Contrasting root overwintering strategies of perennial wetland monocots

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Contrasting root overwintering strategies of perennial wetland monocots

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Abstract

Root turnover is an important contributor to ecosystem nutrient and carbon cycling, but seasonal aspects of root mortality are not well known. This study tests the hypothesis that in strongly seasonal climates, such as in Northern Ontario, Canada, perennial wetland monocots fall into two distinct categories with respect to their root overwintering strategy: complete senescence, or survival over the winter. Root survival in late winter and early spring was tested for a total of 26 species using vitality staining with tetrazolium chloride (TTC). Root survival in spring was either over 85% (18 species), or 0% (8 species). Lateral root survival was marginally lower than that of basal roots. In some species low nutrient supply slightly increased root winter mortality, but did not change the seasonal pattern. We conclude that in a northern temperate climate, the overwintering strategies of roots of herbaceous monocots are binary: either avoidance or tolerance of the long unfavourable season, similar to deciduous and evergreen leaves among woody plants. Roots do not gradually die during the unfavourable season, but either completely senesce in the autumn, or survive the winter. The distinct root overwintering strategies presumably affect the species' resource economics and ecosystem processes.

Key words

phenology, root mortality, root turnover, seasonal climate, wetland

48 **Introduction**

49 Understanding the relationships between plant species' functional traits and their
50 ecological behaviour is necessary to predict species' responses to different environmental
51 conditions, and to be able to develop appropriate management practices under changing
52 environmental conditions (Aubin et al. 2016). Leaf life span is a central trait in this
53 context, with a major contribution to the species' position along the resource economics
54 spectrum, ranging from acquisitive to conservative strategies (Wright et al. 2004; Shipley
55 et al. 2006; Poorter et al. 2014). The role of the life span of roots, which in many
56 ecosystems compose more than half of primary production, is less known (Finzi et al.
57 2015; Iversen et al. 2015). Below-ground traits do not necessarily mirror the leaf
58 economics spectrum (Weemstra et al. 2016), and seasonal patterns of root production can
59 markedly differ from those observed above ground (Steinaker et al. 2010; Abramoff and
60 Finzi 2015; Blume-Werry et al. 2016; Schwieger et al. 2018). Lack of knowledge of fine
61 root phenology limits our understanding of the relationship between root turnover
62 strategies and environmental conditions (Radville et al. 2016).

63 Above-ground, the overwintering strategy of tree leaves in northern temperate
64 climate is a binary trait – deciduous or evergreen – and it is an important characteristic in
65 determining seasonal aspects of species' resource acquisition and loss (Walters and Reich
66 1999; Givnish 2002). A tradeoff between winter-hardiness and high photosynthetic
67 capacity of the leaves constrains a species' response to climate (Reich et al. 1998;
68 Kikuzawa and Lechowicz 2011). Below-ground, interspecific variation in root life span is
69 usually considered to be a continuous variable. However, for herbaceous wetland species
70 in climates with a long cold season there is evidence that root winter survival may be

classified in two distinct categories. For example, roots of arctic wetland graminoids mostly survive for several years, but *Eriophorum* species in the same environment renew their root systems annually (Shaver and Billings 1975). Of the six northern Ontario wetland graminoids studied by Ryser and Kamminga (2009), five had roots which did not senescence at the end of the growing season, while roots of *Sparganium americanum* completely senesced before the onset of winter.

In the present study we address the question whether root overwintering strategies among perennial deciduous wetland monocots in climates with long and cold winters, such as Northern Ontario, are of a binary nature in general. We hypothesize that roots of a given species either completely senescence before or over the long winter, or mostly survive. Hence, as trees in such a climate can be classified either as deciduous or evergreen, wetland monocots might be classified into two distinct categories based on their root winter survival, either as species with “deciduous roots” or species with “evergreen roots”. Existence of two contrasting root overwintering strategies among these herbaceous species that all have autumn-senescing leaves would further emphasize differences between plant above-ground and below-ground economic strategies.

We limit the study to monocotyledons, as their root systems show no secondary growth and less structural and architectural variation than roots of dicotyledonous species. Monocots also are the dominant growth form in a majority of open wetlands in the region (Harris et al. 1996). The species were selected due to their commonness, representing a variety of different taxonomic groups and wetland types.

We also addressed the questions of whether the nutritional status of a plant influences root winter survival, and whether there is a difference in senescence patterns between basal and lateral roots.

Materials and Methods

Study area

The study area is located towards the northern limit of the deciduous zone (Rowe 1972). Sudbury has on average a growing season of 125-145 days, defined as the period between the last and first frost (OMAFRA 2016). The annual average temperature is 4.1°C, with daily averages of -13°C in January, and 19.1°C in July. The average minimum temperature in January is -17.9°C, the average maximum in July 24.8°C (Environment and Climate Change Canada 2017). In the region, wetland monocots generally become photosynthetic in late May, and their leaves senesce between September and November, depending on species (P. Ryser, personal observation). Hence, for many species the period without photosynthesis is well over six months.

Soil temperatures at 5 cm and 30 cm depths have been measured in 38 wetlands of the region over the winter 2012-2013 (iButton® DS1921G-F5# data loggers; Maxim Integrated, San Jose, USA). The soil did not freeze in any of those wetlands, despite minimum air temperatures of below -40°C, probably as a result from the insulating snow cover and litter layer and often, flowing water (P. Ryser, unpublished data).

Overview of the measurements and growth conditions

Root winter mortality was tested for 26 perennial wetland monocot species of Northeastern Ontario (Table 1). The plants were grown in mesocosms in an experimental garden, and assessed for their winter root mortality after one or two years of growth. These measurements occurred over 10 years and consist of two main parts. In 2007 and 2008, root winter mortality was assessed in an experiment for eight species with successive collections over the entire winter, either from November to March (A; Table 1), or from January to early April (B; Table 1). The plants were grown with two different nutrient supply rates. One additional species with two collected individuals was tested as well. In 2013-2017, root winter mortality was screened for 20 species by testing the proportion of living and dead roots at the end of the winter, before new growth started in the spring. These plants were grown without further nutrient additions. For 16 of these species root mortality was assessed for proximal and distal roots separately (C; Table 1), and for four species it was assessed for all roots together (D; Table 1). Three of the species were investigated in both 2007-2008 and 2013-2017.

The plants were grown in 10-litre mesocosms filled with wetland topsoil from a beaver meadow with pH 4.0 and organic matter content of 26% (Bainbridge construction, North Bay, Ontario). The mesocosms stood in pools filled with ground water about 20 cm deep, with substrate surface 2-5 centimeters above the water. The plants assessed in 2007-2008 were grown for two growing seasons before assessing the percentage of living and dead roots during the winter. The plants screened in 2013-2017 were grown for one growing season and one winter before the measurement. During winter, all plants were protected from freezing by covering them with 20-30 cm of straw and tarpaulins. The overwintering plants did not experience temperatures below zero.

137

138 *The experiments and studied species*

139 The measurements in the winter 2007-2008 to assess root winter mortality was
140 conducted for two species of Poaceae (*Calamagrostis canadensis* (Michx.) P.Beauv. and
141 *Glyceria canadensis* (Michx.) Trin.), and two *Scirpus* species (*S. cyperinus* (L.) Kunth.
142 and *S. microcarpus* J. Presl & C. Presl) by harvesting 10-11 plants at 1-2 week intervals
143 from November 8 to March 24, and for four species of *Carex* (*C. lacustris* Willd., *C.*
144 *magellanica* Lam., *C. oligosperma* Michx., *C. stricta* Lam.) by harvesting 8 plants at 1-2
145 week intervals from January 8 to April 1. Each harvest lasted for several days due to the
146 time-consuming careful washing out of the roots. In addition, root vitality was assessed
147 for two individual plants of a fifth *Carex* species, *C. aquatilis* Wahlenb. in early January,
148 one plant in each nutrient treatment. Data of this species are shown, but not included into
149 the statistical analyses. After collecting plants from their winter storage under snow and
150 straw, the plants were kept outdoors in an insulated box until processing. Substrate
151 temperatures during this storage remained mostly above zero, except for February and
152 March when the soil in some pots froze to temperatures a few degrees below zero.

153 These plants were subjected to two levels of nutrient additions from May to
154 September during two growing seasons, 2006 and 2007, with weekly additions of 250 ml
155 of nutrient solution per mesocosm, containing either high or low concentration of mineral
156 nutrients. The high-level nutrient solution consisted of 17 mM N, 7.6 mM P, 6.0 mM K,
157 0.9 mM Mg, 0.9 mM S, 1.8 mM Ca, 21 μ M Fe, 9 μ M Cu, 9 μ M Zn, 22 μ M B, 0.06 μ M
158 Mo and 11 μ M Mn. The low-level nutrient solution was one tenth of that strength. As an

indicator for the effect of nutrient treatments, at harvest the total root dry mass was measured after drying for at least 72 hours at 75°C.

Between 7 May and 21 May 2013, root mortality was assessed for 16 monocot species collected in the region of Sudbury in spring and early summer 2012: *Alisma triviale* Pursh, *Calla palustris* L., *Carex oligosperma*, *Carex stricta*, *Carex utriculata* Boott, *Dulichium arundinaceum* (L.) Britton, *Eleocharis palustris* (L.) Roem. & Schult., *Juncus brevicaudatus* (Engelm) Fernald, *Juncus tenuis* Willd., *Pontederia cordata* L., *Rhynchospora alba* (L.) Vahl, *Sagittaria latifolia* Willd., *Schoenoplectus acutus* (Bigelov) Á. Löve & D. Löve, *Scirpus microcarpus*, *Sparganium americanum* Nutt., *Typha latifolia* L. In early May 2015, root mortality was assessed for *Carex lasiocarpa* Ehrh. and *Rhynchospora fusca* (L.) W.T. Aiton, originating from Lake Superior Provincial Park (300 km west of Sudbury) and grown in the experimental mesocosms in the experimental garden since spring 2014. In contrast to all previously studied species, these two species grew on sieved artificial blend of loam, peat moss and compost (President's Choice® Black Earth Topsoil; Brampton, Loblaws, Canada). In April 2017, root mortality was assessed for *Triglochin maritima* L. originating from Foleyet (ON, Canada) and *Triglochin palustris* L., originating from Manitoulin Island (ON, Canada), after one year of growth in the mesocosms on a mixture of 20% of the artificial topsoil blend and 80% sand.

Assessment of root survival and mortality

The death of a root is not visible until decomposition processes start. In order to detect root mortality immediately after death we used vital staining with 2,3,5-

triphenyltetrazolium-chloride (TTC) solution (Comas et al. 2000). The samples were incubated in 25 ml vials in a solution of 0.3% TTC and 10 mM glucose for 48 hours at 30°C in total darkness. The colorless TTC functions as an H-acceptor in living cells, producing insoluble red-coloured 2,3,5-triphenylformazan, which can be used as an indication of root viability (Comas et al. 2000). The glucose provides additional substrate for the metabolism, enhancing the coloration in substrate-limited cells (Collet et al. 2002). As the colour of roots considerably varies among the species, for most of the species the colour of the investigated roots was compared with that of roots killed by heating them to 70°C before staining. The heated roots did not develop any red or pink colour, a clear indicator that roots staining red were alive. The degree of redness varied among the species, but any distinct sign of red colour in cortex or stele was interpreted as the root being alive.

For the experiment in 2007-2008, samples of coarse basal roots and fine lateral roots were collected separately. Fifty to two hundred randomly selected 10 mm pieces of basal roots were collected close to the stem base, and five randomly selected samples of lateral roots, approximately 1 g each, were collected at different parts of the root system. For plants screened in 2013, two samples were collected at different depths as 1 cm-wide cross sections across the entire root systems. One of the samples was collected 1 cm below the stem (proximal sample), consisting mostly of coarse basal roots, the other 12 cm below the stem (distal sample) consisting of some basal and mostly lateral roots.

Fine root viability was quantified using the grid-intersection method (Newman 1966; Tennant 1975) in a 140 mm Petri dish with help of a hand-held magnifier (3×). Percentage of basal roots alive was assessed by counting the number of stained pieces of

basal roots as a percentage of the total number of pieces of basal roots in the sample. If no stain could be seen with the magnifier, the samples were observed with a stereo microscope (35×). The rhizodermis does not always allow penetration of TTC into the root, and the section was considered to be alive even if the staining was limited to the section close to the cut cross-sectional surface. In case of a dark rhizodermis, staining of the stele, which usually was the most responsive part of the root to staining, was determined after exposing it by peeling the rhizodermis away with fine forceps.

Data analyses

In the experiment of 2007-2008 the percentage of dead lateral root length, and root dry mass was analysed using a General Linear model with species, nutrient treatment and their interaction as independent factors. The percentage of dead root length was arcsine Box-Cox-transformed to attain normal distribution. The effect of the time of harvest on lateral root mortality was tested with the same model, but adding the date of harvest as a continuous independent variable. Due to the high number of plants with close to 100% of living basal roots, the data on basal root mortality could not be normalized. Hence, statistical analyses for the effect of nutrient supply on root mortality were conducted using Kruskal-Wallis tests. The effect of nutrients was tested separately for each species, and the effect of species separately for each nutrient supply level. Differences between percentages of basal and lateral root mortality were tested with Wilcoxon signed-rank tests for each species separately. For the data of 2013, the effect of the two sampling positions along the root system on the same individuals – proximal and distal to the stem – on arcsine-transformed root mortality was analysed with a repeated measures ANOVA

with the position as a within-subject factor and the species as an independent between-subject factor.

Results

All the species investigated in 2007-2008 had a high percentage of surviving root length, both for lateral and basal roots (Fig. 1). Lateral roots had a slightly lower survival than basal roots in case of *Calamagrostis canadensis* and *Glyceria canadensis*, but even for these species the difference between the two root categories remained well below 10% (Fig. 1; Table 2). Species effect was significant on lateral root survivorship (Table 3), which for *Calamagrostis canadensis* was 85% and for *G. canadensis* 89%, while all the Cyperaceae species had on average a root survival of above 98%. Species effect on basal root survival was significant only at low nutrient supply (Supplementary Material, Table S1¹). The percentage of living roots did not decrease over the winter for any of the species, and the effect of date of harvest on root survival was not significant (Table 3). Nutrient treatment had a clear effect on plant size, root dry mass in the high nutrient treatment being 2-3 times higher than in the low nutrient treatment (Fig. 2; Supplementary Material, Table S2). Nutrient effect on lateral root survival was significant albeit small, with values at the low nutrient supply 5-7% lower for the two grass species and with less than 1% difference for all the Cyperaceae (Fig. 1, Table 3). Species $\times$ Nutrient interaction was not significant (Table 3). Basal root survival was not affected by nutrient supply for any of the species ($p > 0.250$; Fig. 1; Supplementary Material, Table S3).

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2018-0065>.

For the species assessed in 2013-2017, the percentage of roots that stained red in spring was either above 85% or close to zero (Fig. 3). *Calla palustris*, *P. cordata* and *R. alba* had no surviving roots at all, and *A. triviale*, *Sagittaria latifolia* and *Sparganium americanum* had less than 3% of their roots alive in May. For the latter three species a few vigorous, bright white, obviously newly produced roots were observed, indicating that the few roots staining red with TTC in May were the first roots of the new cohort produced early that spring. It is unlikely that these few stained roots had survived over the winter. Nevertheless, for all plants with a complete root winter mortality the rhizomes or other storage organs stained red in spring, indicating that the plants were alive. For all these species individuals not destroyed at harvest continued growing in the following growing season.

For the ten species with a separate assessment of root mortality in proximal and distal samples in 2013, and with the roots mostly surviving the winter, the percentage of living roots was higher when collected 1 cm below the stem base than 12 cm below it. Difference in average survival between the two different root positions was small, 96.2% vs. 94.8%, but significant (Table 4). The extent of root survival significantly varied among the species, being for *Carex oligosperma* and *C. stricta* below 93% while the other species had higher values. Species $\times$ position interaction was significant due to the relatively large difference in survival between the root positions for *C. utriculata*, with 86% and 99% (Fig 3; Table 4).

Discussion

The data support our hypothesis that in a climate with a severe and long winter, wetland monocots can be divided into two distinct categories with respect to their root winter mortality, comparable to evergreen and deciduous habits among trees. Roots either mostly survive the winter, or completely senesce. In the case of species with overwintering roots, average survival was in almost all cases over 90% of the total root length, and never below 85%. The measurements for different species were conducted over several years with some variation in nutrient availability and the procedure of root sampling, but overall, the effects of the year, nutrient supply, type of substrate or root order and position within the root system were minor compared to the clear species-specific binary distinction. Species that were measured in different experimental setups showed consistent behaviour. Furthermore, for several of the species data exist to confirm their either complete root senescence before the winter, or survival of the majority of the roots over the winter: *Carex aquatilis*, *C. oligosperma*, *C. stricta*, *R. alba*, *Sagittaria latifolia*, *Scirpus microcarpus* and *Sparganium americanum* (Shaver and Billings 1975; Ohlson and Malmer 1990; Ryser and Kamminga 2009; Lawrence et al. 2013; Alsahame 2016). These data support our conclusions that root winter survival among perennial temperate wetland monocots is a binary species-specific trait, similar to leaf winter survival in temperate trees. The rate of senescence and mortality rate over the winter may be modified by environmental conditions, but the binary nature of the root survival strategy remains unchanged.

For the species measured during the entire winter, there was no sign of increasing mortality over the winter. Measurements of root mortality in autumn show that roots of species with complete mortality at the end of the growing season such as *Sparganium*

americanum, *Sagittaria latifolia* and *R. alba*, have already senesced by October and November (Ryser and Kamminga 2009; Alsahame 2016). Their early senescence indicates that the mortality is a result of an active senescence as preparation for the oncoming winter, rather than a result of extreme conditions during the winter, comparable to leaf senescence of deciduous trees (Kim et al. 2016). All the investigated species are deciduous perennials, as they overwinter with vegetative tissues, but lose their leaves for the winter. Root survival over the winter in the majority of the species, despite autumn-senescent leaves in all of them, further emphasizes that above-ground data alone is not sufficient to describe ecosystem carbon cycling (Abramoff and Finzi 2015). Several of the species which had both leaves and roots senescing, such as *R. alba*, *Sagittaria latifolia*, and *T. palustris*, can be considered to be functional annuals (Verburg and During 1998), as the overwintering vegetative organ is relatively small and may serve as a propagule potentially aiding in dispersal.

Interspecific variation in root life span is assumed to be associated with species' resource economics: fast-growing roots maximize resource acquisition but have a short life-span, while long-lived roots grow slowly but maximize resource conservation (Ryser 1996; Roumet et al. 2016). Short-lived roots are often associated with species of disturbed and nutrient-rich habitats (Roumet et al. 2016); of the species in the present study *A. triviale* is such a species (Hood 2013). On the other hand, for many of the species the ecological advantage of annually renewed roots is not obvious. *Sparganium americanum*, *P. cordata*, or *Sagittaria latifolia* form stable stands on sheltered and infrequently disturbed shores (Hall and Penfound, 1943; Keddy, 1983; Souch et al., 1998), and *Eriophorum* species with annually renewed root systems are dominant of

many arctic wetlands (Shaver and Billings 1975). *Rhynchospora alba* grows on nutrient-poor sites with otherwise sparse vascular plant vegetation (Ohlson and Malmer 1990).

The binary nature of the winter survival of leaves and roots, however, goes beyond being positions along a continuous gradient of life span. It is not clear what trade offs lead to the binary nature of root winter survival, but it is possible that capacity of roots to survive the unfavourable season restricts their growth response to favourable conditions, in a similar manner to leaves. Deciduous leaves are more efficient in resource acquisition, *i.e.*, photosynthesis, during the favourable season (Reich et al. 1998), while evergreen leaves, able to tolerate freezing temperatures, are more efficient in resource conservation and better able to take advantage of marginal seasons (Givnish 2002). However, temperatures in wetland soils in the study area usually remain above 0°C during the winter (unpublished data, P. Ryser), which means that the risk of frost damage to roots in wetland soils at least in the study region is small. Soil nutrient availability in seasonal wetlands can show a distinct peak in early spring (Edwards and Jefferies 2010), and species with overwintering roots may be better able to utilize such short-term resources. Growth at low nutrient availability slightly increased root winter mortality, but this did not change the general species-specific pattern. Previous investigations on nutrient effects on root life span show inconsistent results: nutrient addition either increases or decreases root life span, or has no effect (McCormack and Guo 2014).

Our data is not conclusive about the actual life span of winter-surviving roots. Survival over one winter does not mean that an organ would live for several years, illustrated by leaves of several ericaceous dwarf shrubs of northern nutrient poor wetlands which senesce during their second growing season while nutrients in them are

being remobilized for new growth (Reader 1978). Nevertheless, roots of several arctic graminoid species are known to live for 6-8 years (Shaver and Billings 1975). The low percentages of dead roots (often <2%) found in the present study for *Scirpus* and *Carex* species that had been growing for two growing seasons in the mesocosms indicate that roots of these species survive at least for two winters. Decomposition could have reduced this percentage of dead roots observed after a second growing season, but it seems unlikely that the thick and robust basal roots of these species would disappear without trace so quickly under the water-logged conditions. Survival rates of lateral roots and basal roots hardly differed, matching the modular structure of monocotyledonous root systems, comprised of numerous adventitious roots (Fitter 1987), and indicates that root turnover happens module by module, in contrast to trees in which fine roots generally show a shorter life span than coarse roots (Wells and Eissenstat 2001). Our measurements were conducted over several years, but the data is very consistent, root winter survival of the species was either over 85% or 0%. The three species which were assessed twice showed similar results in both occasions.

In conclusion, the results show that root turnover among wetland graminoids – in a similar manner to leaf turnover – is a part of a plant's development and the species' adaptive strategy. Roots do not just die of wear and tear, but their mortality is an active response to seasonal pattern of the climate with an adaptive choice – at an evolutionary time scale – between avoidance and tolerance. Roots do not gradually die during the winter: they either senesce in the autumn in a controlled manner, or they mostly survive the winter. This choice is bound to have significant consequences for the species' resource economics, contributing to its performance in different environments and to

seasonal patterns of ecosystem nutrient and carbon dynamics. Constraints underlying the trade off between root tolerance or avoidance of the cold season remain unknown, and further research is needed to understand to which extent these binary strategies in root turnover can be found under different climatic conditions.

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Figure captions

Fig. 1. Percentage of living root length (TTC staining) during the winter for lateral and basal roots of eight wetland monocot species at two nutrient supply levels, investigated in 2007-2008. The two lighter bars to the left for each species show the data for fine lateral roots, the two darker bars to the right the data for coarse basal roots. Within each pair, the darker bar to the left show data at the high nutrient supply, the lighter one to the right at the low nutrient supply. Species without asterisk were harvested between November and March ($n=9-11$), species with asterisk between January and early April ($n=8$, except for *C. aquatilis* $n=1$). The bars represent mean values of all plants harvested in the respective periods ($\pm 1SE$).

Fig. 2. Root dry mass during the winter for eight wetland monocot species at low (light grey) and high (dark grey) nutrient supply levels, and harvested after two growing seasons of growth in November-March (no asterisk; $n=9-11$) or in January-April (asterisk; $n=8$, except for *C. aquatilis* $n=1$). The bars represent mean values of all plants harvested in the respective periods ($\pm 1SE$).

Fig 3. Percentage of living root length (TTC staining) in early May of 16 wetland monocot species. In 2013, the roots were collected either proximally (1 cm below the stem; filled bars) or distally (12 cm below the stem; open bars). For the species with an asterisk only one mixed sample (grey bars) per plant was collected in 2015 or 2017. $n=5$, except for the *Triglochin* species with $n=3$. Mean values $\pm 1SE$.

Table 1. Species investigated in the experiments of this study with the number of replicate plants in each measurement. A) November 2007-March 2008 with two nutrient treatments. Separate analyses for lateral and basal roots. B) January 2007-April 2008 with two nutrient treatments. Separate analyses for lateral and basal roots. C) Early May 2013. Sampled proximally (1 cm below stem, mostly basal roots) and distally (12 cm below stem, mostly lateral roots). D) Early May 2016 or 2017, one sample per plant. All plants were grown in 10 litre mesocosms, either on natural wetland soil (N) or commercially available growth substrate (CGS). The substrate for the two *Triglochin* species contained 80% sand. At harvest, A and B had grown in the mesocosms for two growing seasons, C and D for one growing season. All plants were propagated vegetatively from Northern Ontario populations. LSPP=Lake Superior Provincial Park.

Species	Family	A	B	C	D	Substrate	Origin
<i>Alisma triviale</i>	Alismataceae			5		N	Sudbury
<i>Calamagrostis canadensis</i>	Poaceae	9				N	Sudbury
<i>Calla palustris</i>	Araceae			5		N	Sudbury
<i>Carex aquatilis</i>	Cyperaceae		1			N	Sudbury
<i>Carex lacustris</i>	Cyperaceae		8			N	Sudbury
<i>Carex lasiocarpa</i>	Cyperaceae				5	CGS	LSPP/Manitoulin Island
<i>Carex magellanica</i>	Cyperaceae		8			N	Sudbury
<i>Carex oligosperma</i>	Cyperaceae		8	5		N	Sudbury
<i>Carex stricta</i>	Cyperaceae		8	5		N	Sudbury
<i>Carex utriculata</i>	Cyperaceae			5		N	Sudbury
<i>Dulichium arundinaceum</i>	Cyperaceae			5		N	Sudbury
<i>Eleocharis palustris</i>	Cyperaceae			5		N	Sudbury
<i>Glyceria canadensis</i>	Poaceae	11				N	Sudbury
<i>Juncus brevicaudatus</i>	Juncaceae			5		N	Sudbury
<i>Juncus tenuis</i>	Juncaceae			5		N	Sudbury
<i>Pontederia cordata</i>	Pontederiaceae			5		N	Sudbury
<i>Rhynchospora alba</i>	Cyperaceae			5		N	Sudbury
<i>Rhynchospora fusca</i>	Cyperaceae				5	CGS	LSPP
<i>Sagittaria latifolia</i>	Alismataceae			5		N	Sudbury
<i>Schoenoplectus acutus</i>	Cyperaceae			5		N	Sudbury
<i>Scirpus cyperinus</i>	Cyperaceae	10				N	Sudbury
<i>Scirpus microcarpus</i>	Cyperaceae	10		5		N	Sudbury
<i>Sparganium americanum</i>	Typhaceae			5		N	Sudbury
<i>Triglochin maritima</i>	Juncaginaceae				3	Sand/CGS	Foley
<i>Triglochin palustris</i>	Juncaginaceae				3	Sand/CGS	Manitoulin Island
<i>Typha latifolia</i>	Typhaceae			6		N	Sudbury

Table 2. Results of Wilcoxon signed ranks tests on the difference in percentage of living root length between basal and lateral roots of eight wetland monocot species in the winter 2007-2008. Significant p -values ($p < 0.050$) in bold.

	n	Z -score	p
<i>Carex lacustris</i>	16	0.104	0.918
<i>Carex magellanica</i>	16	0.879	0.379
<i>Carex oligosperma</i>	15	-1.70	0.088
<i>Carex stricta</i>	16	0.83	0.406
<i>Calamagrostis canadensis</i>	18	3.29	0.001
<i>Glyceria canadensis</i>	22	2.16	0.031
<i>Scirpus cyperinus</i>	19	-0.80	0.421
<i>Scirpus microcarpus</i>	20	-1.53	0.126

Table 3. Results of General Linear Models on the percentage of living lateral root length in the winter 2007-2008 for eight wetland monocot species, with species, and nutrient supply during growing season as independent factors (Model 1). Model 2 additionally includes the date of harvest as a continuous independent variable to test for a possible trend over time. Significant *p*-values (*p*<0.050) in bold.

	Model 1; $r^2=0.508$			Model 2; $r^2=0.508$		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
Species	7	17.4	<0.001	7	16.3	<0.001
Nutrients	1	4.7	0.032	1	4.7	0.033
Species × Nutrients	7	0.4	0.884	7	0.4	0.884
Date	–	–	–	1	0.0	0.857

Table 4. Results of the repeated measures ANOVA on percentage of root length alive after the winter in early May 2013. Ten species with the majority of their roots surviving the winter are included in the analysis. Arcsine transformed percentage of alive roots as the independent variable, species as dependent factor, and the position of the root sample (1 cm and 12 cm below the stem) as repeated within-subjects factor. $n=51$. Significant p -values ($p<0.050$) in bold.

	df	<i>F</i>	<i>p</i>
<i>Between subjects</i>			
Species	9	5.0	<0.001
<i>Within subjects</i>			
Position of root sample	1	13.3	<0.001
Species × Position	9	7.4	<0.001

Fig. 1. Percentage of living root length (TTC staining) during the winter for lateral and basal roots of eight wetland monocot species at two nutrient supply levels, investigated in 2007-2008. The two lighter bars to the left for each species show the data for fine lateral roots, the two darker bars to the right the data for coarse basal roots. Within each pair, the darker bar to the left show data at the high nutrient supply, the lighter one to the right at the low nutrient supply. Species without asterisk were harvested between November and March (n=9-11), species with asterisk between January and early April (n=8, except for *C. aquatilis* n=1). The bars represent mean values of all plants harvested in the respective periods ($\pm$ 1SE).

74x23mm (300 x 300 DPI)

Fig. 2. Root dry mass during the winter for eight wetland monocot species at low (light grey) and high (dark grey) nutrient supply levels, and harvested after two growing seasons of growth in November-March (no asterisk; $n=9-11$) or in January-April (asterisk; $n=8$, except for *C. aquatilis* $n=1$). The bars represent mean values of all plants harvested in the respective periods ($\pm 1SE$).

110x51mm (300 x 300 DPI)

Fig 3. Percentage of living root length (TTC staining) in early May of 16 wetland monocot species. In 2013, the roots were collected either proximally (1 cm below the stem; filled bars) or distally (12 cm below the stem; open bars). For the species with an asterisk only one mixed sample (grey bars) per plant was collected in 2015 or 2017. n=5, except for the Triglochin species with n=3. Mean values $\pm$ 1SE.

83x29mm (300 x 300 DPI)

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