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Short communication

Profiling the below ground biomass of an emergent macrophyte using an adapted ingrowth core method

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ABSTRACT

In the context of our work exploring the role of *Sparganium erectum* as a physical ecosystem engineer, we aimed to test our hypothesis that the root and rhizome biomass of this species would be largely confined to the uppermost sediment layers, thereby having the effect of reinforcing newly deposited material and facilitating the growth of in-channel macrophyte stands and sediment accumulations. Detailed measurements of the below ground structures of linear emergent macrophytes, in terms of their biomass and architecture, are complicated by difficulties associated with sampling in the highly saturated sediments that these morphotypes typically occupy. In this paper, we describe the development of an adapted ingrowth core method, which allows the extrusion of an undisturbed root-soil matrix from highly saturated environments. The approach combines an ingrowth core, which is commonly used to measure fine-root production in forest topsoil, with an outer casing that facilitates the retention of a sample representative of field conditions, and a laboratory protocol that enables extrusion and measurement of biomass at different depth increments. The new approach enabled detailed depth profiling of *S. erectum*, and confirmed our hypothesis by demonstrating that root and rhizome biomass was predominantly located in the 10 cm of sediment closest to the sediment–water interface throughout our study.

1. Introduction

Linear emergent macrophytes are often highly effective physical ecosystem engineers (sensu Jones et al., 1994), capable of altering the geomorphological structure of river channels by changing flow patterns and accumulating fine sediment (Naden et al., 2006; Asaeda et al., 2010), and facilitating colonisation of the retained sediments by other plant species (Gurnell et al., 2007; O'Hare et al., 2012). There is a growing understanding of the morphological and biomechanical traits that facilitate the profound physical effects caused by aquatic macrophytes on their surrounding environment, such as stem density (van Hulzen et al., 2007), stiffness (Bouma et al., 2005), and breaking force (Liffen et al., 2013), and the abundance and distribution of rhizomes (Kotschy and Rodgers, 2008). We hypothesised that rooting depth is also an important factor in the tendency of macrophytes to retain fine sediment, with roots and rhizomes increasing the erosion resistance of sediment via the same mechanisms that the roots of terrestrial vegetation reinforce highly saturated soils (Gyssels et al., 2005; Fan and Su, 2008). Specifically, we hypothesised that the species *Sparganium erectum*, a well documented fluvial ecosystem engineer (Asaeda et al., 2010; Gurnell, 2013) that is found abundantly throughout the UK (O'Hare et al., 2011) and the northern temperate region (Cook, 1961; Riis et al., 2000), will concentrate its root and rhizome biomass in the upper sediment layers, closest to the sediment–water interface, thereby reinforcing newly deposited material and facilitating the growth of in-channel sediment accumulations.

Due to difficulties associated with sampling, there are few detailed measurements of the below ground biomass of aquatic macrophytes in relation to depth. There exist sophisticated methods to measure the below ground portions and growth dynamics of terrestrial vegetation, which include root windows, wall excavations and minirhizotron cameras (Metcalfe et al., 2009), however none of these are suitable for measuring roots growing in highly saturated sediment because water limits visual observations and causes sediments to be unstable. Measurements of the below ground biomass of aquatic plants have typically involved excavating large sediment blocks, from which root and rhizome material is separated (Klopatek and Stearns, 1978; Klimes et al., 1999; Asaeda et al., 2006a,b, 2010; League et al., 2006). This approach allows total biomass to be accurately quantified, but gives no indication of its vertical distribution within the sampled sediment. Darby and Turner (2008) devised a method for investigating root and rhizome profiles in estuarine sediments, which involved extracting cores

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using stainless steel tubes with sharpened edges, which were then extruded and dissected at 10 cm depth increments. However, in that example, the sediments were relatively cohesive and not prone to loss from the bottom of the coring device.

The ingrowth core method, which was established in the 1980s and has been used commonly since then (Persson, 1983; Nadelhoffer and Raich, 1992; Hendricks et al., 2006), involves the placement of a root penetrable structure into soil, the volume of which is usually excavated and replaced with a root free media (e.g. sand or local soil that has been sieved free of root biomass). The cores are then sampled following a period of predicted root development. In addition to its frequent use in forest topsoil, this method has also been used to measure the below ground dynamics of riparian vegetation (Kiley and Schneider, 2005; Boyd and Svejcar, 2009). This paper proposes a new method for assessing the below ground biomass of aquatic macrophytes of a similar morphology to *S. erectum* using a design based on the ingrowth core method, but also incorporates a sharpened outer sleeve and basal cap that retains the integrity of the unconsolidated sediment, rhizomes and fine-root matrix. A sampling and laboratory procedure is proposed to ensure successful extrusion and measurement of below ground biomass at different sediment depths.

In addition to testing the hypothesis that below ground biomass would be concentrated in the uppermost sediment layers, the study was designed to answer the following questions:

i) How does biomass and its vertical distribution vary seasonally, and what are the possible implications for the ecosystem engineering effects of the species?
ii) Do roots and rhizomes vary in terms of their distribution throughout the sediment profile?

2. Materials and methods

2.1. Core design

Each ingrowth core was 50 cm in length and had an inner diameter of 11 cm. These dimensions were chosen because (i) the fine sediment accumulations that overlay the gravel bed of the study reach were generally less than 50 cm in depth, and (ii) 11 cm was deemed sufficiently wide for a large *S. erectum* plant to emerge unrestricted from the top. It was important that the cores were predominantly permeable structures that allowed plants to grow in/out of their boundaries, yet remained strong and rigid. Therefore, holes were drilled in a honeycomb arrangement to minimise the distance between them without compromising strength. Each hole was 2.6 cm in diameter, twice the diameter of the largest rhizomes measured during a study that involved measurements of plant morphology the previous year (Liffen et al., 2011). The prototype was initially made using polyvinyl chloride (PVC), but this was subsequently changed to polypropylene due to concerns about the possibility of chlorinated compounds affecting plant growth (Fyfield et al., 1984). The polypropylene was 3 mm thick, sufficient to remain rigid despite the extensive area of drilled holes.

Given the differences between those environments previously sampled using the ingrowth core method and the fine, submerged sediments present at our study site, adaptations had to be made to ensure that an undisturbed biomass/sediment sample could be successfully extracted. Two specific problems previously encountered during ingrowth core experiments were (i) saturated soils often fall out of the bottom of the core when extraction is attempted, and (ii) roots 'slide' out of the core holes when the core is removed (Boyd and Svejcar, 2009). To minimise the displacement of roots, plant material was cut flush to the edge of the core in the field using a sleeve, made of the same material as the core but 1 mm larger in diameter and with a sharpened circumference on its lower edge. The sleeve was forced directly over the core immediately prior to extraction, which had the effect of severing roots and rhizomes, and retaining their position within the sediment profile. To prevent the loss of material from the bottom, the core was leveraged slightly before a basal cap was inserted underneath. The core could then be removed as a single, undisturbed profile of sediment and biomass. The core was retained within the sleeve and cap during transport back to the laboratory. Fig. 1A shows the components of the ingrowth core, sleeve and basal cap design, and Fig. 1B shows a test core and its effectiveness in capturing the growth of underground biomass and retaining its vertical distribution within the fine sediment profile, with roots (thin) and rhizomes (thick) growing in/out of the core boundaries.

2.2. Core installation and retrieval

The cores were installed throughout a reach of the River Blackwater, UK (51°19 14 N, 0°45 48 W). A survey of the site in summer 2009 informed which areas of the channel exhibited growth of *S. erectum* and fine sediment accumulations. In February 2010, when biomass was predicted to be minimal and thus their introduction would cause relatively less interference, groups of 8–12 ingrowth cores were forced into the fine superficial bed sediment within five discrete 1.5 m × 1.5 m quadrats of the channel bed (Fig. 1C). Deployment was only possible in wadeable depths, which was sufficient as the cores were installed during low flow when the *S. erectum* stands were only found in 1 m of water or less. In total, 50 cores were placed in the reach in February and six to eight cores were removed on seven sampling occasions between May 2010 and January 2011 (1: 25 May; 2: 30 June; 3: 5 August; 4: 3 September; 5: 8 October; 6: 23 November; 7: 14 January). Cores were sampled on multiple occasions in order to gain more detailed understanding of root and rhizome dynamics in relation to depth, and to capture the timing of annual dieback, which could later be linked to conceptual changes in seasonal flow intensity and sediment erosion that are associated with the presence of above ground biomass (approximately March–November in this reach of the River Blackwater). In total, 49 cores were successfully retrieved from the reach.

Previous applications of the ingrowth core method replace the core volume with root free media, principally because they are designed as a means of measuring root production. Given that this was not the purpose of this investigation, the sediment was not replaced, whilst it was also predicted that removal of the sediment could cause the collapse of surrounding material and interference with root and rhizome distribution.

2.3. Laboratory measurements

The experimental cores were transported directly from the field site to the laboratory, where they were frozen to minimise disturbance of the sediment and root-rhizome profile, and any decay of plant material prior to analysis. When the biomass within a core was to be assessed, the core was removed from the freezer and allowed to thaw for 1 h, at which point the outer sleeve was removed and the core placed horizontally on the workbench (Fig. 1D) The total depth of the sediment within the core was measured, extruded in 5 cm depth increments and cut using a hacksaw. An advantage of freezing was that the core could be easily extruded as a single profile, avoiding mixing and collapse of the saturated sediment when laid horizontally. If the total depth was not divisible by five, the final few centimetres of sediment at the base of the core were analysed separately. All cores were dissected within 72 h of retrieval from the field.

Following Darby and Turner (2008), each 5 cm increment was wet sieved in a 2 mm over a 0.5 mm sieve to retain dead and fine