Quantifying root extracts and exudates of sedge and shrub in relation to root morphology

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A B S T R A C T

Roots deposit labile carbon compounds directly into the soil via exudation. Within the root system, exudation rates are not homogenous; often the sites of exudation are spatially constrained to different morphologies. Younger roots, root tips and the fine root fraction are likely the primary sites of exudation, yet few studies have tracked heterogeneities in exudation quantity and quality within the root system. Understanding the vertical gradients in exudation is important for properly representing the interfacial area with the anaerobic zone, and correctly estimating the flux of C compounds available to the methanogenic community. Root exudates from *Eriophorum vaginatum* and *Rhododendron groenlandicum*, grown in ombrotrophic bog cores removed from the field, were sampled by placing individual root segments of excavated roots into custom-designed root traps. Root extracts were also collected to profile the internal cell concentration. Total root exudation rates for all identified compounds varied by species, with exudation by *E. vaginatum* totaling 205.9 nmol g^{-1} dry weight hr^{-1} compared to 365.2 nmol g^{-1} dry weight hr^{-1} for *R. groenlandicum*. For each species, variations in root extract and root exudation rates between identified root classes were statistically significant for a number of compounds, often sugars, lactate, malate, oxalate, succinate and tartarate. The strong dependence of exudation on root morphology was confirmed by TOC analysis, which revealed that carbon exudation in *E. vaginatum* root classes ranged from 1.24 to 2.55 μg C g^{-1} d.wt. hr^{-1} between immature and senior roots, whereas non-haired *R. groenlandicum* roots had relatively low exudation rates between 0.22 and 0.56 μg C g^{-1} d.wt. hr^{-1} compared to 18.22 and 10.47 μg C g^{-1} d.wt. hr^{-1} for fine and coarse haired roots. These finding document important composition differences between bog species and considerable variability in exudates rates between *R. groenlandicum* root morphologies.

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1. Introduction

Plants are a major source for organic carbon in soil (Megonigal and Schlesinger, 2002) and a substantial proportion of carbon fixed during photosynthesis (20—60%) can be translocated into the rhizosphere (Kuzyakov and Domanski, 2000; Grayston et al., 1997). The transfer of carbon from roots to bulk soil is an important, but poorly understood process relative to other pathways in the terrestrial C cycle (Grayston et al., 1997; Jones et al., 2004). In particular, root exudation has been difficult to quantify. While efflux is primarily driven by diffusion through permeable root cells due to the large concentration gradient between the cell cytoplasm (mM concentration) and soil solution (μM concentration) (Nguyen, 2003), active exudation mechanisms can also occur as plants strive to positively affect soil solubility and enhance acquisition of nutrients (Neumann and Martinoia, 2002), or prevent toxins such as aluminum from entering the root (Ryan et al., 1995). Rewards for the plants investment in belowground photosynthate allocation need to be balanced against the risks of microbial proliferation leading to competition for nutrients and the increased likelihood of pathogen attraction, growth and attack (Nguyen, 2003; Liu et al., 1997). As the returns on investment are not clear cut, it is likely that many species have adapted a variety of strategies that likely affect the magnitude and composition of exudates which may further vary over space and time.

Given the range of plant anatomy, physiology and nutrient acquisition strategies, it is not surprising that numerous studies have documented differences in the composition of root exudates between species (Grayston et al., 1997; Fan et al., 2001). Malic and

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citric acid exudation rates were 0.25, 0.0 versus 0.14, 0.13 μmol h^{-1} g^{-1} root fresh weight (f.wt.) for wheat and tomatoes, while under phosphorous deficiency tomato exudation considerably decreased, whereas wheat exudation of malic was minimally attenuated and citric exudation was not affected (Neumann and Römheld, 1999). Exudation rates are temporally variable and can differ by ~50% between flowering and fruiting stages (Lucas García et al., 2001). Many short term temporal trends in the quantity of root exudates have been noted in many flowers and agricultural crops such as lupin (Lucas García et al., 2001), rice (Oryza sativa L.) (Kerdchoechuen, 2005) and maize (Zea mays) (Carvalhais et al., 2011).

Spatial examination of the root system reveals heterogeneity in exudation rates and composition because root functions are not evenly distributed (Hinsinger et al., 2005). Plants allocate physiological tasks to root morphologies that optimize said function. Thick roots with greater diameters have superior performance at penetrating hard soil (Clark et al., 2003), anchor against aboveground forces and act as scaffolding for nutrient acquiring roots. In contrast, the higher surface area of fine diameter roots enhances uptake activities while costing the plant little if conditions become unfavorable and the root must be abandoned (Wells and Eissenstat, 2002). If anatomical differences denote physiological function, then the roots with the finest diameter may play a primary role in exudation rates due to increased surface area and minimization of structural and transport functions (McCormack et al., 2015). Nevertheless, it's unclear whether coarse diameter roots play a secondary role in exudation, and sorting roots into various functional categories is arbitrary when the transition is continuous. Numerous root morphology factors have been shown to influence exudation rates (Nguyen, 2003), including heightened exudation near growth regions (Hinsinger et al., 2005), younger roots (Doussan et al., 2003), specialization of certain root parts (Neumann and Martinoia, 2002) and efflux close to the root apex (McCully and Canny, 1985) in spite of uniform root permeability (Jones and Darrah, 1994, 1996).

To quantify total flux, root exudation is often upscaled simplistically to the plot level (i.e. m^2) by multiplying measured exudation rates from a single root by the plot level root biomass and the number of days in a year (e.g. (Phillips et al., 2008; Aoki et al., 2012)). In ombrotrophic bogs such as Mer Bleue, Ontario Canada, exudation of relevance to methanogenesis is relegated by the interface of the root mass to the anaerobic zone as partitioned by the water table. Vertical gradients in root mass and exudation rates by root morphology must thereby be accounted for to properly estimate the quantity and quality of carbon deposited, yet few studies make effort to spatially resolve exudation despite evidence that exudation rates vary with depth. Utilizing the exudation rate of mycorrhizal seedlings and fine root density, Van Hees et al. (2005) demonstrated that the oxalate production rate would need to range from 0.1 to 0.6 nmol g^{-1} dry weight (d.wt.) soil h^{-1} between the Bs and O horizon in order to sustain the observed soil solution pool size. Vertically, bog sedges and shrubs are reported to be quite heterogeneous with respect to root morphology, potentially driving vertical gradients in root exudate composition. Methanogens are quite sensitive to the form of root exudate carbon. Monocarboxylic acids such as acetate and formate can directly serve as alternative electron acceptors in methaneogenesis. Other organic acids, sugars and amino acids must be reduced by fermentive microbial communities who incorporate a percentage of the carbon into microbial products (Allison, 2014), increasing the difficulty of establishing the transformation efficacy of individual compounds into methane (CH4). However at the sediment-atmosphere interface, many authors speculate that unusual CH4 fluxes are related to a variable supply of organic carbon from the root exudation pathway (Neue

and Sass, 1994; Lindau et al., 1991; Minoda and Kimura, 1994).

Unfortunately there is a paucity of data on root morphology and corresponding exudation rates at the species level for ombrotrophic bogs. Since the magnitude and composition of exudates are not universal, in order to quantify the potential influence of root exudation on methanogenesis, empirical studies of individual species and root morphologies that may influence flux rates must be undertaken. In order to provide baseline documentation for bog plants, this research will quantify methanogenic carbon substrates in root extracts and exudates at identified root morphologies of cottongrass (*Eriophorum vaginatum*) and an evergreen shrub (*Rhododendron groenlandicum*). These species are model organisms since *R. groenlandicum* features short taproots while the ~1 m long roots of *E. vaginatum* are considered likely to deposit root exudates directly into the anaerobic zone (Saarinen, 1996). The use of microcosms employing localized sampling was chosen as this method offers a hybrid semi-sterile (roots remain exposed to microflora while the trap itself is sterile) solution that permits the use of soil grown plants. To our knowledge, this is the first time anions of root extracts of these bog species have been profiled and localized measurement of exudation by root morphologies have been attempted. Greater fidelity in characterizing exudation of the typically inundated root morphologies will provide better insight into whether root exudation is a driver of the seasonal and inter-annual variations that contribute to the uncertainty in the estimates of total CH4 flux from wetlands.

2. Material and methods

2.1. Study site

Mer Bleue (Fig. 1) is a 28 km^2 raised ombrotrophic bog 15 km east of Ottawa, Ontario (45.41 N, 75.52 W). The climate is cold humid continental climate with a mean annual temperature of 6.0 ± 0.8 °C and mean annual precipitation of 943.5 mm (Fraser et al., 2001). The region lies in a postglacial channel system that transformed into a bog around 7000 years ago. Peat depths vary from 5 to 6 m near the center, decreasing to 0.3 m at the margins (Roulet et al., 2007). Mer Bleue has a typical microtopography with hummocks comprising approximately 70% of the surface. Due to the convex mesoscale topography, hydrological connectivity to the surrounding uplands is negligible and the water supply is restricted to atmospheric inputs.

Bog soils are non-optimal for methanogenesis. Average CH4 flux rates of inundated bog top soil were 0.0007 g m^{-2} per day^{-1}, lower than fen top soil (0.028 g m^{-2} per day^{-1}) (Moore and Knowles, 1989). At Mer Bleue, seasonal flux rates range from 0.01 to 0.03 g m^{-2} per day^{-1} in the spring, to a maximum in mid-summer (0.1–1 g m^{-2} per day^{-1}) and are minimal overwinter (Moore et al., 2011). In addition to temporal variability, Mer Bleue fluxes exhibit strong spatial variability associated with microtopography and plant species composition. Per growing season (May–November), fluxes were generally lower for hummocks than lawns (1.9–8.3 g CH4 m^{-2} per growing season for hummocks versus 8.7–26.5 g CH4 m^{-2} for lawns per growing season), likely due to the thicker oxic layer in hummocks that consumes CH4. Furthermore, the highest CH4 fluxes have been observed from *E. vaginatum* chambers (11.6–76.8 g CH4 m^{-2} per growing season) (Moore et al., 2011).

The vegetation community at Mer Bleue is a multilayered canopy consisting of an upper layer of various trees, shrubs or sedges and a ground layer dominated by mosses. The dominant moss species are mainly Sphagnum (*Sphagnum capillifolium, Polytrichum strictum, Sphagnum magellanicum, Sphagnum angustifolium and Sphagnum fuscum*). The majority of the upper layer is comprised of the evergreen shrubs *Chamaedaphne calyculata, R. groenlandicum,*