.18 cm, which was used when estimating the amount of peat to be used for filling each 10-cm section. Yet, the peat inside the cores maintained the target bulk density, possibly aided by the pressure of ambient soil. The dry sedge peat used in the other sites, which did not allow filling into target density, resulted in higher-than-ambient densities inside the cores in all cases. Steingrobe et al. (2000) found that root ingrowth into cores was not altered by lower-than-ambient soil density in the core, but higher-than-ambient density reduced root ingrowth significantly. If this were the case also in our study, then our estimates obtained with sedge-peat cores would be underestimates. This would mean that the difference between the drained bog and drained fen forest would be even greater than observed. Further, it might mean that the difference between peat and the inorganic substrates would also have been greater if the cores had been filled into ambient density. In any case, we recommend the

use of fresh (“non-dried”) peat, which allows for filling the cores to target densities determined by the recipient sites.

Comparison between the different substrates used is to some extent hampered by the fact that they were on-site during different years (peat over 2011, glass and plastic over 2012). Year 2012 was somewhat cooler and wetter than 2011 (Figs 2 and 3), so the values for the glass- and plastic-filled cores may be somewhat underestimates as compared to the peat-filled cores. There is no information available yet on the effects of soil temperature or water-level on sedge root production at these ranges, however.

With certain reservations, crushed glass could be a feasible substrate for ingrowth cores installed in wet sedge fens, if the only goal were to estimate total production. The advantage obtained is that even very thin roots, that are difficult to separate from peat (Sjörs 1991; Finér and Laine 2000), can be much more easily separated. However, it seems that an inorganic substrate attracts less roots than peat at the very surface. This may be because its porosity and pore-size distribution, and, consequently, water-holding capacity differ from those of peat, so the very surface may get too dry even in a wet site. Consequently, if the depth distribution of root production is of interest, the glass substrate may not be recommended. The performance of plastic granules was overall poor. The different performance of glass and plastic may be due to different pore sizes of these materials. Crushed glass pieces were bigger and had irregular shapes which caused that pores were generally much larger in their size than with the smaller plastic granules. These larger pores when water-filled could have attracted the roots in the glass cores especially if the nutrients are mainly obtained from the water. The lower performance of the plastic substrate may also be because plastic is less inert than glass; the plastic granules may have dissolved compounds that inhibit root growth.

The first attempt to estimate total root production based on the IR spectra of unseparated ingrowth core samples gave promising results. The different plant species found in our peatland sites are chemically heterogeneous (Straková et al. 2010); yet, their roots that altogether formed 5% or less of the total mass of the samples separated well from the peat substrates that formed the bulk of the samples. The fact that the performance of the combined model for both sites was not as good as the site-specific models, in spite of the bigger n , may depend on the peat substrates used. The *Sphagnum*-dominated peat used at the drained bog site, and the *Carex*-dominated peat used at the drained fen site are chemically rather different (Bohlin et al. 1989), and their overall contribution to the sample spectra is considerable (Fig. 9). Thus, it seems that a limited number of standard peat materials should be used in inventory studies to avoid preparation of several calibration models. Two peat types may be sufficient in the boreal zone, since the majority of the peats are predominantly either *Sphagnum* or *Carex* origin, with varying admixtures of other constituents. Even though the different species within these genera – *Sphagnum* and *Carex* – differ to some extent from each other chemically, they form rather homogeneous groups when compared to other plant species or functional type groups (Straková et al. 2010). Further testing is still needed to confirm whether general models developed for the different peat substrates perform well in different sites, or whether some calibration data are needed from all sites. If the latter option were confirmed, it would

then depend on how many samples per site need to be added to the calibration data, as to whether the model approach is feasible in practical scale.

We will apply the methodology presented here primarily in an inventory of root production in drained peatland forests across Finland. This information is urgently needed for greenhouse gas reporting, where currently the biggest uncertainty concerning forest land derives from peatland forests (Statistics Finland 2012), and is largely caused by uncertainty related to fine root production that is estimated using data from mineral soil forests. There is currently no information concerning whether the fine root dynamics of a certain species growing on either mineral soil forest or organic (peat) soil forest are similar. Since the nutrient regime, water regime, and soil texture differ greatly between these broad soil types (Westman and Laiho 2003), it may be assumed that root systems respond differently.

Conclusions

Based on the experience from the first-year cores, the following guidelines may be given for inventorying root production in peatland sites with the modified ingrowth core method:

1. Twenty ingrowth cores per site and recovery may generally be recommended for obtaining reliable mean production values.
2. Fresh peat should be used as the substrate, and the cores should preferably be filled into target densities determined by the recipient sites.
3. After each recovery, the actual core diameters need to be measured and used for the calculations of area-based production values.
4. The height growth of the moss layer may be several cm per year even in drained peatlands (Laiho et al. 2011). This must be considered when installing ingrowth cores for a period longer than one year.
5. Crushed glass could be used as a substrate in wet fens, if the only goal were to estimate total production without paying heed to the depth distribution.
6. A limited number of standard peat substrates should be selected when IR-spectroscopy is used, since for each peat type, a specific calibration model may be needed.

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