New Phytologist

Research

Life span and structure of ephemeral root modules of different functional groups from a desert system

Bo Liu[1,2], Junxia He[1,2], Fanjiang Zeng[1,2,3], Jiaqiang Lei[1,2] and Stefan K. Arndt[4]

[1]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China; [2]Cele National Station of Observation and Research for Desert Grassland Ecosystem in Xinjiang, Cele, 848300 Xinjiang, China; [3]Key Laboratory of Biogeography and Bioresource in Arid Land, Chinese Academy of Sciences, Urumqi 830011, China; [4]School of Ecosystem and Forest Sciences, University of Melbourne, 500 Yarra Boulevard, 3121 Richmond, Vic, Australia

Author for correspondence:
Fanjiang Zeng
Tel: +86 0991 7885442
Email: zengfj@ms.xjb.ac.cn

Received: *15 October 2015*
Accepted: *23 December 2015*

New Phytologist (2016)
doi: 10.1111/nph.13880

Key words: anatomical traits, ephemeral root module, fine roots, functional groups, life span, modified rhizotron, nitrogen concentration, respiration.

Summary

- The terminal branch orders of plant root systems have been proposed as short-lived 'ephemeral' modules specialized for resource absorption. The occurrence of ephemeral root modules has so far only been reported for a temperate tree species and it is unclear if the concept also applies to other woody (shrub, tree) and herb species.
- Fine roots of 12 perennial dicotyledonous herb, shrub and tree species were monitored for two growing seasons using a branch-order classification, sequential sampling and rhizotrons in the Taklamakan desert.
- Two root modules existed in all three plant functional groups. Among the first five branch orders, the first two (perennial herbs, shrubs) or three (trees) root orders were ephemeral and had a primary anatomical structure, high nitrogen (N) concentrations, high respiration rates and very short life spans of 1–4 months, whereas the last two branch orders in all functional groups were perennial, with thicker diameters, no or collapsed cortex, distinct secondary growth, low N concentrations, low respiration rates, but much longer life spans.
- Ephemeral, short-lived root modules and long-lived, persistent root modules seem to be a general feature across many plant functional groups and could represent a basic root system design.

Introduction

Plants acquire water and nutrients through the root system and the bulk of this uptake is through fine roots, which has historically been defined as all roots < 2 mm in diameter. To achieve this uptake function, plants invest substantial amounts of carbon to build the fine roots, and up to 30% of the global annual terrestrial net primary production (NPP) may be devoted to fine root production, if assuming roots turn over once per year (Jackson *et al.*, 1997).

However, it has increasingly been recognized that the traditional fine root concept is deeply flawed (Pregitzer, 2002; McCormack *et al.*, 2015). Smaller diameter roots in trees had much shorter life spans than larger roots (Wells & Eissenstat, 2001; King *et al.*, 2002), which implied that the life expectancy of an individual root is related to its position on the branching root system, contrary to the fine root concept in which all individual roots have the same life span (Pregitzer, 2002). The link between branch order and root anatomy was further explored by several studies (Withington *et al.*, 2006; Guo *et al.*, 2008a), which established a potential relationship between root branch order and physiological function. As such, treating all fine roots (≤ 2 mm) as those that turn over rapidly is an oversimplification (Guo *et al.*, 2008b; Espeleta *et al.*, 2009) and we now need more sophisticated approaches to understand root turnover (Lynch *et al.*, 2013; Warren *et al.*, 2015). One approach is to separate roots into groups that differ in both function and turnover rates, such as the ephemeral root module approach proposed by Xia *et al.* (2010). In this approach, Xia *et al.* (2010) studied demography and physiology of different root branch orders of a temperate tree species and demonstrated that the three most distal branch orders of the root system operated like modular units. These distal root branch orders had similar life spans, but much shorter life spans (1–2 yr) as a group than the higher-order woody roots (multiple years) (Xia *et al.*, 2010). Additionally, these distal branch orders displayed similar nitrogen (N) concentrations and respiration rates (Xia *et al.*, 2010). Because the distal branch orders turned over frequently as intact lateral branches on their higher-order mother roots, the authors termed these structures ephemeral root modules.

It is possible that this modular organization of root system structure and function is widespread in perennial plants, but this has not been widely tested. There are similarities in the relationship between anatomy and branching architecture across the woody species examined so far, but these species were either temperate tree species (Guo *et al.*, 2008a) or a temperate shrub (Valenzuela-Estrada *et al.*, 2008). We still know very little about the structure–function relationship and about the form and

longevity of the ephemeral root modules (if they do exist) in perennial plants of other biomes and plant functional groups.

The investigation of root demography is methodologically challenging. One method used frequently is minirhizotrons. Minirhizotrons can be used to directly monitor the growth and deaths of fine roots (Yuan & Chen, 2012). The widespread use of minirhizotrons has greatly improved our understanding of the production and mortality processes of fine roots in various ecosystems such as grasslands, forests, farmlands and deserts (McCormack *et al.*, 2013; Taylor *et al.*, 2014), but their small viewing windows limit the ability to observe higher-order roots, some of which may be larger than the viewing area (typically *c.* 1.3 × 2.0 cm). Isotopic methods are also frequently applied but are very costly, among other limitations (Guo *et al.*, 2008b; Strand *et al.*, 2008). One method that might work well involves large rhizotrons in combination with sequential sampling of a root cohort. This method is inexpensive and allows observations of thousands of individual roots for several years and thus allows direct observations of the full development of fine root systems for small-statured plants (Xia *et al.*, 2010).

The motivation for the current study was to test if ephemeral root modules also exist in different functional groups of perennial plants in a desert biome. Water availability is an important factor that could have both positive and negative effects on root life span (Eissenstat *et al.*, 2013). Deserts cover large areas of the planet and contain a vast array of different plant functional groups that display different ecological strategies and might therefore also exhibit different root demographics. Trees and shrubs in deserts maintain above-ground biomass throughout the year, whereas herbaceous perennials maintain their root system but grow a new shoot every year. It is therefore possible that perennial desert species also exhibit different strategies with regard to their root demography. Thus, our main objectives for this study were to test if ephemeral root modules exist in trees, shrubs and perennial herbs of a desert ecosystem in the Taklamakan desert; to assess if root life spans of ephemeral roots were different across these different functional groups; and to evaluate whether ephemeral root modules had similar structural and physiological traits (diameter, tissue density, stele: diameter ratio, N concentration and respiration) in comparison to the perennial roots in these three plant functional groups.

Materials and Methods

Study site and species selection

The study site was located at the Cele National Station of Observation and Research for Desert and Grassland Ecosystem (Cele Station; 37°00′56.37″N, 80°43′43.81″E) at the southern fringe of the Taklamakan desert in the Xinjiang Urumqi Autonomous Region of China. Cele oasis is surrounded by a 5–10 km belt of sparse vegetation dominated by phreatophytic species (Bruelheide *et al.*, 2003, 2010). The study site has an elevation of 1366 m above sea level (asl), a mean annual temperature of 11.9°C, a mean annual potential evaporation of *c.* 2600 mm, and a mean annual precipitation of only 35 mm with rare and unpredictable rainfall events (Gui *et al.*, 2013). The climate is thus hyperarid, with hot and dry summers and cold and dry winters, and the growing season typically lasts from April to October. The depth to the groundwater in the study area was 15 m, which fluctuated very little over time. The soils are sandy with low organic matter content and well drained (Arndt *et al.*, 2004). Soil organic matter ranged from 0.24 to 0.48 g kg^{-1} in the top 60 cm. More details of soil properties and study site information can be found in Liu *et al.* (2013).

We selected 12 different plant species from three different plant functional groups that occur in the region (Table 1). The 12 dominant perennial species covered four herbaceous dicots (herbs), four shrubs and four trees from nine families and 10 genera. *Populus alba* var. *pyramidalis* and *Morus alba* L. are two tree species that are grown for commercial reasons in the oasis, and *Populus euphratica* Oliv. and *Eleagnus angustifolia* L. are native trees in the Taklamakan desert. The four shrubs and the four herbaceous perennials were all part of the native vegetation of the plant shelter belt surrounding the oasis. The shrubs may grow up to 2–3 m in height and at various diameters. The leaves of the shrubs are shed in October but the above-ground woody parts persist through the winter. The four herbaceous perennials grow a new nonwoody shoot every April that persists until the onset of autumn in October.

Establishment of rhizotrons

At the beginning of May 2010, we built 12 square concrete pods, with dimension of 3 × 3 × 1 m (length × width × depth). Each concrete pod contained four rhizotron boxes of 1 m^3 in each of the four corners of the pod, and each rhizotron box had two viewing windows (Supporting Information Figs S1, S2). The key steps in building rhizotrons are shown in Figs S3–S7. The specific procedures are detailed in the following.

A level field *c.* 100 m south of Cele Station was selected for this study. The soils within an area of 9 × 22 m were dug to the depth of 1 m. The excavated soil was stacked in layers (20 cm per layer), and any live roots in the soil were removed to ensure that roots scanned were of the target species. On the flat surface of the

Table 1 Taxonomic list of 12 perennial herb, shrub and tree species in this study

Functional group	Species	Family
Herbs	*Alhagi sparsifolia* Shap.	Leguminosae
	Apocynum venetum L.	Apocynaceae
	Scorzonera divaricata Turcz	Asteraceae
	Karelinia caspica (Pall.) Less.	Asteraceae
Shrubs	*Calligonum caput-medusae* Schrenk	Polygonaceae
	Calligonum roborovskii A. Los.	Polygonaceae
	Tamarix ramosissima Lebed.	Tamaricaceae
	Caragana microphylla Lam.	Leguminosae
Trees	*Populus euphratica* Oliv.	Salicaceae
	Populus alba var. *pyramidalis*	Salicaceae
	Elaeagnus angustifolia L.	Elaeagnaceae
	Morus alba L.	Moraceae

excavated area, 12 square pods were built with four walls made of concrete with a strength grade of C20 (compressive strength 20 N mm^{-2}). The area of each pod was 3 × 3 m, with the depth of 1 m and the wall width of 20 cm.

When the concrete pods were built, square steel frames of 1.02 × 1.02 m were welded together and contained toughened glass. Each of the two frames were welded vertically at the edge of frames and fixed on the concrete wall with setscrews. An open-top box structure of 1.02 × 1.02 × 1.02 m was formed by the iron frame and concrete wall. After the toughened glass was installed (Fig. S3), the soil was backfilled according to the original layers (Fig. S4). The same glass was installed on the four sides of each pod. The steel frames were painted white to minimize solar heating (Fig. S5). Curtains were installed outside the rhizotron in order to shelter against the sunshine. We also installed polystyrene boards with a thickness of 10 cm (density = 15 kg m^{-3}) close to each curtain (Fig. S6). One 2-yr-old seedling that was grown from seed and raised in pots was transplanted into each rhizotron box (Fig. S7) on 30 May 2010. We planted a total of 12 species, and each species had four replicates. All 48 rhizotron boxes were watered by sprinklers at a rate of 0.1 m^3 m^{-2} $month^{-1}$ during the growing season from April to October. The quantity of water was measured by a flow meter that was attached to a water tap. Any emerging weeds were removed within 2 d. Clear root hairs and different orders of roots emerged on the glass windows of the rhizotrons *c.* 50 d after the plants were transplanted (Figs S8–S10).

Collection and analysis of root images

From April 2011 to October 2012, all root images of the 96 rhizotron windows were collected by a flat scanner (FileScan 1600xl plus; Microtek, Shanghai, China) of A3 size at intervals of 13–46 d (15 d mostly) during the growing season (April–October). Scanning usually began at *c.* 21:00 h at night and lasted for *c.* 10 h, which helped to reduce the impact of sunlight on the roots in the rhizotrons. The root images were scanned a total of 21 times during the observation period.

The roots on the collected images were classified into different orders, as described by Pregitzer *et al.* (2002), and analyzed using root analysis software (DIGIROOT 2.6; Digital Processing Solutions, Melville, NY, USA) to track the life histories of individual roots of the first four or five orders. The life spans of individual roots were defined as the time elapsed from the first appearance to death. A root was considered dead when it turned black, shriveled up or disappeared (Wells & Eissenstat, 2001; Matamala *et al.*, 2003), and these visual cues were subsequently confirmed by root anatomy and vital staining (stains in 0.05–0.25% Evans blue aqueous solution for 5 min, living cells remain unstained, dead cells become blue; de Neergaard *et al.*, 2000).

Measurements of root anatomical trait, N concentration and respiration

We established an additional 12 plots close to the rhizotron zone for destructive harvesting on 30 May 2010. The plot size for each species was 10 × 10 m and we planted 25 individuals of each species with individuals 2 m apart from each other. We also established a 3 m buffer zone between plots. The transplanted seedlings had the same age and were subjected to the same irrigation scheme as those established in the rhizotrons. The roots for anatomical traits, N concentrations and respiration analyses were sampled at those additional 12 plots at the same time the root scans were taken, a total of 21 times. We sampled roots only on 20 July 2012 for the anatomical analysis in this study considering that root diameter, cortex thickness and stele diameter showed strong phylogenetic signal across species (Kong *et al.*, 2014). Once sampled, roots were brushed free of adhering soil and organic matter, and each root sample was divided into two subsamples. One was gently washed in deionized water and immediately fixed in formalin-aceto-alcohol solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid and 5 ml of 37% methanol) for anatomical analysis. The other was immediately put in a plastic bag and transported to a 4°C refrigerator in the laboratory, where respiration measurements were performed within 2 h (Pregitzer *et al.*, 1998; Xia *et al.*, 2010; Lynch *et al.*, 2013). In the laboratory, roots were divided by different orders, using tweezers and scissors as described by Pregitzer *et al.* (2002), and thoroughly rinsed in deionized water. Roots were carefully examined to be soil-free by visual inspection (Polverigiani *et al.*, 2011; Chen *et al.*, 2013). Subsamples of at least 0.5 g (FW) were prepared for respiration measurements (Xia *et al.*, 2010; Jia *et al.*, 2013). Root respiration was measured until a steady slope displaying the O_2 consumption rate could be identified at 24°C using liquid-phase O_2 electrode (Oxytherm, Hansatech, Norfolk, UK; Clark *et al.*, 2010). Following respiration measurements, root subsamples were dried in an oven (70°C, 48 h) and weighed, then transported to the Elementary Analysis Laboratory at Xi'an where measurements of N concentration were carried out using an elemental analyzer (Vario Micro Cube; Elementar, Hanau, Germany).

Data analysis

A total of 43 435 individual lateral roots were tracked across > 8000 A3 size images (*c.* 700 images per species, 300 dpi), and the detailed information of observed roots for each species is shown in Table S1. Roots with unclear visual images (*c.* 2.6% of the total number) were not included in this analysis. The detailed survival probability of 12 species is shown in Fig. S11.

A Kaplan–Meier model was used for survivorship analysis, from which the median life span was estimated (Kaplan & Meier, 1958; Majdi *et al.*, 2001). The log-rank test was used to compare the survival distributions of different branch orders. Differences in root diameter, secondary growth rate and cortex thickness were analyzed using one-way ANOVA with Dunnett's test for multiple comparisons, and by a nonparametric test (Kruskal–Wallis test) when the normal assumption was not met. Pearson correlation coefficients were calculated for N concentration and respiration among different branch orders. All statistical analyses were performed using SPSS software (v.20.0; SPSS Inc., Chicago, IL, USA).

Results

Root median life span

The root branch orders of herbs, shrubs and trees displayed fundamental differences in traits. The first two root orders of all herb and shrub species showed the traits of ephemeral roots (short life span, no secondary growth; Fig. S12; Table 2), whereas root orders three and four displayed the traits of perennial roots (longer life span, secondary growth, no cortex; Fig. S12; Table 2). By contrast, the tree species all had at least five root orders, with the first three orders displaying traits of ephemeral root and the last two orders displaying perennial root characteristics.

The first two root orders in herbs and shrubs were similar in their mortality patterns (Fig. 1). The cumulative survival rate of first- and second-order roots decreased rapidly with increasing time; 98% of these roots died by the end of the study (Fig. 1). The first three orders of roots in trees were similar in their mortality patterns (Fig. 1). The cumulative survival rate of first-, second-, and third-order roots decreased rapidly with increasing time, and 95% of these roots died at the end of the study (Fig. 1). The median life span for the first two orders of roots of herbs was 31–48 d, that of shrubs was 76–116 d and that of the first three orders of roots of trees was 95–154 d (Fig. 1; Table 2).

By contrast, the third- and fourth-order roots in herbs were similar in their mortality patterns. They showed a much higher cumulative survival rate than roots of the first two orders in all herb species (Fig. 1). However, the cumulative survival rate of third- and fourth-order roots also declined sharply with time; 93% of these roots died at the end of the study (Figs 1, S11), and the median life spans were between 188–423 d. The third- and fourth-order roots in shrubs were similar in their mortality patterns; they consistently had a much higher cumulative survival rate than roots of the first two orders (Figs 1, S11). The cumulative survival rate of third- and fourth-order roots of shrub species declined moderately with time and only 5% of these roots died in the end of the study (Fig. 1); the median life spans were > 550 d. A similar situation was observed in trees, where the fourth- and fifth-order of roots were also very similar in their mortality patterns: they consistently showed a much higher cumulative survival rate than roots of the first three orders (Fig. 1). By the end of this study, the mortality rate of two higher-order root classes was $< 6\%$; as a consequence, the median life spans were > 550 d (Table 2).

Root anatomical traits

The mean root diameter increased significantly with root order in all species, but herbaceous perennials overall had thicker roots compared with shrub and tree species (Table 2; Figs S12, S13). The mean root diameter of the first- to fourth-order roots of herbs was between 0.75 ± 0.07 and 3.95 ± 0.33 mm, that of shrubs was between 0.25 ± 0.10 and 1.35 ± 0.13 mm, and that of the first- to fifth-order roots of trees was between 0.36 ± 0.17 and 1.27 ± 0.27 mm (Table 2; Fig. S12). We observed similarities in anatomical structures in the first two orders of roots of herbs and shrubs (Figs S12, S13). Both branch orders maintained primary structure and cortex, without secondary growth and continuous cork layer. By contrast, the third- and fourth-order roots of herbs and shrubs developed secondary structure and continuous cork layer, and lacked cortex. In trees we also observed primary structure and cortex in the first two root orders of trees, and found no secondary growth and continuous cork layer. Cork layer, cortex, secondary growth and continuous cork layer existed in the third-order roots, and obvious secondary structure, secondary growth and continuous cork layer existed in the fourth- and fifth-order roots in trees (Table 2; Fig. S12).

The root diameter of ephemeral root modules was negatively correlated to root life span in all three plant functional groups ($R^2 = 0.39$, $P < 0.05$; Fig. 2). The variation across functional groups was higher than the variation of root diameter within a group (Table S2). By contrast, root tissue density of ephemeral root modules was positively related to life span (herbs, $R^2 = 0.56$; shrubs, $R^2 = 0.74$; trees, $R^2 = 0.64$; total species, $R^2 = 0.69$, Fig. 3). The regression analysis showed that tissue density, diameter, functional group accounted for 43%, 39% and 14% of total variation in median life span, respectively, while tissue N explained the residual variation (0.71%) (Table S2).

Root N concentration and respiration

The mean N concentration declined significantly with increasing root branch orders of herbs, shrubs and trees (Fig. 4); however, the trend of decline was not consistent. In herbs and shrubs, the N concentration of the first two root orders was significantly higher than that of the third and fourth orders. In trees, the N concentration of the first three orders of roots was significantly higher than the fourth and fifth orders. The mean N concentration of nonwoody distal lateral branch orders of herbs was 22.85 ± 0.89 g kg^{-1}, that of shrubs as 19.00 ± 0.36 g kg^{-1}, and that of trees was 21.37 ± 0.51 g kg^{-1} (Fig. 4; Table 2). The mean N concentration of woody distal lateral branch orders of herbs was 11.76 ± 0.41 g kg^{-1}, that of shrubs was 10.66 ± 0.21 g kg^{-1}, and that of trees was 12.10 ± 0.27 g kg^{-1} (Fig. 4; Table 2).

Similar to N concentration, the mean respiration of roots declined significantly with increasing branch order of herbs, shrubs, and trees (Fig. 4); however, the trend of decline was not consistent. In herbs and shrubs, the respiration of the first two orders of roots was significantly higher than that of the third and fourth orders. In trees, the respiration of the first three orders of roots was significantly higher than that of the fourth and fifth orders. The mean respiration of nonwoody distal lateral branch orders of herbs was 47.87 ± 3.97 nmol O_2 g^{-1} s^{-1}, that of shrubs was 35.70 ± 1.12 nmol O_2 g^{-1} s^{-1} and that of trees was 35.09 ± 1.14 nmol O_2 g^{-1} s^{-1} (Fig. 4; Table 2). The mean respiration of woody distal lateral branch orders of herbs was 20.06 ± 1.25 nmol O_2 g^{-1} s^{-1}, that of shrubs was 16.89 ± 0.64 nmol O_2 g^{-1} s^{-1}, and that of trees was 18.48 ± 0.68 nmol O_2 g^{-1} s^{-1} (Fig. 4; Table 2).

Table 2 Root traits of different branch orders of 12 perennial species

Functional group	Species	Root order	Median life span (d)	Root diameter (mm)	Stele : root diameter ratio	Tissue N (g kg^{-1})	Respiration rate (nmol O_2 g^{-1} s^{-1})
Herbs	*Alhagi sparsifolia* Shap.	1	32	0.64 ± 0.02^{c}	0.26 ± 0.01^{b}	24.24^{a}	50.13^{a}
		2	32	0.83 ± 0.04^{c}	0.33 ± 0.01^{b}	22.60^{b}	45.22^{a}
		3	362	1.42 ± 0.05^{b}	0.61 ± 0.03^{a}	11.30^{c}	22.36^{b}
		4	379	3.47 ± 0.10^{a}	0.82 ± 0.04^{a}	10.29^{d}	16.99^{b}
	Karelinia caspica (Pall.) Less.	1	31	0.81 ± 0.05^{d}	0.24 ± 0.01^{b}	25.39^{a}	54.42^{a}
		2	32	1.36 ± 0.08^{c}	0.35 ± 0.02^{b}	23.72^{b}	51.25^{a}
		3	188	2.69 ± 0.09^{b}	0.65 ± 0.03^{a}	12.63^{c}	25.73^{b}
		4	347	4.23 ± 0.09^{a}	0.79 ± 0.04^{a}	10.97^{d}	20.16^{b}
	Apocynum venetum L.	1	37	0.75 ± 0.04^{d}	0.27 ± 0.01^{c}	22.19^{a}	40.56^{a}
		2	48	1.21 ± 0.04^{c}	0.40 ± 0.02^{c}	20.68^{b}	38.70^{a}
		3	278	2.41 ± 0.08^{b}	0.58 ± 0.03^{b}	13.11^{c}	18.81^{b}
		4	423	3.98 ± 0.10^{a}	0.85 ± 0.04^{a}	11.27^{d}	17.17^{b}
	Scorzonera divaricata Turcz	1	34	0.78 ± 0.04^{d}	0.21 ± 0.01^{b}	23.09^{a}	45.94^{a}
		2	46	1.28 ± 0.06^{c}	0.32 ± 0.01^{b}	21.37^{b}	43.18^{a}
		3	294	2.53 ± 0.09^{b}	0.60 ± 0.03^{a}	11.23^{c}	19.23^{b}
		4	331	4.10 ± 0.11^{a}	0.75 ± 0.04^{a}	10.27^{d}	16.35^{b}
Shrubs	*Tamarix ramosissima* Lebed.	1	76	0.39 ± 0.02^{c}	0.23 ± 0.01^{b}	20.24^{a}	37.45^{a}
		2	84	0.44 ± 0.02^{c}	0.34 ± 0.02^{b}	18.80^{b}	34.91^{a}
		3	>550	0.86 ± 0.04^{b}	0.62 ± 0.03^{a}	10.48^{c}	18.08^{b}
		4	>550	1.50 ± 0.06^{a}	0.85 ± 0.04^{a}	9.84^{c}	16.42^{b}
	Caragana microphylla Lam.	1	96	0.23 ± 0.01^{c}	0.33 ± 0.01^{b}	19.59^{a}	35.40^{a}
		2	108	0.28 ± 0.01^{c}	0.45 ± 0.02^{b}	17.78^{b}	33.33^{a}
		3	>550	0.72 ± 0.04^{b}	0.66 ± 0.03^{a}	11.37^{c}	17.51^{b}
		4	>550	1.23 ± 0.06^{a}	0.79 ± 0.04^{a}	10.38^{d}	15.83^{b}
	Calligonum caput-medusae Schrenk	1	92	0.20 ± 0.01^{c}	0.31 ± 0.01^{c}	21.18^{a}	40.28^{a}
		2	116	0.25 ± 0.01^{c}	0.55 ± 0.03^{b}	19.50^{b}	37.40^{a}
		3	>550	0.78 ± 0.05^{b}	0.66 ± 0.03^{a}	10.80^{c}	18.74^{b}
		4	>550	1.26 ± 0.07^{a}	0.81 ± 0.04^{a}	9.93^{c}	16.86^{b}
	Calligonum roborovskii A. Los.	1	78	0.18 ± 0.01^{c}	0.27 ± 0.01^{b}	18.28^{a}	32.35^{a}
		2	108	0.22 ± 0.01^{c}	0.37 ± 0.01^{b}	17.13^{b}	30.35^{a}
		3	>550	0.82 ± 0.04^{b}	0.65 ± 0.03^{a}	11.80^{c}	15.62^{b}
		4	>550	1.41 ± 0.07^{a}	0.78 ± 0.05^{a}	11.02^{d}	13.84^{b}
Trees	*Populus alba* var. *pyramidalis*	1	125	0.24 ± 0.01^{d}	0.17 ± 0.01^{c}	20.96^{a}	37.66^{a}
		2	140	0.30 ± 0.01^{d}	0.41 ± 0.02^{b}	19.52^{ab}	34.91^{ab}
		3	154	0.39 ± 0.01^{c}	0.49 ± 0.03^{b}	17.80^{b}	33.07^{b}
		4	>550	0.79 ± 0.03^{b}	0.70 ± 0.03^{a}	11.48^{c}	18.77^{c}
		5	>550	1.10 ± 0.05^{a}	0.77 ± 0.04^{a}	10.52^{c}	16.43^{c}
	Populus euphratica Oliv.	1	124	0.22 ± 0.01^{c}	0.35 ± 0.01^{c}	19.57^{a}	32.62^{a}
		2	133	0.25 ± 0.01^{c}	0.42 ± 0.02^{b}	18.03^{a}	30.38^{ab}
		3	147	0.34 ± 0.01^{c}	0.53 ± 0.02^{b}	16.26^{b}	28.46^{b}
		4	>550	0.71 ± 0.03^{b}	0.71 ± 0.03^{a}	9.98^{c}	17.16^{c}
		5	>550	0.98 ± 0.07^{a}	0.77 ± 0.04^{a}	8.83^{c}	15.42^{c}
	Morus alba L.	1	133	0.40 ± 0.01^{d}	0.25 ± 0.01^{c}	25.47^{a}	37.15^{a}
		2	139	0.47 ± 0.02^{d}	0.28 ± 0.01^{c}	23.90^{b}	35.37^{ab}
		3	150	0.68 ± 0.04^{c}	0.35 ± 0.01^{c}	22.31^{c}	32.64^{b}
		4	>550	0.95 ± 0.04^{b}	0.58 ± 0.02^{b}	14.58^{d}	19.53^{c}
		5	>550	1.49 ± 0.07^{a}	0.84 ± 0.04^{a}	13.18^{e}	18.33^{c}
	Elaeagnus angustifolia L.	1	95	0.58 ± 0.01^{d}	0.21 ± 0.01^{b}	25.37^{a}	39.68^{a}
		2	108	0.67 ± 0.02^{cd}	0.25 ± 0.01^{b}	23.94^{a}	37.75^{a}
		3	122	0.83 ± 0.04^{c}	0.28 ± 0.01^{b}	22.47^{b}	35.42^{a}
		4	>550	1.16 ± 0.05^{b}	0.62 ± 0.03^{a}	14.91^{c}	20.94^{b}
		5	>550	1.52 ± 0.07^{a}	0.74 ± 0.04^{a}	13.59^{c}	19.49^{b}

Mean values ± SE are shown; different superscript letters indicate a significant difference among values for Dunnett's *t*-test, two-tailed, at $P < 0.05$.
Root nitrogen concentration and respiration rate are shown as means ± SE across all 21 sample dates during the study period from April 2011 to October 2012 ($n = 21$).
Root secondary growth rate, diameter and cortex thickness are shown as mean ± SE of six replicates sampled on 20 July 2012 ($n = 6$).

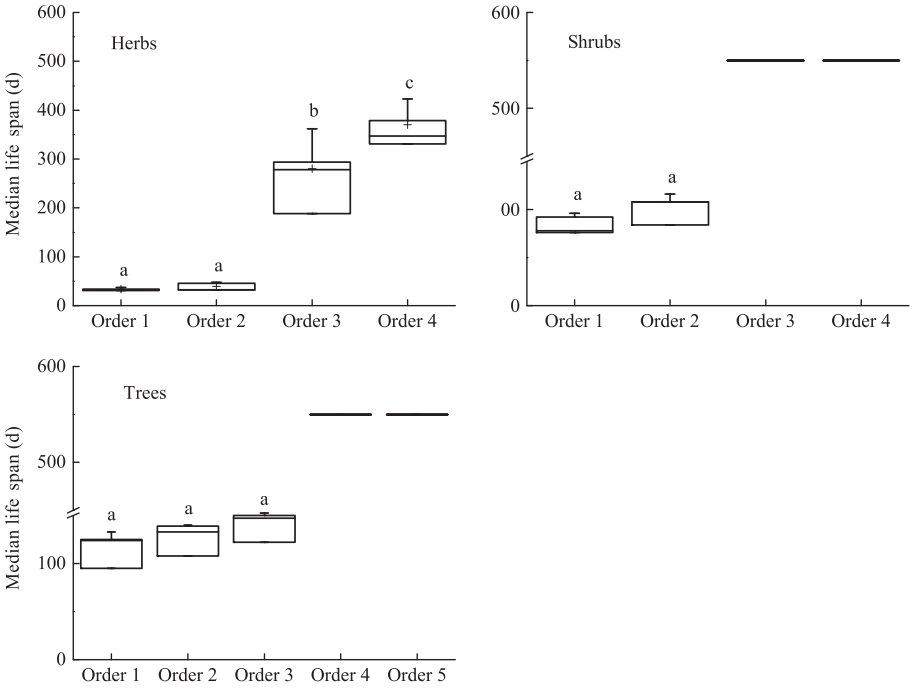

Fig. 1 Root median life span of the first four orders in herbs and shrubs, and the first five orders in trees during the study period from April 2011 to October 2012. Box plots showing mean (+), median (—), quartiles, and outliers (-) of each order. Few of the roots of the third- and fourth-order in herbs and shrubs, the fourth- and fifth-order in trees died in the end of the study, the median life spans were > 550 d. Letters indicate significant differences between orders for each groups ($n = 4$, $P < 0.05$).

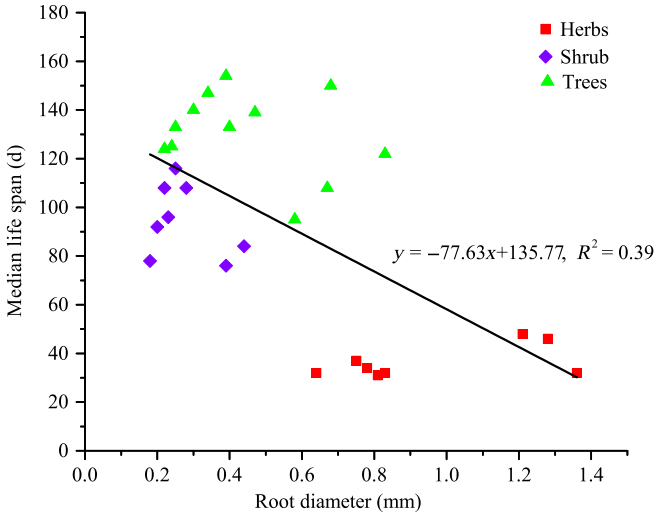

Fig. 2 The correlation between ephemeral root diameter and median life span across three functional groups. The eight points for each functional group (red, purple) represent the first two orders of roots in herbs and shrubs, and the 12 points (green) represent the first three orders of roots in trees.

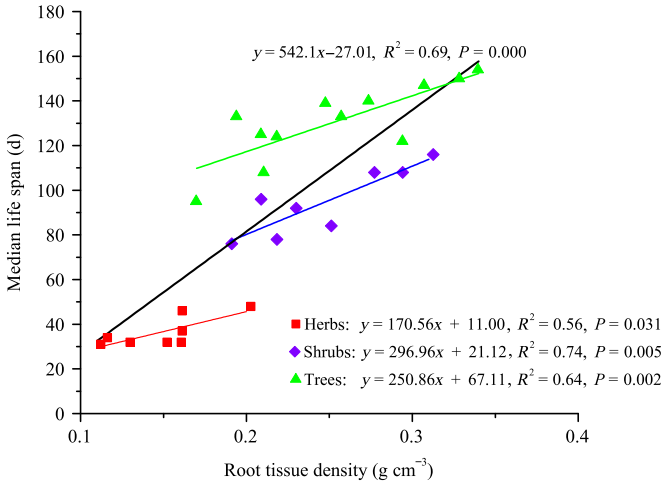

Fig. 3 The correlation between ephemeral root tissue density and median life span across three functional groups. The eight points (red, purple) for each functional group represent the first two orders of roots in herbs and shrubs, and the 12 points (green) represent the first three orders of roots in trees.

Discussion

Our data support the hypothesis that ephemeral root modules exist in all three plant functional groups tested. We could clearly distinguish short-lived, ephemeral roots at the distal end of the root system from longer-lived, perennial roots at the higher branch orders. This was apparent for all species and there were no exceptions. Ephemeral roots were in the form of lateral branches composed of the first two branch orders in perennial herbs and shrubs, and of the first three branch orders in tree seedlings (Fig. 1). Given that the occurrence of ephemeral root modules has so far only been reported for one temperate tree species (Xia *et al.*, 2010) (although the concept was also implied for 23 temperate tree species in Guo *et al.*, 2008b), our findings on arid woody and herbaceous plants greatly expand the generality of ephemeral root module concept.

Ephemeral root modules in all plant functional groups had significantly greater root N concentrations and faster respiration rates compared with perennial root modules. Ephemeral and perennial root modules therefore formed two distinct groups in regard to N concentration and respiration, confirming the functional similarities within each module group. In all the species we

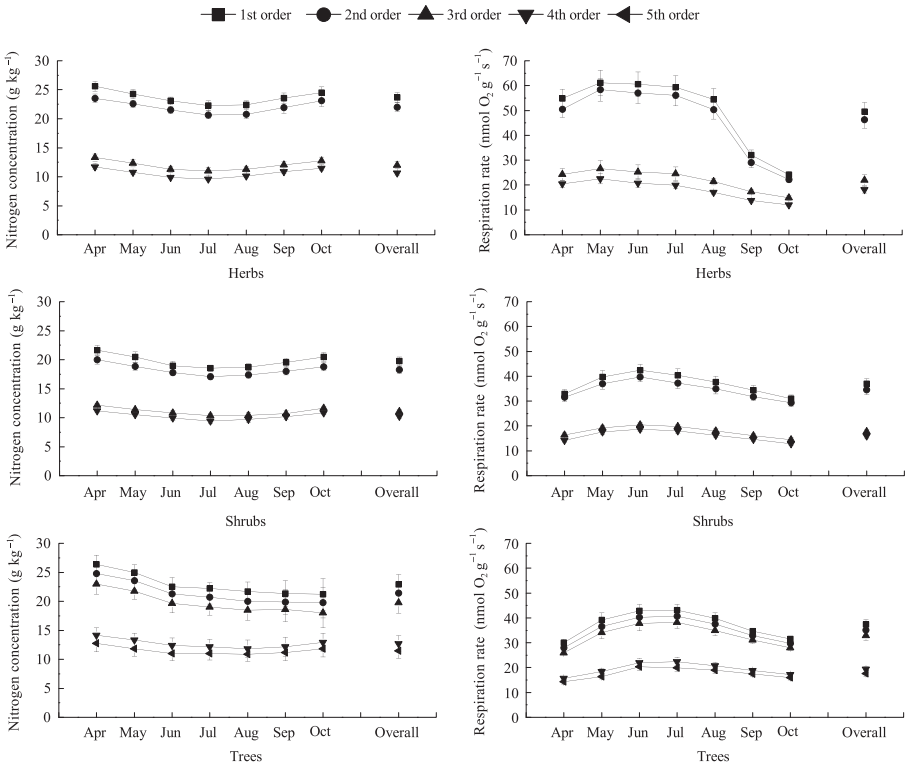

Fig. 4 The mean nitrogen (N) concentrations and respiration rates of different fine root orders in herbs, shrubs and trees. Each point connected by a line represents the mean ± SE of four species in a functional group. The separated points on the right show the means ± SE of 21 means of four replicates in a functional group sampled during the study period from April 2011 to October 2012.

studied, ephemeral roots had a large proportion of cortical cells (Fig. S12; Table 2), which generally have greater metabolic activity, as indicated by fast respiration rates (Fig. 4; Table 2). Similar results have been observed frequently before (Pregitzer *et al.*, 2002; Guo *et al.*, 2008c). By contrast, the permanent root modules were composed of secondary tissue, with no cortical cells, and had a greater proportion of cork layers, which could explain the lower N concentrations and lower respiration rates. In addition, these perennial woody roots had much longer life spans, as the secondary features can protect the roots from environmental pressures and herbivory (Guo *et al.*, 2008b).

The median life spans of ephemeral branch orders were 31–48 d in herbs, 76–116 d in shrubs, and 95–154 d in tree seedlings (Fig. 1; Table 2), and the differences between different functional groups were significant ($P < 0.001$, Table S2). The perennial root branch orders had median life spans of between 188 and 423 d in herbs and over 550 d in shrubs and trees. However, the life spans of the ephemeral root modules were always much shorter than 1 yr for all functional groups, whereas the median life spans reported for fine roots are often > 1 yr (see Finér *et al.*, 2011; Chen & Brassard, 2013). The differences in life spans between the two types of modules are another clear indication of functional differentiation in perennial plant root systems, as has reported previously for temperate species (Guo *et al.*, 2008b).

The life span of the ephemeral root module of the three functional groups found in our study is generally shorter than those reported for woody species in the temperate zone (Valenzuela-Estrada *et al.*, 2008; Xia *et al.*, 2010), probably indicating an adaptation to the harsh desert environment (annual mean precipitation 35 mm) and the pulse supply of critical resources such as water in the form of rainfall or temporary floods resulting from snow melt of the nearby mountains. In the natural desert environment, soil water content is often very low because flood and precipitation inputs are erratic pulse events. The perennial herbs have to grow fast in order to use soil water quickly and efficiently (Verburg *et al.*, 2013). The distal roots are likely to die with the reduction of the soil water in desert environments (Salguero-Gómez & Casper, 2011). Most of the trees and shrubs examined in this study occur mainly near river beds where groundwater table and soil water content are high. As such, the distal roots of those plants had longer life spans than did perennial herbs.

The systematic variation of root tissue density and root diameter in different functional groups appear to be an important factor correlating with the life spans, as proposed previously (Bardgett *et al.*, 2014). The mean root tissue density of ephemeral roots of four herbs was lower (0.15 ± 0.01 g cm^{-3}) than in the eight woody species (0.25 ± 0.02 g cm^{-3}). The low dry matter content of herb ephemeral roots may be advantageous under persistent water stress, where water capture efficiency is likely to be maximized by large vessels, thick cortical cells (Fig. S12; Table 2), low roots tissue density, high N concentrations and fast respiration rates. These functional traits may facilitate greater water uptake capacity (Guo *et al.*, 2008a), but with high root maintenance costs (Postma & Lynch, 2011; Lynch, 2015). Therefore, it may be cost-efficient for herbs to shed roots and regrow them when favorable conditions return, rather than sustaining the maintenance respiration of inefficient roots (Lynch, 2015), as has been reported for a desert perennial in the southwestern US (Salguero-Gómez & Casper, 2011). In addition, continuous replacement of ephemeral roots allows plants to explore the soil

more thoroughly (Janssens *et al.*, 2002). By contrast, high tissue density of woody ephemeral roots would be more favorable under moister environments. High construction costs are associated with the longer life spans of distal roots. Building new roots not only requires carbon skeletons to produce cellulose, lignin and other structural compounds, it also requires metabolic energy (Janssens *et al.*, 2002).

Overall, the variation of root diameter and tissue density across different functional groups may represent distinct strategies of root construction, maintenance, and persistence. Herbs with thicker absorbing roots devote more N per unit mass. These species may be more efficient in assimilating nutrients at higher respiration rates in their short life histories. By contrast, shrubs and trees with thinner absorbing roots devote less N per unit mass. These species may be more efficient in assimilating nutrients by lengthening life spans. It has long been recognized that root life spans were positively related to functional traits, such as root diameter, calcium content, and tree wood density (McCormack *et al.*, 2012). However, in our study, diameter of ephemeral root modules could not predict life span across different functional groups. It appears that the linkage between root life spans and traits related to construction (e.g. diameter and root N concentrations) might apply only in the same functional group with similar habitat and life form.

Implications and uncertainties

Our study clearly demonstrated that two different classes of root modules exist in fine roots in a variety of plant functional groups of desert species. The first two or three root orders in perennial herbs, shrubs and trees are constrained to primary growth and may be shed within a few weeks to 6 months in this desert environment and form an ephemeral root module. Although they differed in their size dimensions (second- and third-order roots are thicker than first-order roots), all root orders within the ephemeral root module had similar basic anatomical features and physiological functions, which differ fundamentally from woody persistent transport roots. It is clear that these ephemeral roots function mainly in resource acquisitions. By contrast, the woody roots had greater diameters and no cortex, showed secondary growth and cork cells, and had lower N concentrations and respiration rates but much longer life spans. In addition, the perennial root modules serve as a mother root for new ephemeral modules and thus can persist for long periods of time. Our data also demonstrate that the classification of the perennial root systems into ephemeral and perennial root modules may apply to more perennial plants than previously recognized. This classification should also simplify the sampling of roots of different functions as compared with coarse–fine root dichotomies (which do not accurately differentiate roots of different physiology and function) and finely defined branch orders (a method that is highly labor-intensive) (McCormack *et al.*, 2015). Thus far, ephemeral root modules have only been observed in temperate species (Guo *et al.*, 2008a; Valenzuela-Estrada *et al.*, 2008; Xia *et al.*, 2010). However, it is likely that ephemeral root modules are actually widespread in perennial plants, given that we observed ephemeral root modules in this extreme desert environment and across all plant functional groups studied. It is possible that the concept of 'absorb fast and die young' ephemeral roots is ecologically advantageous across a larger range of environments, because the availability of essential resources will fluctuate in almost every environment.

Our experiment was conducted on seedlings and it is currently unknown whether the expression of ephemeral root modules will change as plants mature. The plasticity of root trait expression has not been widely studied and there are indications that some traits – like specific root length – indeed change with ontogeny, while branching intensity are more conservative (Tobner *et al.*, 2013). The plasticity of ephemeral root expression with regard to different environmental conditions (soil type, water or nutrient availability) is also currently unknown and needs further study.

Our results also highlight that the life spans of three plant functional groups across 12 species were much lower than the estimation of Jackson *et al.* (1997). This means that the fine root turnover rates of desert species could be underestimated.

The final implication of our study is that single fine-root diameter class cannot be used to define functional root groups across multiple species. All roots in shrubs and trees would have been classed as fine roots under the < 2 mm classification, because all root branch orders in these functional groups were < 2 mm. Moreover, there were wide variations in root diameter within the same order. The herbs had first-order root diameters of between 0.6 and 0.8 mm, which would equate to third- (shrubs) or fourth-order (trees) roots that belong to the permanent module. Hence our data provide further evidence supporting the conclusions of McCormack *et al.* (2015) that branch order classification of root systems should give much better insights into root functionality than diameter classes regardless of how finely divided these diameter classes are.

Acknowledgements

We thank Dali Guo, Zeqing Ma, Yan Li, Xiaopeng Gao, Chengen Ma and Dongwei Gui for valuable comments and modifications regarding this paper; Gang Huang, Xinjun Zheng, Huiliang Liu, Xiaobing Zhou and Bo Zhang for valuable assistance with the data analysis; and Weicheng Luo, Changjun Li and Zichun Guo for assistance in the field and laboratory. We are also grateful to three anonymous reviewers for their helpful comments. This work was supported by the Key Program of Joint Foundation of the National Natural Science Foundation and the Government of Xinjiang Uygur Autonomous Region of China (no. U1203201), the National Natural Science Foundation of China (no. 31100144, 41371516), China Postdoctoral Science Foundation General Financial Grant (no. 2014M552523), and Xinjiang Postdoctoral Science Foundation General Financial Grant.

Author contributions

B.L., F.Z. and J.L. planned and designed the research. B.L. and J.H. performed experiments, conducted fieldwork, and analyzed data. B.L. and S.K.A. wrote the manuscript.

References

Arndt SK, Kahmen A, Arampatsis C, Popp M, Adams M. 2004. Nitrogen fixation and metabolism by groundwater-dependent perennial plants in a hyperarid desert. *Oecologia* **141**: 385–394.

Bardgett RD, Mommer L, De Vries FT. 2014. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution* **29**: 692–699.

Bruelheide H, Jandt U, Gries D, Thomas FM, Foetzki A, Buerkert A, Wang G, Zhang XM, Runge M. 2003. Vegetation changes in a river oasis on the southern rim of the Taklamakan Desert in China between 1956 and 2000. *Phytocoenologia* **33**: 801–818.

Bruelheide H, Vonlanthen B, Jandt U, Thomas FM, Foetzki A, Gries D, Wang G, Zhang XM, Runge M. 2010. Life on the edge–to which degree does phreatic water sustain vegetation in the periphery of the Taklamakan Desert? *Applied Vegetation Science* **13**: 56–71.

Chen HYH, Brassard BW. 2013. Intrinsic and extrinsic controls of fine root lifespan. *Critical Reviews in Plant Sciences* **32**: 151–161.

Chen WL, Zeng H, Eissenstat DM, Guo DL. 2013. Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Global Ecology and Biogeography* **22**: 846–856.

Clark NM, Apple ME, Nowak RS. 2010. The effects of elevated CO_2 on root respiration rates of two Mojave Desert shrubs. *Global Change Biology* **16**: 1566–1575.

Eissenstat DM, McCormack ML, Du Q. 2013. Global change and root lifespan. In: Eshel A, Beeckman T, eds. *Plant roots: the hidden half*, 4th edn. New York, NY, USA: CRC Press, 1–11.

Espeleta JF, West JB, Donovan LA. 2009. Tree species fine-root demography parallels habitat specialization across a sand hill soil resource gradient. *Ecology* **90**: 1773–1787.

Finér L, Ohashi M, Noguchi K, Hirano Y. 2011. Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. *Forest Ecology and Management* **262**: 2008–2023.

Gui DW, Zeng FJ, Liu Z, Zhang B. 2013. Characteristics of the clonal propagation of *Alhagi sparsifolia* Shap. (Fabaceae) under different groundwater depths in Xinjiang, China. *The Rangeland Journal* **35**: 355–362.

Guo DL, Li H, Mitchell RJ, Han WX, Hendricks JJ, Fahey TJ, Hendrick RL. 2008b. Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotope methods. *New Phytologist* **177**: 443–456.

Guo DL, Mitchell RJ, Withington JM, Fan PP, Hendricks JJ. 2008c. Endogenous and exogenous controls of root lifespan, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. *Journal of Ecology* **96**: 737–745.

Guo DL, Xia MX, Wei X, Chang W, Liu Y, Wang ZQ. 2008a. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist* **180**: 673–683.

Jackson RB, Mooney HA, Schulze ED. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences, USA* **94**: 7362–7366.

Janssens IA, Sampson DA, Curiel-Yuste J, Carrara A, Ceulemans R. 2002. The carbon cost of fine root turnover in a Scots pine forest. *Forest Ecology and Management* **168**: 231–240.

Jia SX, McLaughlin NB, Gu JC, Li XP, Wang ZQ. 2013. Relationships between root respiration rate and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus mandshurica*. *Tree Physiology* **33**: 579–589.

Kaplan EL, Meier P. 1958. Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* **53**: 457–481.

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist* **154**: 389–398.

Kong DL, Ma CE, Zhang Q, Li L, Chen XY, Zeng H, Guo DL. 2014. Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist* **203**: 863–872.

Liu B, Zeng FJ, Arndt SK, He JX. 2013. Patterns of root architecture adaptation of a phreatophytic perennial desert plant in a hyperarid desert. *South African Journal of Botany* **86**: 56–62.

Lynch DJ, Matamala R, Iversen CM, Norby RJ, Gonzalez-Meler MA. 2013. Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots. *New Phytologist* **199**: 420–430.

Lynch JP. 2015. Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. *Plant, Cell & Environment* **38**: 1775–1784.

Majdi H, Damm E, Nylund JE. 2001. Longevity of mycorrhizal roots depends on branching order and nutrient variability. *New Phytologist* **150**: 195–202.

Matamala R, Gonzalez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science* **302**: 1385–1387.

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist* **195**: 823–831.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo DL, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB *et al.* 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207**: 505–518.

McCormack ML, Eissenstat DM, Prasad AM, Smithwick EAH. 2013. Regional scale patterns of fine root lifespan and turnover under current and future climate. *Global Change Biology* **19**: 1697–1708.

de Neergaard E, Lyshede OB, Gahoonia TS, Care D, Hooker JE. 2000. Anatomy and histology of roots and root–soil boundary. In: Smit AL, Bengough AG, Engels C, eds. *Root methods: a handbook*. Berlin, Germany: Springer-Verlag, 33–74.

Polverigiani S, McCormack ML, Mueller CW, Eissenstat DM. 2011. Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits. *Tree Physiology* **31**: 1228–1237.

Postma JA, Lynch JP. 2011. Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiology* **156**: 1190–1201.

Pregitzer KS. 2002. Fine roots of trees – a new perspective. *New Phytologist* **154**: 267–270.

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine North American trees. *Ecological Monographs* **72**: 293–309.

Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* **18**: 665–670.

Salguero-Gómez R, Casper BB. 2011. Introducing short roots in a desert perennial anatomy and spatiotemporal foraging responses to increased precipitation. *New Phytologist* **191**: 173–183.

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R. 2008. Irreconcilable differences: fine-root life spans and soil carbon persistence. *Science* **319**: 456–458.

Taylor BN, Beidler KV, Strand AE, Pritchard SG. 2014. Improved scaling of minirhizotron data using an empirically-derived depth of field and correcting for the underestimation of root diameters. *Plant and Soil* **374**: 941–948.

Tobner CM, Paquette A, Messier C. 2013. Interspecific coordination and intraspecific plasticity of fine root traits in North American temperate tree species. *Frontiers in Plant Science* **4**: 242.

Valenzuela-Estrada LR, Vera-Caraballo V, Ruth LE, Eissenstat DM. 2008. Root anatomy, morphology, and longevity among root orders in *Vaccinium corymbosum* (Ericaceae). *American Journal of Botany* **95**: 1506–1514.

Verburg PSJ, Young AC, Stevenson BA, Glanzmann I, Arnone JA III, Marion GM, Holmes C, Nowak RS. 2013. Do increased summer precipitation and N deposition alter fine root dynamics in a Mojave Desert ecosystem? *Global Change Biology* **19**: 948–956.

Warren JM, Hanson PJ, Iversen CM, Kumar J, Walker AP, Wullschleger SD. 2015. Root structural and functional dynamics in terrestrial biosphere models – evaluation and recommendations. *New Phytologist* **205**: 59–78.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. *Ecology* **82**: 882–892.

Withington JM, Reich PB, Oleksyn J, Eissenstat DM. 2006. Comparisons of structure and life span in roots and leaves among temperate trees. *Ecological Monographs* **76**: 381–397.

Xia MX, Guo DL, Pregitzer KS. 2010. Ephemeral root modules in *Fraxinus mandshurica*. *New Phytologist* **188**: 1065–1074.

Yuan ZY, Chen HYH. 2012. Indirect methods produce higher estimates of fine root production and turnover rates than direct methods. *PLoS ONE* **7**: e48989.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Diagrammatic sketch of concrete pod with four rhizotrons.

Fig. S2 Diagrammatic sketch of a rhizotron.

Fig. S3 Rhizotron boxes were installed in concrete pods.

Fig. S4 Soils were filled back to rhizotrons.

Fig. S5 Plants were transplanted to rhizotron boxes (*K.* caspica (Pall.) Less.).

Fig. S6 Curtain installation outside the rhizotron box, and then the installation of two polycinnamene boards to each curtain.

Fig. S7 Growth status after 20 d of transplantation.

Fig. S8 Root hairs in rhizotron (*T. ramosissima* Lebed.).

Fig. S9 Root branch orders in rhizotron (*M. alba* L.).

Fig. S10 Roots image of *K. caspica* (Pall.) Less.

Fig. S11 Root survival probability of the first four orders in herbs and shrubs, and the first four orders in trees during the study period from April 2011 to October 2012.

Fig. S12 Anatomical traits of 12 species (anatomical traits of the first four orders of roots in herbs and shrubs, the first five orders of roots in trees).

Fig. S13 Representative examples of fine root systems of 12 species.

Table S1 The detailed amount of analyzed roots of 12 species in all rhizotrons

Table S2 Regression analysis: life span vs tissue density, diameter, functional group, tissue

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.