Zostera marina root demography in an intertidal estuarine environment measured using minirhizotron technology

Mark G. Johnson*, Christian P. Andersen, Donald L. Phillips, James E. Kaldy

US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, 200 SW 35th Street, Corvallis, Oregon 97333, USA

ABSTRACT: Over the last 4 decades there have been major advances in our understanding of the biology, ecology and physiology of seagrasses and their environmental interactions. Despite these advances, there has been relatively little advancement in our understanding of seagrass belowground dynamics. Minirhizotron tubes are a terrestrial ecology method used to visualize root birth, growth and death to evaluate root deployment, development and demography. Our objectives were to adapt the use of minirhizotrons in an intertidal seagrass bed, and to quantify root standing crop, production, mortality and life span of *Zostera marina* roots in a northwest US estuary. A total of 204 individual roots were observed and tracked over 18 monthly sampling periods. Roots exhibited marked vertical distribution in the sediment with peak root numbers at 9 and 25 cm depth. The median life span for roots deeper than 10 cm in the sediment was 75 d and only 48 d for roots in the top 10 cm of sediment. Root biomass turnover was estimated to be 56.9 g m^{-2} yr^{-1}, while root carbon mass turnover estimate was 16.6 g C m^{-2} yr^{-1}. Although significant logistical obstacles remain (e.g. minirhizotron use in subtidal beds), development of a non-destructive sampling technique for seagrass belowground root dynamics will provide better insight into seagrass root–sediment interactions that cannot be captured using traditional destructive sampling methods.

KEY WORDS: Seagrass · Root turnover · Carbon dynamics · Root life span · Climate change · Pacific Northwest

INTRODUCTION

Seagrasses are marine monocotyledonous angiosperms that occupy both the water column and sediments in low-energy estuarine environments. These communities provide habitat for many commercially important fish and shellfish species and provide other important ecosystem services (e.g. improved water quality, sediment stabilization, carbon sequestration, etc.) and nutrient cycling (Hemminga & Duarte 2000, Orth et al. 2006). Globally, seagrasses annually add ~27 Tg of carbon to marine sediments, roughly 10% of the total annual carbon burial in world oceans (Fourqurean et al. 2012). Thus, belowground dynamics of seagrasses are critical to understanding sediment biogeochemical processes, but there have been few studies due to logistical difficulties.

In many regions of the world, seagrass communities are in decline, and no single causative agent has been identified (Orth et al. 2006). Both natural and anthropogenic stresses may be altering seagrass distribution and growth, but our limited understanding of the environmental dynamics of seagrasses has hampered determining underlying mechanisms (Touchette & Burkholder 2000). In particular, stresses affecting root and rhizome growth and metabolism are poorly understood, due to the lack of methodological approaches for non-destructive, *in situ* sediment process studies.

Plant ecologists have made great progress in developing techniques to evaluate belowground root dy-

namics of terrestrial plant ecosystems, including the use of root windows (Egli & Kälin 1990) and minirhizotron tubes (Johnson et al. 2001). Both methods allow visualization and measurement of root genesis, root life span, demography and development. Further, terrestrial plant ecologists have developed a classification system for roots and mathematical equations to calculate fine root demographics. In contrast, plant belowground (i.e. sediment) dynamics are a poorly understood component of seagrass ecosystems. Consequently, application of methods pioneered by terrestrial ecologists can provide valuable insight to seagrass belowground dynamics, particularly with regard to root demographics (e.g. birth, life span and mortality).

Seagrass scientists have long relied on marking techniques to measure leaf and rhizome growth rates (Short & Duarte 2001), while destructive harvest methods are commonly used to estimate above- and belowground biomass. Leaf marking methods are generally accepted to provide good quantitative estimates of leaf growth rates, although some methods provide better estimates than others (e.g. leaf marking versus clipping techniques; Hauxwell et al. 2001, Kowalski et al. 2001). Rhizome marking methods have been used to estimate seagrass rhizome growth rates (Kaldy & Dunton 2000). Unfortunately, these techniques cannot be used to measure root growth rates, life span or other demographic parameters. The large surface area and anatomical adaptations (e.g. root hairs, lacunae, etc.) associated with seagrass roots facilitate exchange of nutrients and labile organic constituents between plants and sediment. Consequently, sediment microbial communities can use plant-derived carbon to fuel biogeochemical cycling (Devereux 2005, Kaldy et al. 2006). Therefore, quantifying dynamics of root growth and biomass *in situ* may provide more direct insight into linkages between plants and sediment microbial communities.

The goal of this study was to address these knowledge gaps using minirhizotrons to quantify root standing crop, production, mortality and life span in a seagrass bed in Yaquina Bay, Oregon, USA. We had 3 general hypotheses: (1) root standing crop, production, mortality and life span are independent of seabed elevation; (2) root standing crop production, mortality and life span are similar throughout the sediment profile and (3) life span of individual root cohorts is similar regardless of when the cohort developed. The results provide new information on root distribution and also potential carbon fluxes to these highly reduced marine sediments, which may be used to develop better management and protective measures for seagrasses.

MATERIALS AND METHODS

Site description

All fieldwork was conducted in Yaquina Bay, Oregon, USA, in a monotypic *Zostera marina* meadow (44° 37′ N, 124° 2′ W). Detailed information on *Z. marina* characteristics and environmental factors at the site can be found in Kaldy (2006), Kaldy & Lee (2007) and Kaldy et al. (2013). In general, *Z. marina* has strap-like leaves up to 2 m in length anchored to the sediments, with rhizomes with fine roots emerging at nodes spaced 0.5 to 5 cm apart. The study site is characterized by sediments composed of 69% sand, 25% silt and 6% clay and about 1.4% C (by weight) with a C:N ratio of 15.8. The site is macrotidal (~2 m range) and ocean-dominated, characterized by cold water temperatures and high nutrient concentrations derived from N fixation in the watershed and oceanic upwelling (Brown & Ozretich 2009). It is a low-gradient bed, with the intertidal portion of the bed being relatively flat with ~0.46 m of relief between the river channel and the uppermost extent of the *Z. marina* plants.

Minirhizotrons

Adapting minirhizotron technology to the estuarine environment posed a number of technical and logistical problems. The minirhizotron tubes used in this study consisted of extruded polybutyrate plastic tubes, with an inside diameter of ~5 cm and wall thickness of 0.3 cm, that were ~1.83 m in length. The portion of the tubes that were exposed to light during low tides were painted first with a layer of black paint and then a layer of reflective white paint to exclude light (Fig. 1A) (Johnson et al. 2001) which could affect root growth or encourage algal growth within the tubes. One end of each tube was fitted with a sharpened plastic plug glued in place to seal the end of the tube. This pointed end facilitated placement of the tube, which was driven into the sediment with a rubber mallet (Fig. 1A). Twelve tubes were installed in May, 2002 (Fig. 1B) at an angle 16° off the horizontal sediment surface, to ~30 cm depth. This angle was chosen to optimize viewing the shallow roots that *Z. marina* produces. Approximately 40 cm of each tube was left projecting above the sediment to provide above-water access at low tide for the minirhizotron camera. The tubes were attached to a 70 cm ground anchor with a metal hose clamp. Fig. 1C shows the location of the *Z. marina* bed and minirhizotron placement.
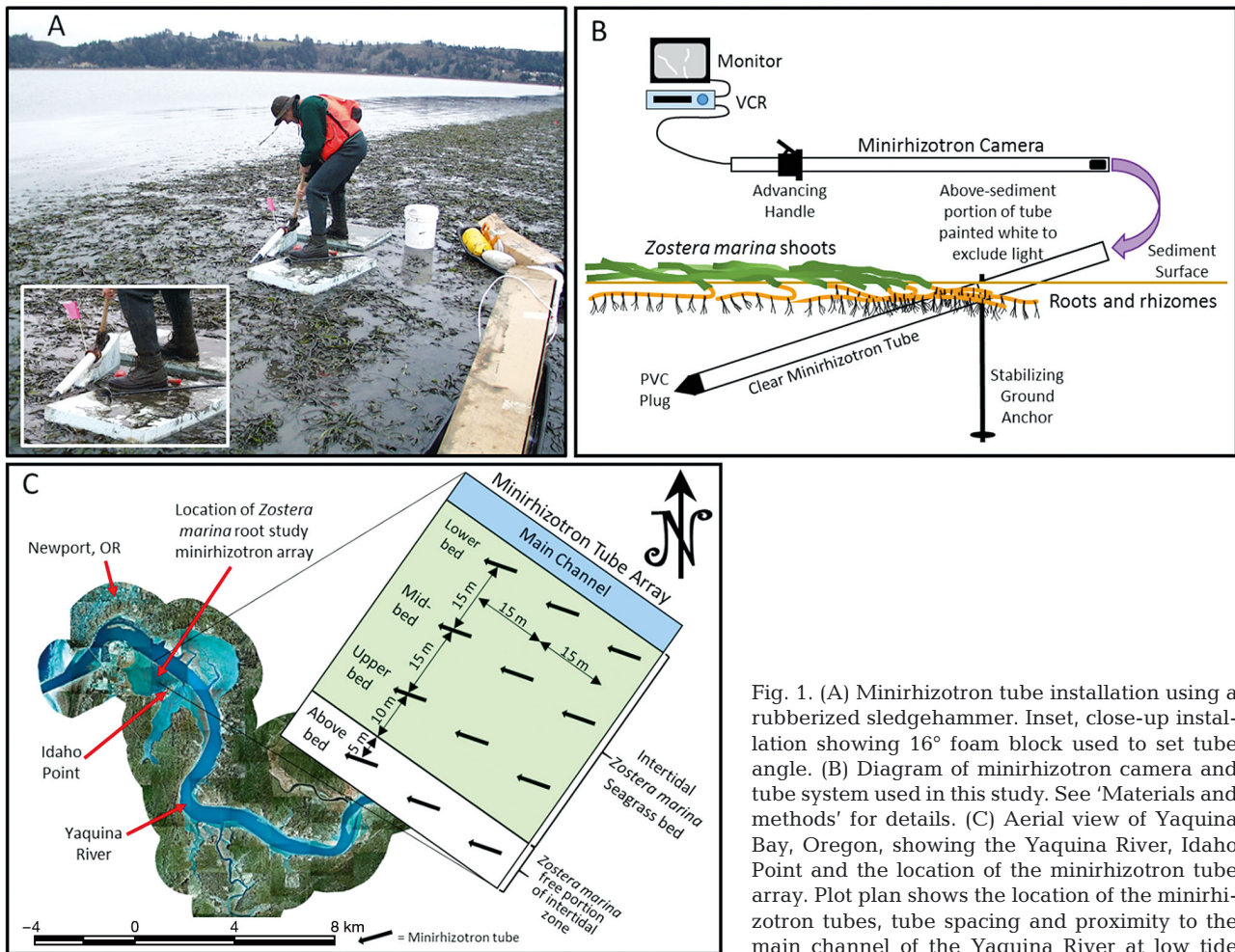
Fig. 1. (A) Minirhizotron tube installation using a rubberized sledgehammer. Inset, close-up installation showing 16° foam block used to set tube angle. (B) Diagram of minirhizotron camera and tube system used in this study. See 'Materials and methods' for details. (C) Aerial view of Yaquina Bay, Oregon, showing the Yaquina River, Idaho Point and the location of the minirhizotron tube array. Plot plan shows the location of the minirhizotron tubes, tube spacing and proximity to the main channel of the Yaquina River at low tide

The seagrass bed exposed at mean low water at Idaho Point was ~35 m wide (mean low water line to uppermost extent of the seagrass). The seagrass bed exposed at mean low water was divided into 3 elevations relative to mean lower low water (MLLW). The lowest was called the 'Lower bed' at a tidal elevation of about 0 m MLLW. Three minirhizotron tubes were installed in the Lower bed, ~2–3 m from the mean low water line, and parallel to it. The 'Mid-bed' was upslope and above the Lower bed at a tidal elevation of about +0.26 m MLLW. Three minirhizotron tubes were installed in the Mid-bed 15 m away from those installed in the Lower bed. Three minirhizotron tubes were also installed in the 'Upper bed' at a tidal elevation of +0.39 m MLLW, 15 m upslope and parallel to the tubes in the Mid-bed, 2–3 m from the upper margin of the seagrass bed. A fourth elevation was delineated above the permanent seagrass bed (i.e. un-vegetated sediment) at about +0.51 m MLLW. This was designated 'Above bed' and 3 minirhizotron tubes were installed at this elevation. After place-

ment of the tubes, a plastic mechanical pipe plug (Cherne Industries, model 270538) was used to seal the end of the tube protruding from the sediment surface to prevent water from entering the tube while submerged at high tide.

Image collection

Beginning in June 2002, we collected root images and sampled shoot, rhizome and root tissue proximal to each tube at monthly intervals through December 2003 (i.e. 18 image collection events). Sampling occurred at low tides of at least −0.15 m MLLW to have sufficient time to complete image acquisition. At each sampling interval, the mechanical pipe plug was removed from the end of the minirhizotron tube and a small manual pump was used to remove any water that had entered. Each tube was swabbed dry with a clean cotton cloth, and a specialized digital camera was used to record root images along the

length of the tube. Images were recorded on digital video using a 5 cm O.D. color minirhizotron video camera (Bartz Technology). Each image covered a field of view of ~1.8 cm^2, and 3 images were taken at each location along the tube — pointed upwards (12:00 position) and to both sides (09:00 and 03:00). The camera was locked in place with an indexing handle that allowed repeated observations at the same location at each sampling event (Johnson & Meyer 1998). There were 83 detents (locations) along each tube where images were taken between the sediment surface and a vertical depth of ~30 cm.

Root digitization and survival analysis

Root data and other information were extracted from the videos back in the laboratory using ROOTs digitizing software (Enslin et al. 1994). Length and width of each root were measured by manual tracing with a computer mouse. Error-checking computer programs were written in SAS to examine 14 types of classification, measurement and recording errors for each image at a single sample time and across sample times (Johnson et al. 2001). Previous studies have found very high data quality and repeatability of root measurements using these techniques (Phillips et al. 2006a,b).

A data set was constructed from the minirhizotron images that included, for each fine root: depth in sediment, zone (position on gradient from deepest to shallowest end of *Z. marina* bed; Lower bed, Mid-bed, Upper bed and Above bed), time of appearance, time of disappearance and life span (interval from appearance to disappearance). Because there is no way to determine root physiological activity, all roots observed in the photographs were considered to be 'live', unless they disappeared from view during the study period. All calculations and analyses were carried out using SAS Version 8 (SAS Institute 1999). Root depth was binned into 2 sediment depth classes for analysis: 0–10 cm and >10 cm. There was also an indicator variable for right-censoring (Allison 1995), which occurred when a root had not disappeared by the end of the study and thus had an indeterminate life span. A seasonal cohort variable was also created to indicate the 3-mo season (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec) in which each root first appeared. A Wilcoxon rank-sum test (PROC NPAR1WAY in SAS) was used to test for differences in root width (mean of all observations for each root) between sediment depth classes (0–10 cm and >10 cm).

Proportional hazards regression (Cox 1972) was used to analyze the effect of root depth in sediment on root survivorship. This procedure has been used extensively in agricultural studies (Baddeley & Watson 2005) and forest tree studies (Phillips et al. 2009). This analysis evaluates the hazard function $h(t)$, which indicates the instantaneous risk of mortality at time t, given survival to time t (Allison 1995):

$$h(t) = \lim_{\Delta t \to 0} \frac{\Pr\{t \leq T < t + \Delta t \mid T \geq t\}}{\Delta t} \quad (1)$$

where T is the time of death. For an individual root i, the hazard is modeled as the product of an unspecified baseline hazard function and an exponentiated linear function of the k predictor variables (Allison 1995):

$$h_i(t) = h_0(t) \exp(\beta_1 x_{i1} + \ldots + \beta_k x_{ik}) \quad (2)$$

The βs are estimated coefficients in the model that provide weights for the effects of each of the predictor variables (x), and χ^2 tests are performed to determine whether the coefficients are significantly different from 0. Since this linear function of predictors is exponentiated, the model assumes that effects of the predictors on the hazard are multiplicative; thus the hazard for one group is proportional to that of another group, where this proportionality is assumed constant over time. Hazard ratios are computed to compare hazards between different levels of the categorical predictor variables. Subtracting 1 from the hazard ratio and multiplying by 100% gives the percent mortality increase between predictor levels (Allison 1995).

PROC LIFETEST in SAS was used to produce root survival curves (proportion of roots surviving versus time since first appearance) by plotting Kaplan-Meier estimates of the survivor function versus time for various predictor variables (Kaplan & Meier 1958). From these estimates, median life spans were computed, which designate the point at which half of the roots originally present have disappeared. Diagnostic tests indicated that there was not constant proportionality of hazards over time among the 4 seasonal cohorts or among the elevations. Therefore, these variables were not included in the proportional hazards model described above, but equality of their survival functions was tested with log-rank and Wilcoxon tests (Allison 1995), and median life spans and their 95% confidence intervals were compared among cohorts and among zones. The proportional hazards analysis was carried out in PROC PHREG of SAS, testing for the effect of root depth in sediment on root survival. The hazard ratio was determined for deeper roots (>10 cm) versus shallower roots (0–10 cm).

Root biomass and carbon turnover

Biomass and carbon standing stocks were estimated using empirical data collected from the same *Z. marina* bed during this study (Kaldy 2006). Each month, 10 replicate 15-cm diameter cores inserted to a depth of 20 cm were collected monthly from March 2001 to November 2002. The number of shoots was recorded and leaf blades, rhizomes and roots were washed, separated, dried and weighed. Percent carbon (C) was determined on dried sub-samples using a Carlo-Erba CHN analyzer and replicate analyses were pooled for monthly values (n = 21). To estimate root biomass turnover rate, we multiplied mean root biomass on an area basis (g m^{-2} to 20 cm) by the estimated mortality rate (d^{-1}) from the minirhizotron survival analysis. A similar calculation was made for root C mass turnover rate. Using all roots from the minirhizotron analyses, the overall median life span (d) represented the half-life of a negative exponential survival curve and the overall mortality rate (d^{-1}) was calculated as ln(2)/half-life. Mortality was re-expressed on a yearly basis (yr^{-1}), resulting in root biomass and C mass turnover rates in g m^{-2} yr^{-1} to a depth of 20 cm.

Root length dynamics

As a complement to the survival analysis of individual fine roots, the dynamics of root length were also examined. At each sampling time, the following dynamic metrics using root length were quantified: (1) standing crop was estimated from the length of all roots present (mm/tube); (2) production over the sampling interval was estimated from the length of newly appeared roots plus length extension of roots already present (mm/tube over the sampling interval); and (3) mortality over the sampling interval was estimated from the length of disappeared roots plus length decreases of roots already present (mm/tube over the sampling interval). Repeated-measures ANOVAs using PROC GLM in SAS (SAS Institute 1999) were performed to test for effects of elevation and sediment depth (between-subjects effects), time (within-subjects effect) and their interactions on root length standing crop, production and mortality. Greenhouse-Geisser corrections were used for time and time interaction tests to adjust for possible non-sphericity (Greenhouse & Geisser 1959).

RESULTS

Root characterization

A total of 204 individual roots were observed in the 12 minirhizotron tubes over the 18 sampling periods. Equal numbers of roots were observed at 0–10 cm and >10 cm depths (Table 1). The largest number of roots were first observed in the Apr–Jun cohort (Table 1); there was an obvious seasonal component to root emergence, with fewer new roots observed during the October–March time period. More roots were observed in intermediate elevation zones (Mid- and Upper bed) compared with Lower bed and

Table 1. Numbers of roots observed and results of tests for effect of seasonal cohort (time of first appearance), seagrass bed zone and root depth in sediment on survivorship curves. See 'Materials and methods' for specific details of statistical methods. Root depth was tested using a proportional hazards model (PROC PHREG), and had a significant effect on survival. Seasonal cohort and seagrass bed zone did not meet assumptions of constant proportional hazards over time, and were tested using PROC LIFETEST; they did not have significant effects on survival. The censored root refers to one that had not disappeared by the end of the study and thus had an indeterminate life span. NA: not applicable

Variable	p-value	Class	No. of roots, total = 203 (+1 censored)	Median life span in days (95% confidence limits)	Hazard ratio compared with reference class
Root depth in sediment	<0.0001 (chi-square)	0–10 cm >10 cm	101 (+1) 102	48 (44–51) 75 (57–95)	Reference 0.436
Seasonal cohort	0.5956 (log-rank) 0.3833 (Wilcoxon)	Jan – Mar Apr – Jun Jul – Sep Oct – Dec	11 84 65 43 (+1)	57 (35–92) 57.5 (56–66) 48 (48–75) 47 (47–104)	NA NA NA NA
Seagrass bed zone	0.7224 (log-rank) 0.6091 (Wilcoxon)	Lower bed Mid-bed Upper bed Above bed	46 67 69 (+1) 21	49.5 (29–58) 58 (51–75) 51 (48–75) 51 (47–76)	NA NA NA NA

Above bed zones (Table 1). It is important to note that *Zostera marina* generally produces adventitious roots off the rhizome, with no branching or lateral roots (Tomlinson 1982). Terrestrial ecologists classify roots based on diameter; fine roots are considered those <2 mm diameter (Johnson et al. 2000). All of the roots observed in the present study fell within the <2 mm 'fine root' classification. Overall, roots had a median width of 0.332 mm (5%ile = 0.196 mm, 95%ile = 1.145 mm). Roots at >10 cm depth in the sediment (median = 0.442 mm, 5%ile = 0.208 mm, 95%ile = 1.291 mm) had significantly greater widths (Wilcoxon rank-sum test p < 0.001) than those at 0–10 cm depth (median = 0.290 mm, 5%ile = 0.193 mm, 95%ile = 0.657 mm). Occurrence of roots within the sediment exhibited marked structure with a bimodal distribution (Fig. 2), with peak root numbers at 9 and 25 cm depth.
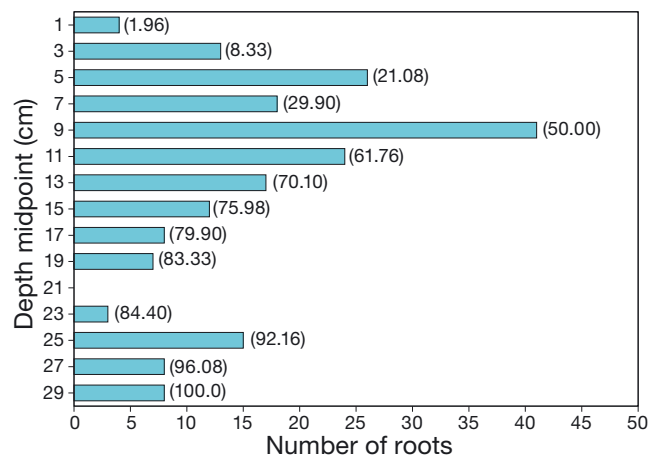

Fig. 2. Depth distribution of all roots (n = 204) observed during the 18 mo of sampling. Numbers in parentheses indicate cumulative percentage of roots observed with depth

Survival analysis

Of the 204 roots observed during the experiment, one was right-censored and life span could not be determined. Compared with shallow roots (0–10 cm), deeper roots (>10 cm) had a hazard ratio of 0.436, corresponding to a 56.4% decrease in mortality rate, which can clearly be seen in comparing the 2 survival curves (Table 1, Fig. 3). The median life span for deeper roots was 75 d compared with 48 d for shallower roots (Table 1), and one root lived for almost a year (Fig. 3). Calculated life spans based upon minirhizotron observations may be overestimated because of inability to determine whether an observed root is still living. When a *Zostera marina* fine root dies it rapidly loses soluble organic matter but root structural constituent decomposition is slow (Kenworthy & Thayer 1984). In contrast, neither seasonal cohort nor elevation zone had any significant effect on root survival (Table 1).

Root biomass and carbon turnover

Over the 21 mo core sampling period, average root biomass was 12.58 g m^{-2} to a depth of 20 cm. With an average 29.12% C concentration, this represents a stock of root carbon of 3.66 g C m^{-2} to 20 cm. Overall, median life span was 56 d, which corresponds to a mortality rate of 0.0124 d^{-1}. Root biomass turnover is therefore estimated to be 0.156 g m^{-2} d^{-1} or 56.9 g m^{-2} yr^{-1}, and root C mass turnover is estimated to be 0.045 g C m^{-2} d^{-1} or 16.6 g C m^{-2} yr^{-1}.
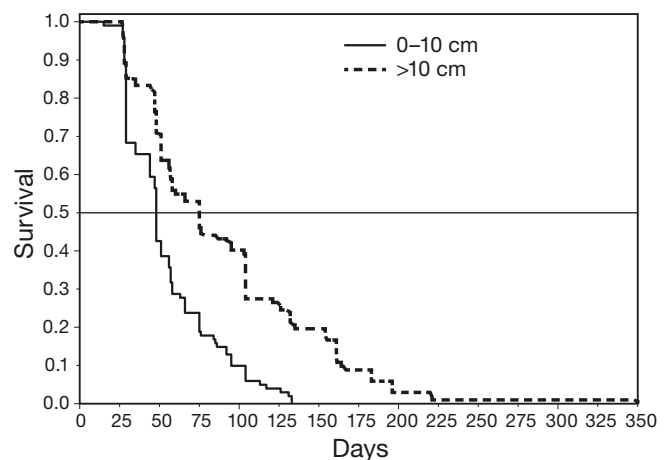

Fig. 3. Root survival (proportion of original cohort) over time as a function of 2 sediment depths: 0–10 cm and >10 cm. The number of days at 0.5 survival (horizontal line) indicates median life span

Root length dynamics

Fine root length standing crop fluctuated from one sampling period to the next, reflecting the balance between root length added (production) and root length that disappeared (mortality) between samples (Fig. 4). However, ANOVAs revealed no significant differences in fine root length standing crop, production or mortality due to time (18 sampling times), elevation zone (4 gradient positions), sediment depth (2 classes) or their interactions (Table 2), although the time effect was nearly significant (p = 0.056) for standing crop.
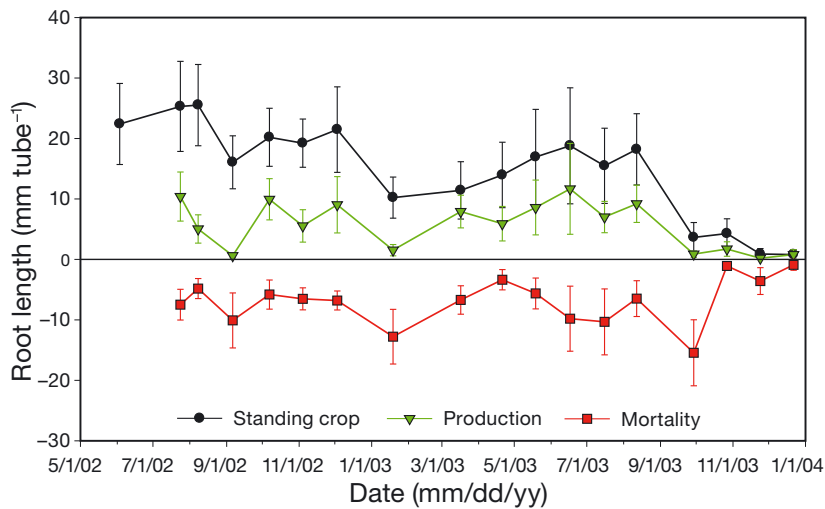
Fig. 4. Temporal dynamics of *Zostera marina* roots observed over monthly sampling interval (mm of root per minirhizotron tube): mean root standing crop, mean root production and mean root mortality rate. Error bars are ±1 SE

Table 2. Results of repeated-measures ANOVAs on fine root length standing crop, production and mortality over 18 sampling events, 4 seagrass bed zones (positions along the depth gradient within the *Zostera marina* bed) and 2 sediment depth classes (≤10 cm, >10 cm). p-values are for the *F*-test with Greenhouse-Geisser correction for time and time interaction tests

Dependent variable	Source of variation	df	p-value
Root standing crop	Elevation	3	0.58
(mm tube^{-1})	Depth	1	0.16
	Elevation × Depth	3	0.82
	Error (between subjects)	15	
	Time	17	0.056
	Time × Elevation	51	0.52
	Time × Depth	17	0.11
	Time × Elevation × Depth	51	0.96
	Error (within subjects)	255	
Root production	Elevation	3	0.46
(mm tube^{-1})	Depth	1	0.78
	Elevation × Depth	3	0.86
	Error (between subjects)	12	
	Time	16	0.19
	Time × Elevation	48	0.55
	Time × Depth	16	0.42
	Time × Elevation × Depth	48	0.99
	Error (within subjects)	192	
Root mortality	Elevation	3	0.38
(mm tube^{-1})	Depth	1	0.97
	Elevation × Depth	3	0.52
	Error (between subjects)	13	
	Time	16	0.16
	Time × Elevation	48	0.39
	Time × Depth	16	0.22
	Time × Elevation × Depth	48	0.88
	Error (within subjects)	208	

DISCUSSION

Root demographics

Analysis of over 200 roots showed distinct seasonal patterns, but overall, standing crop, production and mortality did not vary statistically with seabed elevation or sediment depth, consistent with our hypotheses. However, median root life span did vary significantly depending on where in the sediment profile the root was observed (Table 1). Our estimates of 48 and 75 d median root life span for roots in the top 10 cm of sediment and >10 cm sediment depth, respectively, indicate that root turnover is greater than 7 times per year in the 0–10 cm sediment depth, and less than 5 times per year in sediment deeper than 10 cm. At both depths, our results are within the range of root turnover estimates of 2–10 per year that Duarte et al. (1998) found for most seagrass species and sites that they reviewed. Differences in life span with sediment depth (Table 1) may be related to reduced bioturbation or decomposition at depth or may be an artifact of the inability to differentiate live and dead tissue from visual appearance. Our median estimates are also well aligned with rough projections developed from plastochrone intervals and the documented physiological integration of *Zostera marina* rhizome nodes. *Z. marina* roots are adventitious and develop from rhizome nodes, which are formed by leaf initiation at the meristem (Tomlinson 1982). Consequently, the leaf plastochrone is the same as the rhizome plastochrone, with new leaves initiated about every 14 d (Kaldy 2006). Kraemer & Alberte (1993) showed that rhizome metabolism decreases with increasing node rank (i.e. older tissue) and was almost negligible at node rank 10, suggesting that roots might be expected to be metabolically active (i.e. alive) for up to 140 d. Our calculated median values are well within this range. The presence of a single root after 1 yr is likely a methodological artifact or indicates the inability of this method to definitively distinguish between live and dead seagrass roots.

The time between tube installation and image collection in this study was approximately 1 mo. Joslin & Wolfe (1999) and Johnson et al. (2001) recommend a longer waiting period to avoid

observing potential root growth artifacts due to tube installation. Seasonal variation was observed, but there were no readily apparent qualitative differences in production and mortality in the second year versus the first. Consequently, the early root data were not excluded. The 18 mo sampling period was likely sufficient time for all existing roots at the time of tube installation to have completed their life span, as well as for new cohorts of roots to complete their life cycle. Perhaps new rhizome and root growth was primarily into new areas rather than backfilling the areas recently vacated. This is consistent with observations that ramets can have life spans of 2 yr or less (Olesen & Sand-Jensen 1994).

New root production varied by season, with the greatest flush of new roots occurring in April through June, and the least occurring January through March (Table 1). These patterns are generally consistent with previous observations of seasonal lateral shoot formation (Olesen & Sand-Jensen 1994). Ruesink et al. (2010) observed a ~50% increase in shoot density during February attributed to recent branching. The subsequent development of roots along rhizome nodes could easily account for the observed numbers of new roots. Likewise, new root production during summer was also elevated (Table 1) and may be associated with high rhizome branching rates (~30%; Ruesink et al. 2010, Olesen & Sand-Jensen 1994) or seasonal growth patterns (Kaldy & Lee 2007). The flush of new roots is clearly correlated with seasonal light availability and thermal patterns in the region and likely reflects plant response to these drivers.

Observation of individual root mortality combined with core samples of roots allowed estimation of *Z. marina* root biomass turnover and its associated carbon content. Although relatively little information is available on belowground dynamics in seagrasses, Duarte et al. (1998) calculated root turnover as production/biomass, essentially equating production and turnover under the assumption of steady-state biomass. Their estimates of root biomass production and turnover rates varied from 210 to 1427 g m^{-2} yr^{-1}. Our estimate of 57 g m^{-2} yr^{-1} is considerably lower, but is consistent with the values of 55−182 g C m^{-2} yr^{-1} estimated by Kenworthy & Thayer (1984) for a warm temperate system. Duarte et al. (1998) reviewed data from 3 tropical and 1 warm temperate system, in contrast to our cool temperate system, where water temperatures are 9−12°C year-round, and the system can be light-limited with annual underwater irradiance of only 1200 mol photons m^{-2} yr^{-1} (Kaldy & Lee 2007). *Z. marina* shoot density in monotypic stands in Yaquina Bay (301 shoots m^{-2}) is also much lower than total sea-grass shoot densities (800 to 4449 shoots m^{-2}) across multiple species reported in Duarte et al. (1998). Likewise, the allocation of belowground biomass only accounts for 20−40% of total biomass for *Z. marina* in Yaquina Bay (Kaldy & Lee 2007, Kaldy 2012), which is lower than many species examined by Duarte et al. (1998). Methodological differences also likely contributed to differences in estimates, such as core sampling (our 20 cm depth versus their 35 cm depth), and our direct minirhizotron observation of root birth and death versus their calculation of root production based on allometric relationships with rhizomes.

Minirhizotron application

Minirhizotrons provide a nondestructive, *in situ* method for viewing roots and are one of the best tools available for directly studying roots. They permit the simultaneous measurement of fine root production and disappearance, which cannot be accomplished using coring or in-growth cores (Majdi 1996). Minirhizotron techniques have improved significantly since they were first proposed (Bates 1937) and are widely utilized to study the dynamics and functions for fine roots in agricultural and natural plant communities (e.g. Taylor 1987, Hendrick & Pregitzer 1996). Their greatest strength is their ability to monitor (from birth to death) specific root segments at frequent time intervals without significantly impacting fine root processes. They can be used to characterize fine root production, phenology, growth, mortality and life span, and are useful in developing ecosystem carbon budgets (Hendrick & Pregitzer 1996, Majdi 1996).

The system used to anchor the minirhizotrons was effective against large tidal changes characteristic of Yaquina Bay, often in excess of 2 m. The use of only one minirhizotron tube was lost during the 2 yr study. This loss was likely due to physical perturbation rather than to tides. In other systems with less tidal influence, deployment of this technology will be even easier. The means of plugging the tubes was only partly effective as water was often found in tubes after the roughly 60−90 tidal changes that occurred between sample intervals. Nonetheless, tubes remained clear and did not foul below the sediment line, allowing root and rhizome image collection throughout the study period.

The estimates of root life span and growth rates generated with minirhizotrons will be useful for making more accurate estimates of blue carbon contributions. Although significant logistical obstacles remain (e.g. use in subtidal beds), development of a non-destructive sampling technique for seagrass

belowground (i.e. within sediment) dynamics will provide better insight into seagrass root–sediment interactions that cannot be captured using traditional destructive sampling methods. The importance of belowground dynamics and interactions to seagrass ecosystem health and structure are generally under-represented in the literature, even though they are widely acknowledged as a critical component of the system (Hemminga & Duarte 2000).

Better quantitative estimates of root dynamics also will likely enhance seagrass modelling capabilities. For example, the relationship between sediment sulfide concentration and seagrass toxicity is not well understood, in part because of complex sediment chemistry (Hasler-Sheetal & Holmer 2015) and inter-action with anatomical and physiological adaptations of the plants. A better understanding of root dynamics (e.g. production and mortality) may facilitate the capacity to accurately model belowground biogeo-chemical processes. It is well established that sea-grass exudates and detritus influence sediment bacterial remineralization rates (Boschker et al. 2000, Holmer et al. 2001, Kaldy et al. 2006), which can, under some conditions, lead to toxic sulfide conditions (Terrados et al. 1999, Welsh 2000).

To date, there have been very few studies tracking belowground dynamics in marine seagrass beds, in part due to challenges of studying belowground dynamics in the relatively harsh conditions of an estuary with macro-tidal swings. We demonstrated that root minirhizotrons, which were initially developed for use in terrestrial soils, are also effective for tracking root production, growth, turnover and life span in intertidal beds of *Z. marina*. This information is useful for understanding not only dynamics of root growth in relation to aboveground productivity, but also how these systems respond to stress. Root minirhizotrons could be coupled with rhizotron windows (Egli & Kälin 1990) to assess sub-tidal dynamics; however, our results across a depth profile should provide reasonable estimates for submerged portions of the bed. There are numerous opportunities to expand the scope of information obtained using minirhizotrons in estuarine systems. For example, in terrestrial systems mini-rhizotrons have been used to help elucidate competitive interactions among roots; to track root associations with symbionts such as bacteria and fungi (Rygiewicz et al. 1997); and, coupled with stable isotope assessments (Hobbie et al. 2001), to identify carbohydrate partitioning within and among roots in a community (Hobbie et al. 2002, Andersen et al. 2010). In addition, it may be possible to couple root minirhizotrons with planar optodes (Frederiksen & Glud 2006) or other de-vices to track sediment chemistry. The information presented here, coupled with other approaches to focus on more refined aspects of belowground dynamics, will help modelers and others interested in assessing susceptibility of these systems to large-scale stresses such as nutrient loading and climate change.

LITERATURE CITED

Allison PD (1995) Survival analysis using the SAS system: a practical guide. SAS Institute, Cary, NC

Andersen CP, Ritter W, Gregg J, Matyssek R, Grams TEE (2010) Below-ground carbon allocation in mature beech and spruce trees following long-term, experimentally enhanced O_3 exposure in southern Germany. Environ Poll 158:2604–2609

Baddeley JA, Watson CA (2005) Influences of root diameter, tree age, soil depth and season on fine root survivorship in *Prunus avium*. Plant Soil 276:15–22

Bates GH (1937) A device for the observation of root growth in the soil. Nature 139:966–967

Boschker HTS, Wielemaker A, Schaub BEM, Holmer M (2000) Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* spp. meadows. Mar Ecol Prog Ser 203:181–189

Brown CA, Ozretich RJ (2009) Coupling between the coastal ocean and Yaquina Bay, Oregon: importance of oceanic inputs relative to other nitrogen sources. Estuaries Coasts 32:219–237

Cox DR (1972) Regression models and life tables (with discussion). J R Stat Soc B B34:187–220

Devereux R (2005) Seagrass rhizosphere microbial communities. In: Kristensen E, Haese RR, Kostka JE (eds) Interactions between macro-and microorganisms in marine sediments. American Geophysical Union, Washington, DC, p 199–216

Duarte CM, Merino M, Agawin NSR, Uri J and others (1998) Root production and belowground seagrass biomass. Mar Ecol Prog Ser 171:97–108

Egli S, Kälin I (1990) The root window — a technique for observing mycorrhizae on living trees. Agric Ecosyst Environ 28:107–110

Enslin WR, Machemer P, Hendrick RL, Pregitzer KS (1994)

MSU ROOTs Manual—Version 1.5. Center for Remote Sensing, Michigan State University, East Lansing, MI

Fourqurean JW, Duarte CM, Kennedy H, Marba N and others (2012) Seagrass ecosystems as a globally significant carbon stock. Nat Geosci 5:505–509

Frederiksen MS, Glud RN (2006) Oxygen dynamics in the rhizosphere of *Zostera marina*: a two-dimensional planar optode study. Limnol Oceanogr 51:1072–1083

Greenhouse SW, Geisser S (1959) On methods in the analysis of profile data. Psychometrika 24:95–112

Hasler-Sheetal H, Holmer M (2015) Sulfide intrusion and detoxification in the seagrass *Zostera marina*. PLOS ONE 10:e0129136

Hauxwell J, Cebrian J, Herrera-Silveira JA, Ramirez J, Zaldivar A, Gomez N, Aranda-Cirerol N (2001) Measuring production of *Halodule wrightii*: additional evidence suggests clipping underestimates growth rate. Aquat Bot 69:41–54

Hemminga MA, Duarte CM (2000) Seagrass ecology. Cambridge University Press, Cambridge, UK

Hendrick RL, Pregitzer KS (1996) Applications of minirhizotrons to understand root function in forests and other natural ecosystems. Plant Soil 185:293–304

Hobbie EA, Olszyk DM, Rygiewicz PT, Tingey DT, Johnson MG (2001) Foliar nitrogen concentrations and natural abundance of ^{15}N suggest nitrogen allocation patterns of Douglas-fir and mycorrhizal fungi during development in elevated carbon dioxide concentration and temperature. Tree Physiol 21:1113–1122

Hobbie EA, Tingey DT, Rygiewicz PT, Johnson MG, Olszyk DM (2002) Contributions of current year photosynthate to fine roots estimated using a C-depleted CO_2 source. Plant Soil 247:233–242

Holmer M, Andersen FO, Nielsen SL, Boschker HTS (2001) The importance of mineralization based on sulfate reductions for nutrient regeneration in tropical seagrass sediments. Aquat Bot 71:1–17

Johnson MG, Meyer P (1998) Mechanical advancing handle that simplifies minirhizotron camera registration and image collection. J Environ Qual 27:710–714

Johnson MG, Phillips DL, Tingey DT, Storm MJ (2000) Effects of elevated CO_2, N-fertilization, and season on survival of ponderosa pine fine roots. Can J Res 30:220–228

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. Environ Exp Bot 45:263–289

Joslin JD, Wolfe MJ (1999) Disturbances during minirhizotron installation can affect root observation data. Soil Sci Soc Am J 63:218–221

Kaldy JE (2006) Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (*Zostera marina*) sink in a temperate estuary? Mar Pollut Bull 52:342–353

Kaldy JE (2012) Influence of light, temperature and salinity on dissolved organic carbon exudation rates in *Zostera marina* L. Aquat Biosyst 8:19

Kaldy JE, Dunton KH (2000) Above- and belowground production, biomass and reproductive ecology of *Thalassia testudinum* (turtle grass) in a subtropical coastal lagoon. Mar Ecol Prog Ser 193:271–283

Kaldy JE, Lee KS (2007) Factors controlling *Zostera marina* L. growth in the eastern and western Pacific Ocean: comparison between Korea and Oregon, USA. Aquat Bot 87:116–126

Kaldy JE, Eldridge PM, Cifuentes LA, Jones WB (2006) Utilization of DOC from seagrass rhizomes by sediment bacteria: ^{13}C-tracer experiments and modeling. Mar Ecol Prog Ser 317:41–55

Kaldy JE, Brown CA, Andersen CP (2013) *In situ* ^{13}C tracer experiments elucidate carbon translocation rates and allocation patterns in eelgrass *Zostera marina*. Mar Ecol Prog Ser 487:27–39

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat Soc 53:457–481

Kenworthy WJ, Thayer GW (1984) Production and decomposition of the roots and rhizomes of seagrasses *Zostera marina* and *Thalassia testudinum* in temperate and subtropical marine ecosystems. Bull Mar Sci 35:364–379

Kowalski JL, DeYoe HR, Allison TC, Kaldy JE (2001) Productivity estimation in *Halodule wrightii*: comparison of leaf-clipping and leaf-marking techniques and the importance of clip height. Mar Ecol Prog Ser 220:131–136

Kraemer GP, Alberte RS (1993) Age-related patterns of metabolism and biomass in subterranean tissues of *Zostera marina* (eelgrass). Mar Ecol Prog Ser 95:193–203

Majdi H (1996) Root sampling methods—applications and limitations of minirhizotron technique. Plant Soil 185:255–258

Olesen B, Sand-Jensen K (1994) Demography of shallow eelgrass (*Zostera marina*) populations—shoot dynamics and biomass development. J Ecol 82:379–390

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM and others (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996

Phillips DL, Johnson MG, Tingey DT, Catricala CE, Hoyman TL, Nowak RS (2006a) Effects of elevated CO_2 on fine root dynamics in a Mojave Desert community: a FACE study. Glob Change Biol 12:61–73

Phillips DL, Johnson MG, Tingey DT, Storm MJ, Ball JT, Johnson DW (2006b) CO_2 and N-fertilization effects on fine root length, production, and mortality: a 4-year ponderosa pine study. Oecologia 148:517–525

Phillips DL, Johnson MG, Tingey DT, Storm MJ (2009) Elevated CO_2 and O_3 effects on fine-root survivorship in ponderosa pine mesocosms. Oecologia 160:827–837

Ruesink JL, Hong JS, Wiseheart L, Hacker SD, Dumbauld BR, Hessing-Lewis M, Trimble AC (2010) Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Biol Invasions 12:1773–1789

Rygiewicz PT, Johnson MG, Ganio LM, Tingey DT, Storm MJ (1997) Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels. Plant Soil 189:275–287

SAS Institute (1999) SAS/Stat User's Guide, Version 8. SAS Institute, Cary, NC

Short FT, Duarte CM (2001) Methods for the measurement of seagrass growth and production. In: Short FT, Coles RG (eds) Global seagrass research methods. Elsevier Science Amsterdam, p 155–182

Taylor HM (1987) Minirhizotron tubes: methods and applications for measuring rhizosphere dynamics. Book ASA Spec Publ 50. American Society of Agronomy, Madison, WI

Terrados J, Duarte CM, Kamp-Nielsen L, Agawin NSR and others (1999) Are seagrass growth and survival constrained by reducing conditions of the sediment? Aquat Bot 65:175–197

Tomlinson PB (1982) Anatomy of the Monocotyledons. VII. Helobiae (Alismatidae). Oxford University Press, NY

Touchette BW, Burkholder JM (2000) Overview of the physiological ecology of carbon metabolism in seagrasses. J Exp Mar Biol Ecol 250:169–205

Welsh DT (2000) Nitrogen fixation in seagrass meadows: regulation, plant–bacteria interactions and significance to primary productivity. Ecol Lett 3:58–71