

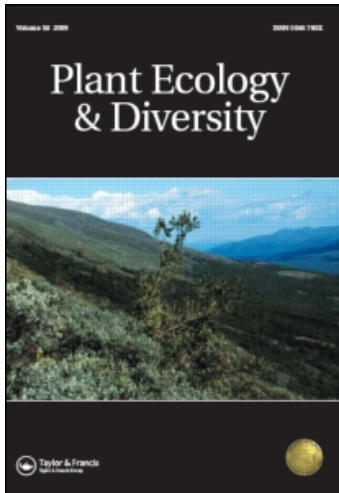
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Root survival of six cool-temperate wetland graminoids in autumn and early winter

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Background: Timing of leaf senescence is an important ecological trait of species in cool-temperate climates. In contrast to above-ground biomass, not much is known about timing and extent of root mortality of herbaceous perennials at the end of the growing season.

Aims: We undertook to assess patterns of end-of-season root mortality in species of temperate wetlands, investigate if the interspecific variation in the pattern of leaf senescence in autumn is reflected in patterns of root mortality, and determine if coarse axile roots and fine lateral roots differ in their autumn mortality pattern in wetland graminoids.

Methods: For six cool-temperate perennial wetland graminoids, we assessed whether their roots die back for the winter, if the species-specific timing of senescence corresponds to interspecific variation in leaf senescence, and if fine lateral roots and axile roots differ in their mortality pattern. Survival and mortality of lateral and axile roots, and above-ground senescence was assessed in Sudbury, Ontario, Canada, from 11 September to 11 December, 2007, a period during which most of the above-ground biomass died. Root mortality was assessed using 2,3,5-triphenyltetrazolium chloride.

Results: In *Carex oligosperma*, *Carex stricta*, *Phalaris arundinacea* and *Scirpus microcarpus* the majority of lateral and axile roots showed little mortality until mid-December. Axile roots of *Dulichium arundinaceum* showed little mortality, but its lateral roots died in October. Both lateral and axile roots of *Sparganium angrocladum* died in October.

Conclusions: These results indicate that fine roots of many species do not senesce along with the leaves for winter in cool-temperate climates. In some species, however, all roots senesce in autumn.

Keywords: autumn senescence; axile roots; fine roots; root mortality; triphenyltetrazolium chloride; wetland

Introduction

Seasonal variation in leaf biomass, i.e. growth in spring and senescence in autumn, is an important aspect of biomass turnover of deciduous and herbaceous species in climates with a pronounced winter. The overall significance of this phenomenon can be seen, for example, in the seasonal fluctuations of the atmospheric CO₂ concentrations it causes (Keeling et al. 1996). In contrast to leaf turnover, less is known about seasonal patterns in root senescence. In deciduous trees, the seasonal variation in fine root turnover has been found to be less pronounced than that in leaf turnover, even though root mortality often increases at the end of the growing season (Hendrick and Pregitzer 1996; Wells and Eissenstat 2001; Wells et al. 2002; Kern et al. 2004; Baddeley and Watson 2005; Satomura et al. 2006). In herbaceous species of pastures and lawns the amount of live roots decreases for the winter, mostly as a result of decreased production (Fitter et al. 1998; Liu and Huang 2002), but sometimes due to increased mortality in late summer (Volder et al. 2007). The relatively low seasonal variation in mortality has been attributed to the short life spans of fine roots, which results in a continuous mortality over the growing season (Eissenstat and Yanai 1997; Zak et al. 2000; Gregory 2006). However, average root life spans show large interspecific variation, which may be associated with the ecological strategies of the species

(Ryser 1996; van der Krikt and Berendse 2002). Information on interspecific variation in timing of root senescence in herbaceous species of natural environments, and its possible significance on the ecological requirements of the species is still lacking.

The majority of the studies that include seasonal aspects of root mortality have been conducted with trees, or plants in agricultural ecosystems. Root turnover studies in temperate wetlands are rare in general (Gill and Jackson 2000). Shaver and Billings (1975) compared three graminoids in arctic wetlands and found that one of the species annually produced a new root system, whereas the others kept their roots for several years. Root biomass has been found to decrease for the winter in a *Cladium mariscoides* fen in New York State (Bernard et al. 1985), but to increase in a *Carex rostrata* wetland in Minnesota (Bernard 1974).

Timing of leaf senescence is an important part of the ecological strategy of species (Watson and Lu 2004; Kahlert et al. 2005). Seasonal pattern of above-ground biomass and shoot demography show large interspecific differences in graminoid macrophytes of temperate wetlands (Bernard and Bernard 1973, 1977; Gorham and Somers 1973; Bernard 1976). In northeastern Ontario, at the northern edge of the deciduous biome with a growing season length of around 180 days (Watson and McIver 1995), species of

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wetland habitats with recent disturbance, such as *Sparganium* sp. or *Alisma triviale* senesce in September, whereas many large sedges of less disturbed wetlands, such as *Carex lacustris* and *Carex stricta*, senesce only after severe frosts in November. *Carex oligosperma*, a species of nutrient-poor fens, may remain partially green long into the winter (P. Ryser, pers. obs.).

Understanding the pattern of fine-root turnover is as important as that of leaf turnover, as it forms a considerable part of the resource balance of a plant and of the carbon cycle of an ecosystem. For example, in arctic sedge ecosystems fine root production may account for one-third of the net primary production (Nadelhoffer et al. 2002). Fine root turnover also shows sensitive responses to environmental conditions (Guo et al. 2008). In the present study, the timing of root senescence at the end of the growing season was assessed for six ecologically contrasting perennial wetland graminoids, and compared with patterns in above-ground senescence. As different root branch orders differ in function (Gill et al. 2002; Pregitzer et al. 2002; Wells and Eissenstat 2003), we examined separately the mortality of the coarse axile roots, and the attached fine lateral roots of 2–3 orders.

The present study had three aims: (1) to assess patterns of end-of-season root mortality in species of temperate wetlands, (2) to investigate if the interspecific variation in the pattern of leaf senescence in autumn is reflected in patterns of root mortality, and (3) to determine whether coarse axile roots and fine lateral roots differ in their autumn mortality pattern in wetland graminoids.

Root viability cannot be readily judged based on appearance, and in the anoxic and cool wetland soils any decomposition processes leading to visual changes in dead roots would be slow. We used staining with 2,3,5-triphenyl-tetrazolium chloride (TTC; Comas et al. 2000) to assess the percentage of the root length alive at any given time. In living tissue, the colourless TTC is reduced to red formazan. We assessed the mortality of lateral and axile roots from mid-September to mid-December, a period during which for all of the species the above-ground parts mostly senesced.

Material and methods

Species studied

The study was conducted with six graminoid wetland monocots from the region of Sudbury, Ontario, Canada (46°30'N; 81°00'W) – four species of the family Cyperaceae, one species of Sparganiaceae and one species of Poaceae. Nomenclature follows Flora of North America Editorial Committee (1993–). *Carex oligosperma* was collected from a *Sphagnum*-dominated nutrient-poor fen, *C. stricta* from a tall sedge meadow marsh, and *Scirpus microcarpus* from an abandoned agricultural grassland flooded by beavers. Tillers were collected in spring 2006 and planted in organic wetland soil in 0.5-l plastic beakers. The plants were grown outdoors until 15 May 2007,

when they were planted in 18-l pots. Three more species were planted in spring 2007. Rhizomes of *Dulichium arundinaceum* were collected on a lakeshore in April 2007, before any leaf development. Rhizomes of *Sparganium androcladum* were collected at a muddy creek-bottom in April 2007 before they had leafed out. Seeds of *Phalaris arundinacea* were collected in summer 2005 in a grassland flooded by beavers, and germinated on soil in trays in April 2007. All these species were grown for a few weeks in plastic beakers before planting them in 18-l pots in late May 2007.

Growth conditions

The plants grew in 18-l plastic pots, 30 cm in height and 28 cm in diameter, with holes at the bottom. The pots were filled with topsoil, rich in organic matter, from a beaver meadow (pH = 4.0; measured in 1:1 mixture of soil and distilled water). No fertilizer was added. The pots stood outdoors in pools filled with 20–25 cm groundwater, continuously filled using a solar pump, resulting in about two-thirds of the soil volume being under water. The pots were regularly weeded to prevent other species from becoming established.

From 11 September to 11 December, nine plants of each of the six species were harvested at intervals of 6 to 14 days. The harvests were conducted on 11–13 September, 21–25 September, 3–4 October, 17–19 October, 29–31 October, 12–19 November, 26–28 November, 4–5 December and 10–11 December.

To minimize frost exposure of the roots, the pots were moved to the Laurentian University campus on 15 November 2007, where they were stored outdoors in a box with insulated walls until harvest. The three last harvests were conducted using these plants.

Microclimatic measurements were conducted adjacent to the pots using a Watchdog Ministation (Spectrum Technologies, Inc, Plainfield, Illinois, USA). Air temperature was recorded 60 cm above soil surface at the level of the canopy in the pots. Soil temperature in a 10-l plastic pot, standing in a pool of 10 cm water, was recorded with an external sensor. Some meteorological data from the garden was lost and to fill the gaps air temperatures at Sudbury Airport, 24 km east-north-east of the experimental garden (Environment Canada, 2007; <http://www.climate.weatheroffice.ec.gc.ca>) are given for the harvesting period.

Until early October, average daytime maximum temperatures at canopy level in the garden reached 20 °C and above (Figure 1). Night frosts were observed in late September and mid-October. In late October night frosts were frequent, and in November the air temperature remained around and below zero for longer periods (Figure 1).

Soil temperatures in the 10-l pot with the sensor fluctuated less. From late October onwards the temperatures remained mostly below 10 °C, but never fell below zero while the pots of this experiment were in the experimental garden.

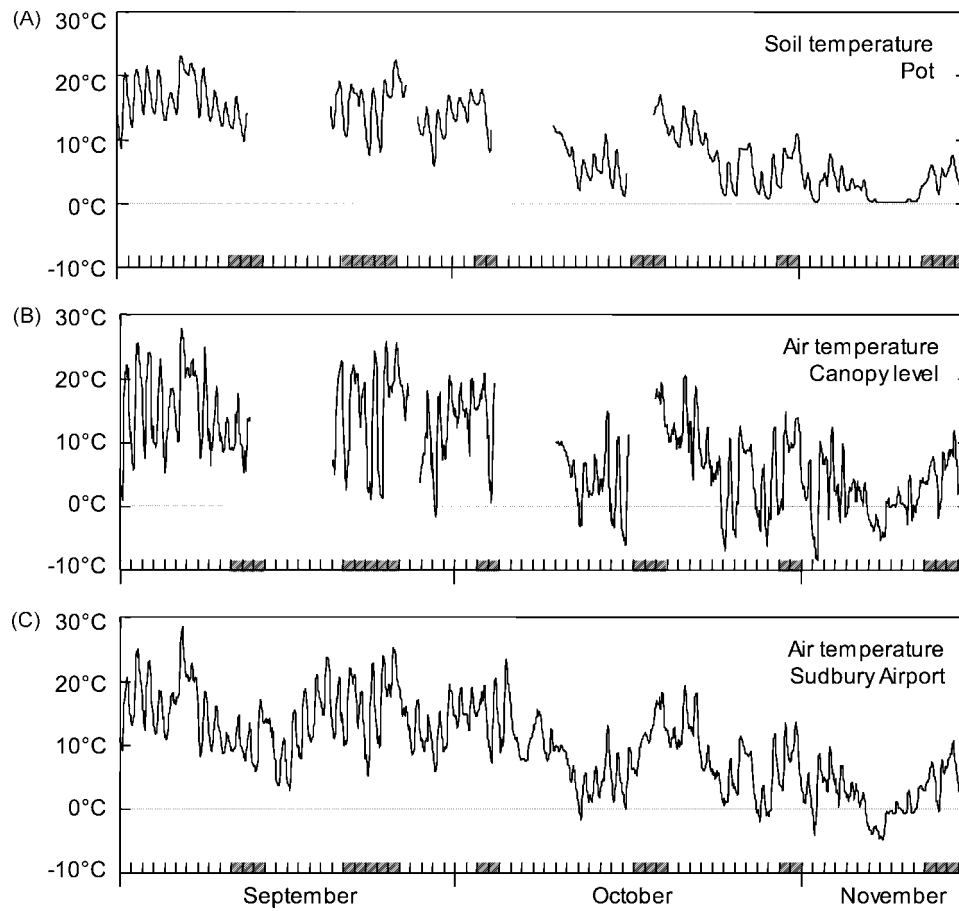


Figure 1. Temperature during the period of harvests from the experimental garden (1 September–15 November, 2007). (A) Soil temperature in one 10-l pot in the garden, standing in a pool about 10 cm deep. (B) Air temperature at the height of the shoots of the studied plants (60 cm). (C) Air temperature at Sudbury airport, measured by Environment Canada (<http://www.climate.weatheroffice.ec.gc.ca>). The striped days indicate the dates of the six first harvests during this period.

In the insulated box where the plants were kept after 15 November, air temperature remained around zero, dropping a few degrees below in December. At the harvest of 4 December the topmost 3–5 cm of the soil in the pots was frozen. At the harvest of 10–11 December the pots were entirely frozen, and had to be thawed out a day before harvesting in the laboratory. Soil temperatures measured in the pots at harvests were never below -3°C . This can be assumed to be similar to natural conditions in wetlands of the area (Amon et al. 2002; McCaughey et al. 2006).

Harvests

At harvest, the above-ground biomass was separated into living (green) and dead fractions, and dried. Roots growing through the holes at the bottom of the pots were discarded, and the soil was washed away. For each plant five axile roots with attached fine lateral roots were collected for determination of the percentage of living roots. The remaining below-ground biomass (roots and rhizomes) was dried. Dry masses were measured after at least 7 days at 75°C .

Diameters of lateral and axile roots were determined using samples from the last two harvests after a 42-day

storage in 50% ethanol. The diameter of at least 50 random lateral roots and 5–10 axile roots in each sample was determined with a microscope at 40-fold magnification, with a resolution of $25\ \mu\text{m}$.

Root mortality

Five entire axile roots with attached lateral roots were collected from each plant. Each of these roots was cut into pieces of 10 to 15 mm in length, and separately placed in 25 ml vials in a solution containing 0.3% TTC and 10 mM glucose. In living tissue the colourless TTC is reduced to insoluble, red formazan, allowing differentiation between live and dead tissue (Kuhn and Jerchel 1941; Comas et al. 2000; Ruf and Brunner 2003). Adding glucose to the solution provides the cell substrate for the metabolism, enhancing the coloration in substrate-limited cells (Collet et al. 2002; Robey 2007). The vial was wrapped in aluminum foil to keep it dark, and incubated at 30°C for a period of 36–48 h.

After the incubation, the contents of each vial were spread on a 20-cm Petri dish with an underlying grid. The vitality of each root crossing a grid line was determined based on its colour, using a 3-fold magnification lens.

Roots with red or pink coloration at the point of intersection were counted as alive. Sections with only stained ends were also considered to be alive, as such a pattern is likely to be a result of impermeability of the rhizodermis to the TTC solution, allowing a reduction only at the cut surfaces. Often only the stele was coloured, and in some cases the brown rhizodermis and cortex had to be peeled away under a microscope to assess this. In the last harvests the steles of axile *Sparganium androcladum* roots were very pale pink, and the roots were regarded as dead.

Fine lateral roots and coarse axile roots were regarded separately in the first harvest for *Dulichium arundinaceum* and *Scirpus microcarpus*, and from the second harvest onwards for all species. For all the species studied, root architecture was simple and the axile roots could easily be distinguished from the finer lateral roots. However, *D. arundinaceum* had a root system consisting of a large number of thin axile roots with very few laterals. For each sampled root, colouring of 100–200 sections was assessed, with the exception of *D. arundinaceum* with often only 20–30 lateral root sections. The staining of all axile root sections in each sample, 30–100, was assessed.

To compare the timing of senescence in different plant parts, for each species an average vitality was calculated for lateral roots, axile roots and above-ground parts as on an integral over the study period. For each harvest interval a product of the length of this period and the average percentage living organ in the two harvests delimiting this period was calculated. All the values over the entire study period were then summed up as the average vitality. Such a value would be low for early senescence, high for late senescence.

In order to investigate the effect of the glucose in the staining solution, five additional roots of each plant were sampled in the first harvest, and assessed for vitality using a TTC solution without added glucose. The effect was investigated for lateral roots, except for *D. arundinaceum*, for which axile roots were used due to the small number of lateral root sections (2) in some of the samples.

Statistical analyses

In the analyses, average percentages of living length of the five roots from each plant were used. Prior to analyses the

values were tested for normality and arcsine square root transformed. Interspecific differences, and the effects of time on the percentage of living roots were analysed using a General Linear Model with the percentage of living roots (nine plants per species) as a dependent variable, species as an independent factorial variable, and the Julian date of the harvest as an independent continuous variable. Species \times date of harvest interaction was included in the model to test for interspecific differences in the temporal pattern of root mortality.

The effect of glucose in the TTC solution in the first harvest was tested using an ANOVA with the percentage of living lateral roots in each of the replicate root samples as dependent variable, and species and the presence of glucose as independent factorial variables. For this test the percentage of living lateral roots was Box–Cox-transformed with $\lambda = 5$ ($y' = ((y^5 - 1) / 5)$) to attain normality. One outlier with a studentised residual >3 was left out.

Results

Plant biomass and above-ground senescence

The below-ground part of the standing crop during the study period exceeded the above-ground mass (dead and alive) for all species (Table 1). At the first harvest on 11 September, most of the above-ground mass was live (Figure 2A). The largest percentages of senesced above-ground mass, around 25%, were found in *Sparganium androcladum* and *Phalaris arundinacea*. By the harvest of 12 November, all species but *Carex oligosperma* had less than 20% of the above-ground mass green. In *S. androcladum* most of the above-ground biomass died by the harvest of 3 October, in *Dulichium arundinaceum* by the harvest of 17 October, and in *Scirpus microcarpus* by 29 October. In *Carex stricta*, *C. oligosperma* and *P. arundinacea* the greatest percent mortality occurred in November. From mid-November to early December *C. oligosperma* had around 45% of its above-ground mass green, but in the plant harvested on 10 December this percentage was only 23%. In the last harvests, leaves of all species but *C. oligosperma* were dead, the living parts being stem bases and buds of new tillers for spring.

Table 1. Total dry mass and below-ground mass fraction for all harvested plants and fine root and axile root diameters of the plants in the last two harvests, 4–5 December and 10–11 December.

	Total dry mass (g)	BMF (g g ⁻¹)	Fine root diameter (mm)	Axile root diameter (mm)
<i>Carex oligosperma</i>	24.8 \pm 3.4	0.67 \pm 0.04	0.15 \pm 0.00	0.7–1.7
<i>Carex stricta</i>	70.0 \pm 9.5	0.83 \pm 0.03	0.13 \pm 0.01	0.8–2.1
<i>Dulichium arundinaceum</i>	41.4 \pm 1.2	0.82 \pm 0.01	0.32 \pm 0.06	0.8–1.2
<i>Phalaris arundinacea</i>	44.2 \pm 2.2	0.92 \pm 0.01	0.15 \pm 0.01	0.7–1.3
<i>Scirpus microcarpus</i>	92.9 \pm 14.9	0.86 \pm 0.02	0.11 \pm 0.00	0.7–1.5
<i>Sparganium androcladum</i>	22.4 \pm 2.0	0.75 \pm 0.03	0.18 \pm 0.01	1.0–1.3

Note: Values are mean \pm 1 standard error, except axile root diameter, which is given as a range. Abbreviation: BMF, below-ground mass fraction (ratio of root and rhizome mass to total mass).

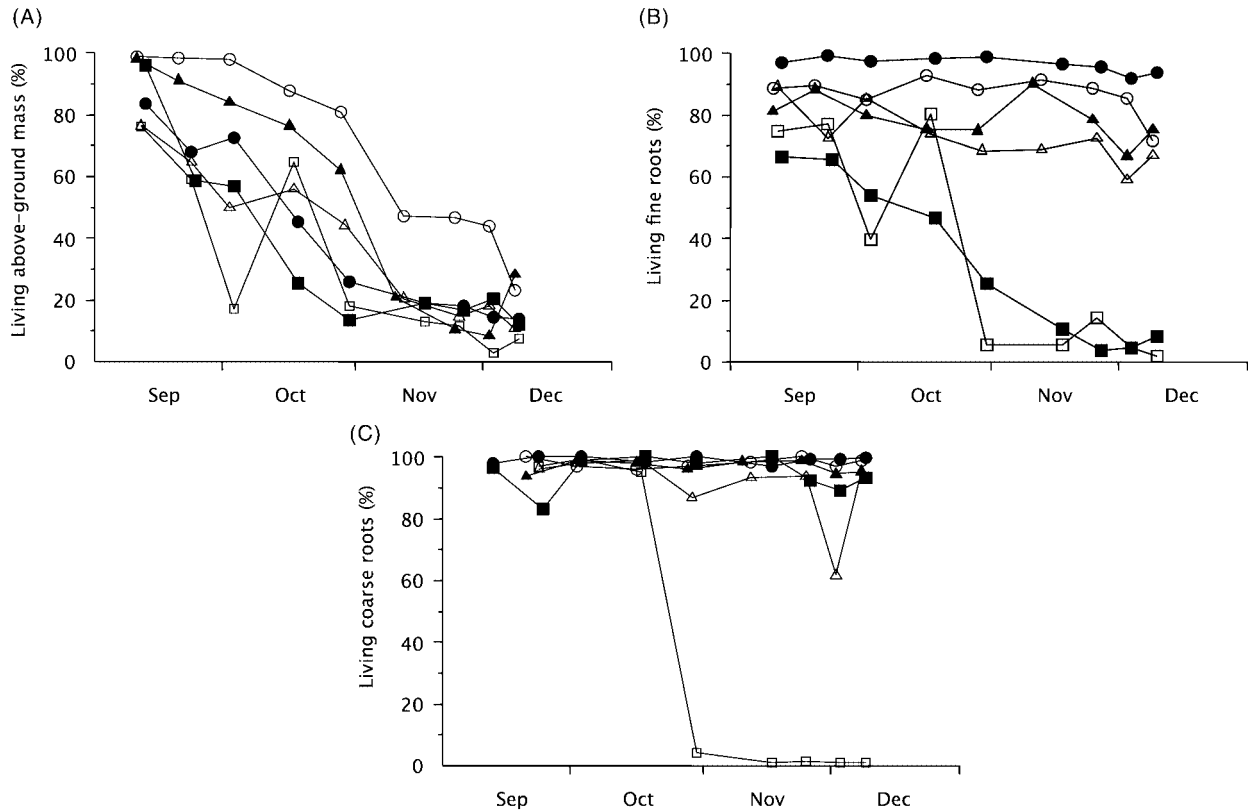


Figure 2. Proportion of live mass of the total above-ground dry mass (A) and the proportions of living fine root length (B) and living axile root length (C) in the nine plants of each species harvested between 11 September and 11 December.

Root diameters and mortality

Lateral roots were clearly finer than axile roots (Table 1). Average diameters of the lateral roots were for all species except *Dulichium arundinaceum* between 0.1 and 0.2 mm, with maximum values not exceeding 0.4 mm. The few lateral roots of *D. arundinaceum* had an average diameter of 0.32 mm. Axile roots of all species ranged from 0.7 to 2.1 mm in diameter, those of *D. arundinaceum* being the thinnest.

The percentage of living fine lateral roots during the study period showed significant interspecific differences, a significant decrease with time, and a significant species \times time interaction (Figure 2B; Table 2). Over 60% of the lateral roots of *D. arundinaceum* and *Sparganium androcladum* were still alive in September, but this percentage decreased to values below 20% in October, and below 10% in December. In contrast, lateral roots of *Scirpus microcarpus* remained mostly alive until the last harvest in December. The average over the whole study period was 97%. *Carex oligosperma*, *C. stricta* and *Phalaris arundinacea* had average values between 73% and 87%, with a slight decline during the study period. General Linear Model conducted with data excluding *Sparganium* and *Dulichium*, the two species with heavy mortality, still showed significant effects of species and time ($P < 0.001$), but the time \times species interaction was not significant ($P = 0.46$).

Coarse roots remained mostly alive during the study period with averages above 90%, except for *Sparganium* that had a sudden mortality of the coarse roots in the second

Table 2. Results of General Linear Models testing the effects of species and date of harvest on percentages of fine roots and axile roots stained red with TTC.

	<i>df</i>	Mean-square	<i>F</i> -ratio	<i>P</i>
Fine roots				
$r^2 = 0.93$				
Species	5	0.10	7.6	<0.001
Date of harvest	1	1.02	80.2	<0.001
Species \times date	5	0.17	13.0	<0.001
Error	42	0.01		
Axile roots				
$r^2 = 0.88$				
Species	5	0.31	12.2	<0.001
Date of harvest	1	0.68	26.4	<0.001
Species \times date	5	0.42	16.1	<0.001
Error	38	0.03		

Note: The r^2 values indicate the variance explained by the models.

half of October (Figure 2C). The effects of species, time, and their interaction were highly significant (Table 2). However, when *Sparganium* was excluded from analysis, only the species effect remained significant ($P = 0.011$).

Lateral root vitality over the study period correlated with axile root vitality among the species (Figure 3A; Spearman's correlation coefficient $r_s = 0.829$; $P = 0.042$; $n = 6$). However, in case of *Dulichium* lateral roots had a clearly higher mortality than coarse roots. Both lateral and

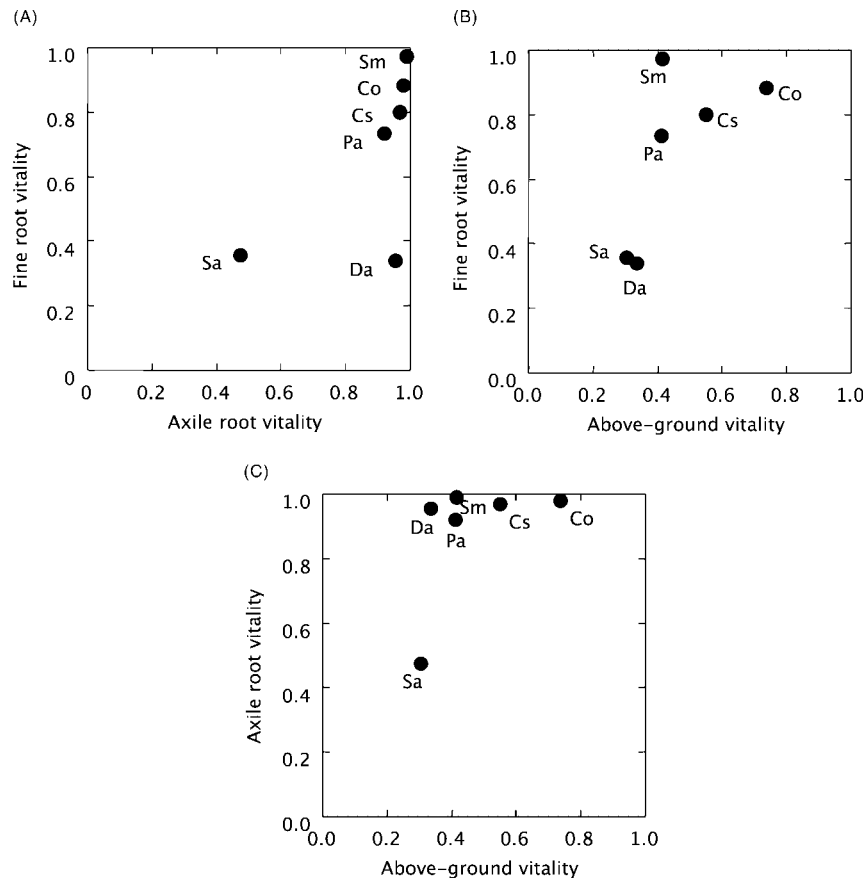


Figure 3. Relationships between the vitalities of fine roots and axile roots (A), fine roots and above-ground parts (B) and axile root and above-ground parts (C) during the study period. The vitalities are calculated as integrals of the proportion of living biomass or living root length between the first and last harvest. Abbreviations: Ca, *Carex oligosperma*; Cs, *C. stricta*; Da, *Dulichium arundinaceum*; Pa, *Phalaris arundinacea*; Sa, *Sparganium androcladum*; Sm, *Scirpus microcarpus*.

axile root vitalities showed a weak, non-significant positive association with the above-ground vitalities of the species (Figure 3B, C; Spearman correlations, $r_s = 0.77$, $P = 0.072$, $n = 6$; data not shown).

Effect of glucose in the staining solution

In *Sparganium* the addition of 10 mM glucose increased the percentage of lateral roots that stained red from 29% to 75% in the first harvest. A similar trend could be observed for the lateral roots of *Dulichium* (from 47% to 66%), but due to the low number of lateral roots (2) in some of the samples the data were discarded and coarse roots were used for the statistical test. According to this test, glucose had no significant main effect (ANOVA; $P = 0.330$), the stained proportion of root length being for all species except *S. androcladum* with and without glucose at least 79%. The species effect ($P < 0.001$) and the species \times glucose interaction ($P = 0.013$) were significant.

Discussion

Data on fine root senescence at the end of the growing season are scarce for herbaceous perennials. As fine roots are

often considered ephemeral, with life spans considerably shorter than a season, seasonal variation in mortality is assumed to be low (Eissenstat and Yanai 1997; Zak et al. 2000; Gregory 2006), and much of the root systems of herbaceous plants are assumed to die back in autumn (Eissenstat and Volder 2005). Our data, however, indicates that this is not necessarily the case for cool-temperate wetland graminoids. In two of the six species investigated a distinct end-of-season mortality of fine lateral roots could be observed along with leaf senescence. In three of the species the fine roots were mostly alive in mid-December, despite a complete senescence of the leaves. In one of the species most of the fine roots and some leaves were alive in December.

How can these contrasting mortality patterns be explained? In leaves, the timing of endogenous senescence is understood as an evolved response to environmental cues to minimise nutrient losses by a predictable disturbance, such as a harsh season (Watson and Lu 2004). Nutrients are reallocated from senescing leaves to storage (Killingbeck 1996), the timing of this procedure being determined by a trade-off between continued use of the nutrients for further growth, and minimised risk of loss (Watson and Lu 2004). In roots, concentrations of mineral

nutrients are generally lower than in leaves, and no nutrient resorption from senescing roots has been found in the few studies addressing the issue in herbaceous plants such as bean (Snapp and Lynch 1996), grasses (Aerts 1990) or sedges (Scheffer and Aerts 2000). There is some evidence for nutrient retranslocation in aging tree roots (Meier et al. 1985; Goldfarb et al. 1990). The reason for root senescence is generally considered not to be recovery of nutrients prior to disturbance, but avoidance of the respiratory costs of maintenance (Pritchard and Rogers 2000). It may be cheaper to produce new roots than maintain old ones over extended periods (Eissenstat et al. 2000).

Based on this, one might expect that in a climate with a long period of no growth, fine roots should not senesce in autumn. In cool-temperate wetlands, the above-ground parts of vegetation can be brown for over 6 months of the year (Bernard 1974), which also is the case in the region of this study (P. Ryser, pers. obs.). During winter, coarse roots of grasses and sedges may serve as storage (Aerts 1989; Thornton and Bausenwein 2000; Gloser 2005). The function of fine roots during this time is not known, but it is unlikely that their maintenance would incur costs that would lead to shedding them. At soil temperatures around zero (Amon et al. 2002; McCaughey et al. 2006), the roots do not require extreme frost tolerance, and respiratory costs of maintenance are likely to be low (Lloyd and Taylor 1994). Root turnover often increases with increasing soil temperatures (Fitter et al. 1998; King et al. 1999; Tierney et al. 2003). The four species with low fine root mortality in this study usually occur in fens, wet meadows or sedge meadows, habitats without regular major soil disturbances. In spring, the presence of established fine roots may be an advantage in the competitive environment of these wetlands, as production of new roots is slow in cool temperatures (Fitter et al. 1998). Hence, the advantage of maintaining roots over winter may exceed the costs. In the arctic, roots of the graminoids *Carex aquatilis* and *Dupontia fisheri* have been described to live for several years (Shaver and Billings 1975). Remarkably, the species with the lowest root mortality in our experiment, *Scirpus microcarpus*, did not produce any fine roots close to the soil surface, but all of them were found below the depth of 5–10 cm. This is possibly to minimize the risk of freezing.

In two of the species, fine roots had completely died by the end of October. One of these species, *Sparganium androcladum* with a complete death of its roots, commonly grows in habitats with open water and open canopy, such as in streams and on muddy shores. Such habitats are less competitive, and more likely to be disturbed in winter or spring by ice or flood, than the characteristic habitats of the species with low root mortality. It is possible that under conditions where the likelihood of root death in winter or spring is high, root death in the autumn is a kind of adaptive trait. The other species with a complete mortality of its fine roots, *Dulichium arundinaceum*, also occurs along shorelines. However, this species has a different root system structure than the other species studied in that it has very few lateral roots, and in contrast to *Sparganium*, its axile roots had a low mortality.

Root mortality in autumn was less pronounced than the mortality of above-ground parts. Despite some association between timing of leaf senescence and fine root mortality among the species, the relationship was not significant. Notably the behaviour of *Scirpus microcarpus*, with rapid leaf senescence, but practically no root senescence, indicates that root senescence is not necessarily associated with above-ground senescence. In general, the fine lateral roots were more likely to die than axile roots, but with the exception of *Dulichium*, interspecific differences were larger than differences between lateral and axile roots. Mortalities of lateral and axile roots correlated across the species. When comparing with other studies, however, one has to be aware that the present data are for graminoid species with a very different root system structure to that found in trees, for which most root order studies have been conducted.

Studies quantifying TTC reduction in senescing roots have found that root death is not abrupt, but they die slowly, with the amount of reduced TTC decreasing with the progress of senescence (Clemensson-Lindell 1994; Comas et al. 2000). TTC reduction correlates with root respiration (Comas et al. 2000). The slow death indicates that there may be no programmed death, but the plant stops supporting the roots once they have outlived their usefulness, resulting in a progression of cell and tissue death (Eissenstat and Volder 2005). This also is the case in annuals such as *Phaseolus vulgaris* with root death only after the shoot has undergone full senescence (Fisher et al. 2002). Our data on *Sparganium*, the only species with complete mortality of its roots, are in agreement with diminishing substrate and gradual death of the roots. The roots of this species gradually became paler until there was only a very pale pink colour, or no colour at all. The addition of glucose increased the amount of stained *S. androcladum* roots in the first harvest mid-September, indicating that substrate may already have been getting low in these roots, which then died in October. The presence of glucose is a requirement of tetrazolium reduction (Robey 2007), and Collet et al. (2002) have shown that addition of glucose increases TTC staining in excised and probably substrate-limited root tips in maize. Fine roots of four of the species remained red until the last assessment in December, indicating that these roots were not slowly dying, but likely to survive all winter. The most extreme case of this behaviour was *Scirpus microcarpus* with practically all roots bright red during the entire study.

We conclude that interspecific variation in root turnover of herbaceous wetland graminoids in cool-temperate climates is more complex than previously thought. Species may differ not only with respect to root longevity in general, but may show distinct seasonal patterns in root mortality. Some species show a rapid mortality of the entire root system along with the senescence of the above-ground structures. In other species no autumn and early-winter root mortality was detected, indicating that fine root turnover may in some species be slower than leaf turnover.

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