Root porosity contributes to root trait space of wetland monocotyledons independently of economics traits

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Abstract

Aims Root aerenchyma, a key adaptive trait to anoxic soils has rarely been integrated into trait-based plant ecology. This study aims to evaluate the relationship between root porosity and root economics-related traits among wetland plants, focusing on the effect of aerenchyma on root tissue density, a central trait in plant economics spectrum.

Methods Root porosity, root tissue density with air-space included (RTD) or excluded (RTDA), and other root traits such as root dry matter content (root dry mass to fresh mass ratio) were measured separately for adventitious and lateral roots of 16 garden-grown Ontario wetland monocots with contrasting root longevities.

Results Principal component analysis showed that in the root adaptive trait space of wetland plants, the first dimension is defined by economics-related traits, the second dimension by lateral root porosity and the ratio of lateral to adventitious root length, and the third dimension by adventitious root porosity.

Conclusions Interspecific variation in root porosity was unrelated to root economics traits. Consequently, root tissue density excluding air space (RTDA) better differentiated between species with contrasting root longevities than RTD did, consistently both for adventitious and lateral roots. Root dry matter content accurately predicted RTDA.

Keywords Aerenchyma · Root longevity · Root tissue density · Root dry matter content · Root economics spectrum · Wetland

Introduction

Root economics spectrum (RES), based on morphological and physiological root traits that are associated with resource acquisition and resource conservation, has been suggested to match the above-ground leaf economics spectrum (Mommer and Weemstra 2012; Reich 2014; Roumet et al. 2016). Traits such as specific root length (SRL), root respiration rate, root tissue density (RTD) and root lifespan have been
shown to co-vary across species and biomes, enabling their potential use to form a synthetic spectrum to represent root economics, i.e., patterns of allocation and turnover of carbon and nutrients (Ryser 1996; Roumet et al. 2016; Liu et al. 2016; Han and Zhu 2020). However, it also has been observed that root economics spectrum is more complex than above-ground trait relationships within the leaf economics spectrum would indicate (Weemstra et al. 2016, 2021; Bergmann et al. 2017, 2020; Freschet et al. 2020). There are several possible reasons for this complexity. The multiplicity of belowground environmental constraints could result in a multidimensional root economics space (Weemstra et al. 2016), and the commonly used root traits may be inadequate for disentangling different adaptations to the multiple constraints (Freschet et al. 2020). Thus, the applicability of root economics spectrum may be ecosystem-dependent, depending on the specific below-ground constraints the plants are subjected to in a given ecosystem (Shipley et al. 2016; Carvajal et al. 2019), and, a functional root economics spectrum may require the use of more specific traits (Poorter and Ryser, 2015; McCormack et al. 2017).

Wetlands are ecosystems that pose such specific constraints on roots. Most importantly, wetland soils are waterlogged, resulting in a reducing hypoxic or anoxic environment (Mitsch and Gosselink 2015). Wetland plants have adapted to such conditions by developing porous roots that allow transport of air from the atmosphere down into the oxygen-limited environment, enabling root respiration and affecting the biogeochemistry of the rhizosphere (Armstrong 1980; Lamers et al. 2013; Marzocchi et al. 2019). Such air-conducting tissue, root aerenchyma, can also be induced in terrestrial plants by flooding or other stresses (Visser et al. 2000; Zhu et al. 2010), but in wetland plants and some flood-tolerant terrestrial plants it is constitutive (Laan et al. 1989; Jackson and Colmer 2005; Mano & Omori 2013). The relative proportion that aerenchyma occupies of the total root volume can be expressed as root porosity, i.e., the fractional air space (Armstrong 1980). Root porosity varies among different species and habitats, reported values ranging from 5 to 55% (Smirnoff and Crawford 1983; Justin and Armstrong 1987; Visser et al. 2000; Pan et al. 2020); root porosity also shows phenotypic plasticity in response to waterlogging (Striker et al. 2007; Ryser et al. 2011). However, despite its importance as an adaptive/functional trait in wetland vegetation and ecosystems, root aerenchyma has rarely been incorporated into the framework of trait-based ecology (Moor et al. 2017; Pan et al. 2019).

Studies on the ecophysiology of root aerenchyma generally have not involved economics-related traits, i.e., traits related to acquisition and conservation of nutrients, or have only included a small number of species at a time, limiting a general understanding of wetland ecology (Moor et al. 2017; Pan et al. 2019).

The aim of the present study is to elucidate the relationship between the interspecific variations in root porosity and in root economics-related traits among wetland plants. As root porosity is mainly an adaptation to oxygen deficiency, and the economics-related traits are adaptations to nutrient availability and disturbance, the question arises, how do these traits interact among wetland plant species (Moor et al. 2017). It has been hypothesized that variation in root porosity might be orthogonal to the expected root economics spectrum in wetland plants, due to the independent nature of the major selective pressures, i.e., survival under oxygen deficiency and growth under contrasting levels of nutrient supply (Pan et al. 2019). On the other hand, synergy or tradeoffs among the traits could lead to correlations between them. For instance, flooding stress has been shown not only to result in changes in root air space, but also in changes in root diameter and RTD (Visser et al. 2000; Striker et al. 2007; Ryser et al. 2011). Purcell et al. (2019) showed that root porosity correlated with SRL but was independent of dry matter content across a gradient of waterlogging duration. Furthermore, trait variation may result in interactions among environmental factors, such as variation in root porosity leading to differences in soil aeration, and consequently, affecting mineralization rates (Ghosh & Kashyap 2003).

RTD is considered to be a key root trait in comparative ecology, as it expresses the amount of structural material (dry mass) invested per unit volume of root, thus having consequences both for growth rate and root robustness, and consequently, on root lifespan (Ryser 1996; Birouste et al. 2014). However, variation in the amount of root air space potentially confounds the association of RTD with other traits, as gaseous volume does not add to tissue dry mass (Eissenstat et al. 2000; Bouma et al. 2003). The confounding effect would be especially pronounced if root porosity is independent of root economics and if it shows
a large interspecific variation. This problem can be solved by measuring the density of the non-gaseous root tissue, i.e., excluding the air space from the volume measurement. Therefore, we propose a modified and more specific variable: Root tissue density with air excluded (RTDA), i.e., root dry mass per root volume without its air space. We investigate whether this new variable enables a functional improvement of the wetland root economics spectrum. In addition, because the measurement of root tissue volume is laborious, we also examine the applicability of root dry matter content (RDMC) as a more easily measurable substitute for RTDA. RDMC has previously been recommended as a decent proxy of RTD among non-wetland plants (Birouste et al. 2014).

This study has two primary objectives: (1) to quantify the confounding effect of root aerenchyma in wetland plants on the relationship between RTD and other root traits, including root lifespan, and (2) to examine the association of root porosity with economics-related root traits in a regional wetland root economics spectrum. To capture interspecific variation in root life-span, we chose species based on their contrasting root overwintering habits. Wetland monocots in Northern Ontario with a strongly seasonal climate show two distinct root overwintering habits; they either have roots that all senesce in late autumn, or they have roots that mostly persist to the next growing season (Nieman et al. 2018; Courchesne et al. 2020). This feature allows us to have a binary measure for root lifespan: short-lived roots with a longevity of one growing season, approximately 5 months at most, or long-lived roots with a longevity of one year, at least. This directly addresses the constraints on root economics spectrum posed by the trade-off between construction cost and longevity. Besides the interspecific variation, root traits also vary within a given root system, e.g., among the branching orders (Picon-Cochard et al. 2012; Rose 2017; McCormack et al. 2017). Therefore, for a comprehensive view of root trait relationships, we investigated the above-mentioned relations separately for roots of the different branching orders, notably, for the axile adventitious roots with the main functions of structure and transport, and for the fine lateral roots with the function of resource uptake (Eissenstat et al. 2000). Furthermore, given the various functional associations of root architecture at the root system level (Freschet et al. 2021), and its phenotypic response to anoxic soil (Pedersen et al. 2021), we characterized root architecture by the ratio of lateral root length to adventitious root length.

Materials and methods

Species selection

For this study, sixteen monocotyledonous plant species from Northern Ontario wetlands were selected (Table 1). These species represent two different root overwintering habits. Roots of ten of the species overwinter, and roots of six of the species completely senesce in late autumn (Nieman et al. 2018). Given the differences in root overwintering habit, roots of these species can be considered to be either short-lived (<5 months) or long-lived (>1 year).

Experimental design and growth conditions

The experiment was conducted outdoors as a pot experiment in Sudbury, Ontario, Canada (46°36’N, 81°06’W). Study plants originated in local wetlands, grown in mesocosms in a common garden for a few years. Plants in this experiment were vegetatively propagated in early summer 2019 and planted in 2.7 L pots, 10 cm in diameter and 34 cm in height, on sieved wetland soil (Bainbridge Construction, North Bay, ON, Canada), or, in case of four of the species (D. arundinaceum, A. triviale, S. latifolia, T. palustre), in 2.3 L pots of 15 cm diameter and 15 cm height on sieved artificial blend of loam, peat moss and compost (President’s Choice® Black Earth Topsoil; Brampton, Loblaws, Canada). The pots were kept in pools filled with groundwater, with the water level at the level of the substrate surface. Iron rods placed in some of the pots did not rust below the substrate surface, indicating hypoxic to anoxic conditions (Owens et al. 2008).

The average growing season in Sudbury lasts 125-145 days, defined as the consecutive number of frost-free days (OMAFRA 2016), between May and October. Temperatures during the growing seasons were the warmest in July with average daily maxima of 26.8 °C (Table S1, in Supplementary Information). Between 5 November 2019 and 1 May 2020.
the plants were covered with straw and tarps to protect against freezing of the roots. During that time pot temperatures remained mostly between 0.5 and 2 °C, similar to soil temperatures in local wetlands during that time of the year (Courchesne et al. 2020).

### Trait measurement

Seventy-one plants from sixteen species in total were harvested, of which eight species were harvested in September 2019 and eight species in August 2020. Species harvested in the second year consisted of the six species with autumn-senescing roots, and two species with overwintering roots (Table 1). Consequently, all collected roots were roots produced during the current growing season, with exception of the two species with overwintering roots collected in the second year. For each species, three to five replicate plants were measured, the variation in the number being a result of early mortality of a few plants, and experimental error when harvesting the first replicate (Table S2, in Supplementary Information).

All species showed similar root system structure, with thicker axile adventitious roots originating from the stem bases, and thinner lateral roots attached to those (Fig. S1, in Supplementary Information). Adventitious roots refer to the axile roots originating from the stem base and in some cases from the rhizome. Lateral roots are the roots originating from other roots. In the studied plants lateral roots did not further branch, with exception of Carex sp. that had two orders of lateral roots, that we pooled in the analyses.

For each individual plant, a slice of the pot content was sampled between 10 and 20 cm depth for the taller pots, and between the 7 to 13 cm depth for the shallower pots. All roots in that sample were collected by carefully washing the growth substrate out. Ten to twenty root segments consisting of adventitious root and all the connected lateral roots were collected for measurement, the number depending on

### Table 1  Species used for this experiment, with the information on their root lifespan based on their root overwintering habit, and characteristic habitats

| Species                        | Family            | Root lifespan a | Habitat                                                      |
|--------------------------------|-------------------|-----------------|--------------------------------------------------------------|
| *Alisma triviale*² Pursh       | Alismataceae      | S               | Ditches, muddy banks, disturbed wet ground, wet thickets     |
| *Carex lasiocarpa*¹ Ehrh.      | Cyperaceae        | L               | Sphagnum bog and fen mats, shallow water                     |
| *Carex magellanica*¹ Lam.      | Cyperaceae        | L               | Sphagnum bogs, coniferous swamps                             |
| *Carex oligosperma*¹ Michx      | Cyperaceae        | L               | Bogs, fens                                                   |
| *Dulichium arundinaceum*² (L.) Britton | Cyperaceae    | L               | Marshy shores, river margins                                 |
| *Eleocharis palustris*¹ (L.) Roem. and Schult. | Cyperaceae       | L               | Shallow waters along rivers and lakeshores                   |
| *Glyceria canadensis*¹ (Michx.) Trin | Poaceae        | L               | Bogs, swamps and wet borders of lakes, ponds and ditches     |
| *Iris versicolor*³ L.           | Iridaceae         | L               | Shores, marshes, river borders, and wet meadows              |
| *Pontederia cordata*² L.        | Pontederaceae     | S               | Marshy borders of lakes and streams                           |
| *Rhynchospora alba*² (L.) Vahl  | Cyperaceae        | S               | Bogs, fens                                                   |
| *Sagittaria latifolia*² Willd. | Alismataceae      | S               | Shores of lakes, ponds, streams                               |
| *Schoenoplectus tabernaemontani*³ (C. C. Gmel.) Palla | Cyperaceae | L               | Shallow water of ponds, lakes, rivers, ditches, and marshes   |
| *Scirpus cyperinus*¹ (L.) Kunth  | Cyperaceae        | L               | Wet meadows, ditches, openings in swamps                     |
| *Sparganium emersum*² Rehm.     | Typhaceae         | S               | Shores, lakes up to 6 dm of water, stream borders.           |
| *Triglochin maritima*² L.       | Juncaginaceae     | L               | Gravelly and marly shores, wet sandy beaches                 |
| *Triglochin palustris*² L.      | Juncaginaceae     | S               | Wet sandy or marly shores and marshy flats                   |

a Root lifespan based on the habits of the species’ root systems during the winter: L, long-lived overwintering roots; S, short-lived roots senescing each autumn (Nieman et al. 2018). Habitat information based on Voss and Reznicek (2012).

The year of harvest is indicated with a superscript, 1 for 2019, 2 for 2020.

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how many of them were required to obtain a sample volume deemed to be practical for the pycnometric measurements. Adventitious roots and lateral roots were separated using a razor blade while floated in a water-filled dish. This was done with great care to minimize crushing and any effects on the volume of these aerenchymatous roots. For all the species the distinction between adventitious and lateral roots was obvious, based on the diameter.

To calculate root porosity and root economics traits, the following variables were measured, in the following order: root fresh mass \((m_{\text{fresh}})\), total root volume \((V_{\text{root}})\), air-excluded root volume \((V_{\text{air-excluded}})\), root length, and root dry mass \((m_{\text{dry}})\) (Table 2). All variables were measured separately for adventitious and lateral roots. The \(m_{\text{fresh}}\) was weighed with a microbalance (MX5; Mettler-Toledo, Greifensee, Switzerland), immediately after removing root surface water by carefully blotting with paper tissues (Visser and Bögemann, 2003). The \(V_{\text{root}}\) and \(V_{\text{air-excluded}}\) were measured with the pycnometer method (Vernescu and Ryser 2009). This method is a kind of Archimedes’ method as described in Birouste et al. (2014), the most direct measurement to \(V_{\text{root}}\) based on displacement of water by submersed roots. To be specific, we measured the mass of the pycnometer filled with water \((m_1)\), filled with water and the roots \((m_2)\), and filled with water and roots after a vacuum treatment at about 7 kPa three times for 5 min in a desiccator \((m_3)\). During the vacuum treatment roots were kept submerged with a weighted mesh, resulting in the vacuumed intercellular space of roots to be filled by water when the air pressure was released. At the end the absence of new gas bubbles at the ends of the vacuumed roots, and the root fragments sinking to the bottom confirmed the efficacy of the air evacuation. \(V_{\text{root}}\) and \(V_{\text{air-excluded}}\) can be calculated with the equations: 

\[ V_{\text{root}} = \frac{(m_1 - m_2 + m_{\text{fresh}})}{\rho} \]

\[ V_{\text{air-excluded}} = \frac{(m_1 - m_3 + m_{\text{fresh}})}{\rho} \]

respectively, in which \(\rho\) is the density of water, 1.00 g cm\(^{-3}\) at 25 ℃, the temperature at which the measurements were conducted (Vernescu and Ryser, 2009). Root length was determined using the grid-intersection method, counting the number of times the root sample crosses lines of a 1-cm grid (Newman 1966; Tennant 1975). The \(m_{\text{dry}}\) was measured after drying in the oven for 48 h at 70℃.

### Calculated traits

Based on the variables measured, root porosity, specific root length (SRL), average root diameter and root density-associated traits were calculated (Table 2). Root tissue is physically composed of three phases: solid, liquid and air (Roderick et al. 1999a); root cellular tissue mainly contains the solid and liquid phase but root aerenchyma mainly the air phases. Density of root tissue can be calculated as the ratio of mass and volume, based on different combinations of root phases. Accordingly, traits that reflect root density include the commonly-used root tissue density, i.e., dry mass per total root volume (RTD; Birouste et al. 2014), root tissue density excluding air (RTDA; new variable), fresh root cellular density as the fresh mass of the cellular tissue per unit of its volume (i.e., the air-excluded volume or solid-tissue volume; Curran et al. 1996), and root dry matter content (root dry mass to fresh mass ratio; RDMC; Birouste et al. 2014) (Table 2).

We also calculated the lateral to adventitious root length ratio, as a trait describing root architecture in terms of branching density and elongation of the lower-order roots (Maurel and Nacry 2020). Ratios of different root entities have been used to describe

| Root traits                              | Abbrev. (unit) | Calculation |
|------------------------------------------|----------------|-------------|
| Root porosity                            | \( (\text{mm}^3 \ \text{mm}^{-3}) \) | \( 1 - \frac{V_{\text{air-excluded}}}{V_{\text{root}}} \) |
| Root diameter                            | \( (\text{mm}^2) \) | \( 2^{(\frac{V_{\text{root}}}{(\pi \times \text{root length})^{0.5}}} \) |
| Root tissue density                      | \( \text{RTD (g cm}^{-3}) \) | \( \frac{m_{\text{dry}}}{V_{\text{root}}} \) |
| Root air-excluded tissue density         | \( \text{RTDA (g cm}^{-3}) \) | \( \frac{m_{\text{dry}}}{V_{\text{air-excluded}}} \) |
| Fresh root cellular density             | \( (\text{g cm}^{-3}) \) | \( \frac{m_{\text{fresh}}}{V_{\text{air-excluded}}} \) |
| Root dry matter content                 | \( \text{RDMC (g g}^{-1}) \) | \( \frac{m_{\text{dry}}}{m_{\text{fresh}}} \) |
| Specific root length                    | \( \text{SRL (m g}^{-1}) \) | root length / \( m_{\text{dry}} \) |
| Lateral to adventitious root length ratio | \( (\text{m m}^{-1}) \) | lateral / adventitious root length |

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305 Plant Soil (2022) 471:301–314

**Table 2** Analysed root traits and their calculations

\( m_{\text{fresh}} \), the root fresh mass; \( m_{\text{dry}} \), the root dry mass; \( V_{\text{root}} \), the root volume; \( V_{\text{air-excluded}} \), the air-excluded root volume.
root architecture (Freschet et al. 2021), either in terms of length, mass, or number of the roots, such as root branching density (Postma et al. 2014) or the mass fraction of specific roots within a root system (Ye et al. 2019).

The accuracy of the root diameter values attained by calculations based on root volume and root length was tested by assessing the average root diameter of a representative sample of 16 plants, one of each species, directly by using a microscope and a measuring ocular, and by a calculation based on the determined sample volume (pycnometer) and length. There was a strong correlation between the calculated and directly measured values, with $R^2$ values of 0.958 ($P<0.001$; Pearson; $N=16$) and 0.881 ($P<0.001$; Pearson, $N=15$) for adventitious and lateral roots, respectively. On average, for adventitious roots the values obtained by using a pycnometer were 4.6% lower than the values obtained with a microscope ($P=0.016$, paired t-test, $N=16$), whereas for lateral roots the difference was not significant ($P=0.361$; paired t-test; $N=15$) (Fig. S2 in Supplementary Information).

Statistical analysis

Statistical analyses were conducted using average values of each of the 16 species. A two-way ANOVA was used to test the effects of root order (adventitious/lateral roots) and root lifespan (short-lived/long-lived roots) on root traits. The difference between RTD and RTDA was examined with a paired $t$-test separately for adventitious and lateral roots. Pairwise trait relationships were assessed using Pearson’s correlations for adventitious and lateral roots respectively. The dominant dimensions of the trait space at the root system level were analyzed with a Principal Component Analysis (PCA), including root porosity and key root economics traits of both adventitious and lateral roots, as well as the lateral to adventitious root length ratio. With the data of adventitious and lateral roots, major axis regression were performed using individual plant values for assessing the relationship of RDMC as proxy to RTDA or RTD (Warton et al. 2006; Birouste et al. 2014). Normality and homoscedasticity of data were tested to satisfy the assumptions of parametric analyses. SRL and root diameter were log transformed for the tests. Statistical analyses were performed using R version 4.0.3 (R Development Core Team).

Results

Trait differences and correlations between adventitious and lateral roots

Across the 16 studied wetland species, aerenchyma occupied on average 44% of the root volume in the adventitious roots, and 13% of the lateral roots, with maximum values of 56% and 30%, respectively (Table 3; Table S3, in Supplementary Information).

| Root types | adventitious roots | lateral roots |
|------------|-------------------|---------------|
| Root lifespan | long-lived | short-lived | long-lived | short-lived |
| Root porosity (cm$^3$ cm$^{-3}$) | 0.43±0.04 | 0.45±0.02 | 0.12±0.02 | 0.14±0.04 |
| SRL (mg$^{-1}$) | 17.4±3.5 | 68.9±17.3 | 173±32.6 | 687±142 |
| Root diameter (mm) | 0.87±0.09 | 0.71±0.10 | 0.21±0.02 | 0.16±0.02 |
| RTD (g cm$^{-3}$) | 0.13±0.01 | 0.05±0.01 | 0.22±0.02 | 0.09±0.01 |
| RTDA (g cm$^{-3}$) | 0.23±0.02 | 0.09±0.01 | 0.25±0.02 | 0.11±0.01 |
| Fresh root cellular density (g cm$^{-3}$) | 1.07±0.01 | 1.04±0.01 | 1.09±0.01 | 1.04±0.01 |
| RDMC (g g$^{-1}$) | 0.22±0.02 | 0.09±0.01 | 0.23±0.02 | 0.11±0.01 |
| Lateral to adventitious root length ratio (m$^{-1}$) | 7.0±2.0 | 4.5±1.3 |

*Ten Species with roots with a lifespan of more than one year; *six species with roots with a lifespan of one growing season approximately 5 months at most. SRL, specific root length; RTD, root tissue density; RTDA, root tissue density air-excluded; RDMC, root dry matter content
Information). Porosities of adventitious and lateral roots did not correlate with each other across the species (Table 4).

Of the traits reflecting root density, RTD was 41% lower in adventitious roots than in lateral roots, but RTDA, RDMC and fresh root cellular density did not differ between adventitious and lateral roots (Tables 3 and 5). Unsurprisingly, lateral roots were significantly thinner than adventitious roots. Adventitious and lateral root diameters did not correlate among the species. In contrast, species averages of RTD, RTDA and RDMC showed strong correlations between adventitious and lateral roots (Table 4). Fresh root cellular density varied only little, ranging for all species and roots between 1.01 and 1.15 g cm\(^{-3}\) (Table S3, in Supplementary Information).

Trait differences between long-lived and short-lived roots

Root porosity did not differ between species with contrasting root longevities (Tables 3 and 5). In contrast, traits reflecting density of root tissues (RTD, RTDA, fresh root cellular density and RDMC) were significantly lower and SRL higher in species with short-lived roots compared to species with long-lived roots (Tables 3 and 5). Diameters of adventitious and

### Table 4 Two-way ANOVA with the main effects root lifespan (long-lived, 10 species; short-lived, 6 species) and root order (adventitious; lateral) on root traits

| Root traits                     | Root lifespan | Root order | Interaction | R²  |
|---------------------------------|---------------|------------|-------------|-----|
|                                 | F   | P    | R² | F   | P   | R² | F   | P    | R² | total |
| Root porosity                   | 0.6 | 0.45 | 0  | 107.3 | *** | 0.79 | 0  | 0.98 | 0  | 0.79  |
| SRL                             | 35.8 | *** | 0.21 | 106.7 | *** | 0.63 | 0  | 0.85 | 0  | 0.84  |
| Root diameter                   | 5.9  | *    | 0.02 | 205.7 | *** | 0.86 | 0  | 0.62 | 0  | 0.88  |
| RTD                             | 40.3 | *** | 0.45 | 20.1  | *** | 0.22 | 2  | 0.16 | 0.02 | 0.69  |
| RTDA                            | 49.4 | *** | 0.63 | 1     | 0.32 | 0.01 | 0  | 0.92 | 0  | 0.64  |
| Fresh root cellular density     | 15.8 | *** | 0.35 | 0.4   | 0.56 | 0.01 | 1.1 | 0.31 | 0.02 | 0.38  |
| RDMC                            | 53.5 | *** | 0.65 | 1     | 0.33 | 0.01 | 0  | 0.99 | 0  | 0.66  |

An equivalent for \(R^2\) was calculated as the sum of the effect in proportion to the total sum of squares. SRL, specific root length; RTD, root tissue density (air-included); RTDA, root tissue density air-excluded; RDMC, root dry matter content. *** \(P<0.001\), * \(P<0.05\)

### Table 5 Pearson correlation coefficients for pairwise trait comparisons of adventitious roots (lower left) and lateral roots (upper right) across 16 wetland monocot species

| Lateral roots Adventitious roots | Root porosity | SRL | Root diameter | RTD | RTDA | Fresh root cellular density | RDMC | Lateral to adventitious root length ratio |
|---------------------------------|--------------|-----|---------------|-----|------|-------------------------------|------|----------------------------------------|
| Root porosity                   | 0.08         | -0.22 | 0.47\*        | -0.17 | 0   | -0.08                         | 0.02 | -0.61\*                               |
| SRL                             | 0.23         | 0.70\* | -0.88\*       | -0.77\* | -0.82\* | -0.60\*                        | -0.83\* | -0.01                                |
| Root diameter                   | 0.02         | -0.81\* | 0.29          | 0.38  | 0.47\* | 0.24                          | 0.49\* | -0.1                                 |
| RTD                             | -0.48\*      | -0.70** | 0.18          | 0.65** | 0.98** | 0.89**                         | 0.98** | 0.13                                 |
| RTDA                            | -0.11        | -0.76** | 0.3           | 0.92** | 0.77** | 0.88**                         | 0.99** | 0.02                                 |
| Fresh root cellular density     | -0.09        | -0.79** | 0.50\+        | 0.79** | 0.86** | 0.28                          | 0.85** | 0.24                                 |
| RDMC                            | -0.11        | -0.76** | 0.29          | 0.92** | 0.99** | 0.84**                         | 0.79** | 0.02                                 |
| Lateral to adventitious root length ratio | 0.07 | -0.36 | 0.51\*        | 0     | 0.09 | 0.12                          | 0.09  |                                       |

Correlations were significant at: **, \(P<0.01\); *, \(P<0.05\); +, \(P<0.1\). SRL, specific root length; RTD, root tissue density; RTDA, root tissue density air-excluded; RDMC, root dry matter content. The values on the diagonal (in bold and italics) indicate the coefficients for comparisons between adventitious and lateral roots.
Lateral roots were smaller in species with short-lived roots than in those with long-lived roots, but the difference was only weakly significant (Table 5). The interaction between root order (adventitious/lateral) and root lifespan (short-lived/long-lived) was not significant for any of the root traits, indicating that the root trait differences between species with either long-lived roots or short-lived roots were not affected by the root order (Table 5).

Influence of root aerenchyma on measures of root tissue density (RTDA vs. RTD)

Root air content causes RTD to be an underestimation of the amount of dry matter required to produce a given amount of cellular root tissue. Reflecting the porosity, RTD was lower than RTDA in adventitious roots by 44% and in lateral roots by 13% (Fig. 1; Table 3); these differences are significant (student t-test; P<0.05). As a result, although both RTD and RTDA were on average different for species with the different root lifespans, this difference was more consistent for RTDA (Fig. 1). In case of RTD, the threshold separating the species with short-lived and long-lived roots was different for adventitious and lateral roots, whereas for RTDA the threshold was the same for all roots (0.15 g cm⁻³; Fig. 1b). In addition, RTDA was more closely correlated with SRL than RTD was, both in adventitious roots as well as in lateral roots (Table 4).

Trait correlations

Root porosity did not show statistically significant relationships with root traits related to resource economics, neither in adventitious nor in lateral roots (Table 4). On the other hand, traits reflecting root density (RTD, RTDA, fresh root cellular density and RDMC) were all positively correlated with each other and negatively with SRL in both root orders. Additionally, root diameter negatively correlated with SRL, both in adventitious and in lateral roots (Table 4).

Multidimensional ‘root adaptive space’ of wetland plants

To compose a trait variation space of root systems, a PCA was conducted using nine traits of adventitious and lateral roots of the 16 studied wetland species with contrasting root lifespans. The included traits were for adventitious and lateral roots root porosity, and the root economics-related traits SRL, root diameter and RTDA, and additionally, lateral to adventitious root length ratio as a root architectural trait. The first dimension (45% of the total variation) was mainly represented by the covariation of RTDA and SRL, in which species with short-lived roots and long-lived roots were clearly clustered to opposite ends along this axis (Fig. 2; Table S4, in Supplementary Information). The second dimension (24% of the

![Fig. 1](image_url)
total variation) was dominated by lateral root porosity and the lateral to adventitious root length ratio, two variables that showed a negative trend with each other (Table 4). In addition, adventitious root porosity dominates the third dimension (12% of the total variation). The loading value of the diameters of adventitious roots and lateral roots were not specific to any dimension (Table S4, in Supplementary Information).

RDMC as the proxy of RTDA

Among the 140 paired measurements for RTDA and RDMC, there was a strong positive correlation between these two variables, both for adventitious and lateral roots (Fig. 3). Their numeric values were almost identical with an $R^2$ of 0.98 in a Pearson correlation (Fig. 3), RDMC being smaller than RTDA by 6% on average (Table 3). The relationship of RDMC with RTD also showed a significant positive correlation, but with a lower correlation coefficient than the relationship with RTDA, especially for adventitious roots ($R^2=0.83$; Fig. S3, in Supplementary Information).

Discussion

Root economics spectrum in wetland plants has rarely been addressed (Moor et al. 2017). Results of the present study demonstrate that interspecific variation in root porosity, an adaptation to oxygen-deficient soils (Justin and Armstrong 1987) is largely independent of root economics traits such as tissue density and root longevity (Ryser 1996; Biroueste et al. 2014), supporting the hypotheses by Pan et al. (2019) and Purcell et al. (2019) that suggest a decoupling of root porosity from plant economics in wetland plants. Among the wetland plants of the present study, the first dimension of the root trait space was defined by traits related to economics, such as RTDA, SRL and root lifespan, both for adventitious and lateral roots, supporting an extrapolation of root economics spectrum, as described by Roumet et al. (2016), into wetlands. Root porosity did not contribute to the first dimension, but the second dimension was determined by lateral root porosity, accompanied by root architecture expressed as the ratio of lateral to adventitious root length, with a negative trend between these traits. A relationship between root porosity and root architecture to match the oxygen-conducting structure with oxygen requirements has been postulated by Pedersen...
et al. (2021). Hence, this dimension seems to be associated with tolerance to oxygen deficiency. Interestingly, there was no significant correlation between lateral and adventitious root porosities, the latter one being dominant in the third PCA dimension, and varying within a relatively narrow range of high values. Large aerenchyma is required in axial roots to conduct air to lateral roots (Armstrong 1980), but the porosity is constrained by requirements for mechanical strength (Striker et al. 2007).

Species with overwintering, long-lived roots clearly had a higher root tissue density towards the end of their first growing season, compared to species with roots that were going to senesce later in the fall. The distinction was especially clear when the confounding effect of aerenchyma was removed. The finding supports previous observations that root tissue density is closely associated with plant economics spectrum, and correlates negatively with SRL (Ryser 1996; Craine et al. 2001; Kramer-Walter et al. 2016; Sun et al. 2016). Root diameter, often considered an important trait for root economics, did not show a clear functional association among the studied wetland plants: Root diameter correlated negatively with SRL, but variation in it further contributed to several dimensions of the trait space, in a different manner for adventitious and lateral roots, and with no crucial role on any of the dimensions. Likewise, Kramer-Walter et al. (2016) found that among 66 tree species, root diameter was loaded on a separate PCA dimension than traits associated with plant economics spectrum. Constraints of root diameter may be order-specific, with independent involvement of the functions of cortical parenchyma, aerenchyma, and stele (Yamauchi et al. 2021), and related to plant size (Ryser 1998), temperature (Weemstra et al. 2021) or drought (Laughlin et al. 2021). Furthermore, the diameter of cortex is known to be associated with the degree of mycorrhizal infection, which has been suggested to form its own dimension in root economics space (Bergmann et al. 2020). We did not investigate this aspect, but published literature indicates that mycorrhizal infection in submerged wetland plants, especially among species of Cyperaceae is mostly absent or weak (Peat and Fitter 1993; Miller and Bever 1999).

Eissenstat et al. (2000) and Bouma et al. (2003) point out that aerenchyma formation in wetland plants may confound relationships between tissue density and root lifespan. The observed lack of correlation between root porosity and root economics shows that this concern is real and that an exclusion of root air space from calculation of root tissue density improves our understanding of the relationships among the economics traits of aerenchymatous roots. A large interspecific variation in root air space may blur relations between the structural costs of root tissue and the species’ physiology and ecology, as indicated by Bouma et al. (2003). These authors did not find any difference in root tissue density between two salt-marsh grasses, the fast-growing Elymus pycnanthus with short-lived roots and the stress-tolerant Spartina anglica with substantially longer-lived roots. Our data supports the authors’ suggestion that the considerably higher porosity in S. anglica (Justin & Armstrong 1987) leads to underestimation of its root tissue density, masking the relation between economics and tissue structure. Furthermore, a difference in root porosity between adventitious and lateral roots creates a difference in RTD between these root orders, whereas RTDA is independent of the order. The latter comparison seems to be more meaningful, given the modular production and senescence of the root systems of herbaceous species, i.e., a adventitious root produces only one cohort of lateral roots (Sun et al. 2016). Consequently, minimal life-span differences between adventitious and lateral roots was observed for Ontario wetland monocots (Nieman et al. 2018).

Hence, we propose RTDA, tissue density excluding air space, as the functionally more meaningful variable in context of root economics. This is of importance especially for wetland species, but may also be of general importance, because many species characteristic of drier environments can also develop considerable aerenchyma when temporarily flooded (MacDonald et al. 2002).

Aerenchyma formation has also been described to develop in response to other stresses, such as drought or P-deficiency, possibly as means to reduce the maintenance respiration (Fan et al. 2003; Zhu et al. 2010; Postma and Lynch 2011). In anoxic soils, however, aerenchyma is a necessity for any oxidative root respiration (Laan et al. 1990). Nutrient and oxygen deficiency may also have synergistic effects, low nitrogen availability strongly increasing proportional aerenchyma in rice, possibly due to the longer roots of the N-deficient plants needing a better oxygen transport capacity (Abiko and Obara 2014).
Direct determination of root volume by the buoyancy-based method is laborious and time-consuming (Birouste et al. 2014). Our results confirmed that the more easily measurable fresh mass can accurately approximate root tissue volume (Shipley and Vu 2002; Birouste et al. 2014), resulting in RDMC being almost identical to RTDA. This is because at least in case of herbaceous tissues the specific weight of fresh plant tissue varies within a very limited range, only slightly above the specific weight of water 1 g cm\(^{-3}\) (Roderick et al. 1999b). Moreover, because the measurement of fresh mass is insensitive to tissue air content, the fresh mass is for porous roots a better equivalent to tissue volume than total root volume is. Hence, we suggest that RDMC is preferable to RTD to quantify the actual root tissue structural costs. Nonetheless, also fresh mass measurement requires great care, with a delicate balance between removal of surface water, and avoiding tissue dehydration due to evaporation (Freschet et al. 2021). For plants of dry environments, presumably with less aerenchyma, RDMC has been shown to correlate closely with RTD (Birouste et al. 2014).

As the main focus of this study was tissue density, we measured root volume directly, rather than by calculating it based on root diameter, a method that is commonly used, but sensitive to errors due to heterogeneity in root diameter (Rose 2017). Average root diameter was then calculated based on the volume of roots of a known length. Results of our additional test supports the validity of the calculated values for average root diameter. Nevertheless, over the 16 species, for adventitious roots the calculated values were slightly (<5 %) lower than the directly measured ones, possibly due to slight crushing of the aerenchymatous roots during the processing.

Our data on interspecific variation of root traits in herbaceous wetland species within a region shows a multidimensional trait space with the main adaptation to oxygen-deficient wetland soils, root aerenchyma, being independent of traits related to economics spectrum. Because aerenchyma can occupy more than half of the root volume, exclusion of this air space from calculation of tissue density is advisable when describing root traits in context of economics. This can be done by using RTDA or RDMC, traits that show a close association with root lifespan, irrespectively of root order. Other root traits, such as porosity and diameter vary independently for adventitious and lateral roots, indicating that constraints on those traits depend on root order.

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**Code availability** Not applicable.

**Declarations**

**Conflicts of interest/Competing interests** None.

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