Ecosystems (2015) 18: 1307–1318
DOI: 10.1007/s10021-015-9900-y

ECOSYSTEMS

Rapid Root Decomposition Decouples Root Length from Increased Soil C Following Grassland Invasion

Vasiliki G. Balogianni,[1]* Scott D. Wilson,[1] Richard E. Farrell,[2] and Andrew S. MacDougall[3]

[1]*Department of Biology, University of Regina, 3737 Wascana Parkway, Regina, Saskatchewan S4S 0A2, Canada;* [2]*Department of Soil Science, University of Saskatchewan, 5E18 - 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada;* [3]*Department of Integrated Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario N1G 2W1, Canada*

Abstract

Plant invasion often increases stand biomass, but higher tissue quality (for example, less lignin and more nutrients) in invasive species might accelerate litter decomposition. This mechanism may minimize increases in soil carbon (C) sequestration despite higher production. Our knowledge about invasion and tissue quality is based on shoots, but roots contribute 50–90% of biomass in vegetation types such as semiarid grasslands. Here we investigate root decomposition rates and tissue quality in the widespread invasive grass *Agropyron cristatum*, which doubles root mass but not soil C in the Great Plains of North America. Root length was significantly greater beneath *Agropyron* than native grassland 7 years after minirhizotron installation. However, CO_2 evolution from decomposing roots was twice as much for *Agropyron* roots as for native grass roots ($P < 0.05$). CO_2 evolution from decomposing native grass roots was not significantly different from controls with no root tissue added, suggesting that *Agropyron* invasion can convert grassland soil to a source of CO_2 to the atmosphere. Rapid root decomposition was associated with significantly lower lignin content in *Agropyron* roots than native grass roots, although root N and lignin:N ratios did not differ. We present the first report of root decomposition rates associated with plant invasion. Increases in root length were accompanied by increased root decomposition rates of low-lignin tissue, such that invasion-driven enhanced productivity did not enhance soil C sequestration. Among-species differences in root tissue quality and decomposition rates could influence soil C dynamics during invasions of systems dominated by belowground production, such as tundra, boreal forests, and semiarid grassland.

Key words: *Agropyron cristatum*; below ground; invasion ecology; lignin; minirhizotron; nitrogen; tissue quality.

Received 5 November 2014; accepted 17 May 2015;
published online 3 July 2015

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-015-9900-y) contains supplementary material, which is available to authorized users.

Author contributions Vasiliki G. Balogianni performed research, analyzed data, and contributed in conceiving and designing of the study and writing the paper. Scott D. Wilson contributed in conceiving and designing of the study and writing the paper. Richard E. Farrell contributed in new methods and Andrew S. MacDougall contributed in conceiving and designing of the study.

*Corresponding author; e-mail: vasiliki.balogianni@gmail.com

Introduction

Plant invasion is often accompanied by an increase in stand biomass (Ehrenfeld 2010; Vila and others 2011; Castro-Diez and others 2014; Maron and others 2014), which may result in increased soil carbon (C) sequestration (Luyssaert and others 2008; Nave and others 2013; Ziter and MacDougall 2013). On the other hand, relative to native vegetation, invader biomass often has higher tissue quality (less lignin and more nutrients) typical of species with high growth rates (Castro-Diez and others 2014; Meisner and others 2014) and associated with faster tissue turnover and decomposition (Dìaz and others 2004; Dijkstra and others 2006; Shipley and others 2006). The faster decomposition rates of invaders potentially result in more rapid loss of organic matter from the ecosystem compared with native systems, which can decrease soil C sequestration (Peltzer and others 2009). Thus, the net effect of increasing both tissue mass and quality is unknown, and increased decomposition rates may offset increased production, minimizing effects on soil C sequestration. Here we explore relationships among tissue quality, decomposition, and soil C in invaded and native semiarid grasslands. Understanding these relationships is important because soil contains about 70% of terrestrial organic C, and belowground production and decomposition regulate CO_2 fluxes to the atmosphere that are approximately 10 times greater than those from anthropogenic sources (Chapin and others 2002).

Roots are the dominant source of soil C in temperate, arid, and arctic soils (Loya and others 2004; Crow and others 2009), accounting for 50–90% of total production (Mokany and others 2006), and decomposing up to 2.8 times more slowly than leaves (Freschet and others 2013). In spite of the importance of roots to ecosystem function (Carrillo and others 2014), most of our knowledge about potential invasion effects on soil C is derived from aboveground tissues (Weidenhamer and Callaway 2010; Hickman and others 2013; Tamura and Tharayil 2014; Zhang and others 2014). Among-species differences in tissue quality and decomposition rates in aboveground tissues tend to reflect similar differences in roots (Freschet and others 2013; Ziter and MacDougall 2013), suggesting that the higher tissue quality of invader leaves may also occur in roots. Root lignin:N ratios were lower for the introduced grasses *Bromus inermis* (15.6) and *Agropyron repens* (11.8) relative to a suite of five native grasses (range 16.8–21.0) in Minnesota (Dijkstra and others 2006). Thus, differences in root tissue quality between invasive and native species might offset differences in productivity in influencing soil C.

Both root and shoot mass of North American grasslands have been reported to significantly increase with invasion by grasses such as *Agropyron cristatum* and *Bromus tectorum* (Belnap and Phillips 2001; Ehrenfeld 2003; MacDougall and Wilson 2011; Balogianni and others 2014). *Agropyron cristatum* is a widely planted for forage production and revegation (for example, following the dust bowl of the 1930s) and readily invades adjacent native grassland (Heidinga and Wilson 2002; Henderson and Naeth 2005) forming stable, low-diversity stands on more than 10 million ha in North America (Lesica and DeLuca 1996). In spite of greater biomass in *Agropyron* stands, soil C is not significantly different between *Agropyron* stands and native grassland (Chen and Stark 2000; Balogianni and others 2014), possibly because the roots of *Agropyron* are dominated by white, nutrient-rich roots with high decomposition rates (MacDougall and Wilson 2011).

Here we report the first test of the hypothesis that more rapid decomposition and higher root tissue quality in invasive grasses, relative to native grasses, offset greater root production minimizing increases in soil C content. Additionally, we compare the roots and shoots of invaded and native grassland under four long-term vegetation treatments to test the generality of greater mass in invaded stands.

Methods

Vegetation

We compared root and shoot mass, tissue quality, and soil C in *Agropyron* stands and native grassland in a field experiment at Medicine Lake National Wildlife Refuge, Montana (48°28′ N, 104°22′ W). Native grassland was dominated by the C_4 grass *Bouteloua gracilis* (Wild. ex Kunth.) Lag. ex Griffiths, the forb *Tragopogon dubius* Scop. and the subshrub *Artemisia frigida* Willd. Average annual precipitation is 34 cm, the average July temperature is 20.8°C, and the average January temperature is −13.1°C (NOAA-Western Regional Climate Center, http://www.wrcc.dri.edu/). The soils are classified as Mollisols, suborder Ustoll (http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051233.pdf), with a well-drained loam A-horizon (0–15 cm) over a clay-loam B-horizon (15–100 cm), greater than 200 cm above the water table (USDA-NRCS, http://websoilsurvey.nrcs.usda.gov).

We sampled 40 plots (5 m diameter, >10 m apart) scattered across 6 ha. Half the plots were randomly located in *Agropyron*-invaded areas (≈70% cover of *Agropyron* in 2013, V. Balogianni, unpublished data) and half in native grassland (≈7% cover of *Agropyron* in 2013). Distance between the native and invaded plots varied from 50 to 200 m. No major changes in species composition occurred during the study. All plots were approximately 200 m from fireguards that had been sown with *Agropyron* during 1940–1960 (J. Rodriguez, unpublished data). Since then *A. cristatum* has invaded from the fireguard into the adjacent uncultivated native grassland of our study site. The uncultivated nature of the invaded grassland is shown by the absence of plowlines in aerial images (for example, http://www.fsa.usda.gov/FSA/apfoapp?area=homeandsubject=prodandtopic=cat), and the presence of native vegetation in patches where *A. cristatum* is absent. The two vegetation types had similar topography and soils. Previous management included occasional moderate grazing and prescribed burning every 5–7 years (M. Borgreen, unpublished data).

To compare root length and shoot mass of *Agropyron* to native vegetation under different management and fertility conditions, we randomly assigned five plots in both *Agropyron* stands and native grassland to each of the following treatments: (1) no treatment (controls); (2) mowing; (3) N addition; and (4) mowing and N addition. Treatments were applied annually from 2006 to 2013. Mowed plots were cut at around 2 cm above the ground every May and again at about 5 cm above the ground in late June. Nitrogen (N) was added twice each year, at the same time as mowing, with each plot receiving 5.4 g m^{-2} of urea (46-0-0) annually. The N application rates mimicked the highest deposition rates of N in the northern Great Plains (22 kg ha^{-1} y^{-1}) (Köchy and Wilson 2001). However, 2 years after initiating the experiment, it was found that N addition had no effect on shoot biomass; thereafter, from 2008 through 2013, N addition rates were increased to 44 kg ha^{-1} y^{-1}.

Roots and Shoots

We measured root length during three growing seasons (2011–2013) using minirhizotrons (Bartz Technology Corporation, Santa Barbara, CA). Although minirhizotrons may underestimate root production due to their small sampling area (Taylor and others 2013), they detect more root length than do harvest methods (Hendricks and others 2006) and allow repeated non-destructive sampling of identical locations without continuing soil disturbance. In each plot, one transparent rhizotron tube (180-cm long, 5-cm internal diameter) was installed at a 45° angle to the soil surface in spring 2006. Tube length within the soil varied (40–160 cm) according to the presence of rocks that prevented deeper installation, but even the shortest length sampled the top layer of grassland soil that contains the bulk of roots (Steinaker and Wilson 2008a) and organic C (Jobbágy and Jackson 2000). Images (each 18 × 14 mm) of the top of each tube were collected annually (in May, June and August) at depths of 3, 8, 13, 18, 23, and 28 cm below the soil surface (BSS, measured vertically from the soil surface). Root length in the images was measured using Rootfly (http://www.ces.clemson.edu/~stb/rootfly/) and was expressed as length of root per area of image ($m \cdot m^{-2}$). Whereas root length per image area is highly correlated with root mass (Balogianni and others 2014) root length is a better index of resource uptake than mass (Jackson and others 1996). Shoot mass was collected from a quadrat (8 × 100 cm) in each plot after application of the treatments in late July 2011–2013, dried to constant mass and weighed.

Agropyron roots showed continuing colonization of minirhizotron tubes over the first 5 years of this study (Balogianni and others 2014), suggesting that a steady state in root dynamics around the minirhizotron tube had not yet been reached. Therefore, here we report only the last three (of seven) years of root and shoot data for both native and *Agropyron*-dominated grasslands.

Decomposition

To test for differences in decomposition rate between *Agropyron* and native grasses, we measured CO_2 evolution in a controlled environment. Roots and shoots for decomposition measures were obtained from a randomly selected control plot in each vegetation type. Roots were collected from the top 20 cm of the soil in July 2011. Fine roots (<0.2 mm) were washed and all tissue was frozen at −10°C. Shoots were collected in September 2012, just after senescence.

The soil used in the decomposition substrate study included the A- and upper B-horizon (0–20 cm BSS) material collected from a randomly selected control plot in each of the *Agropyron* and native grassland sites in May 2012. All visible roots and litter was removed, and soil from the two vegetation types was bulked, homogenized, and sieved at 2 mm to provide a standardized matrix. Soil, roots, and shoots were stored at −10°C for

1 week and then subjected to three 12-h periods of alternating temperature (−5 and +15°C) to simulate winter and spring conditions. We measured CO_2 evolution from 10 g of dry soil incubated in 60-ml gas-tight vials with self-resealable caps (Wheaton, NJ, USA), after adding water to achieve 20% of soil water holding capacity (Fierer and others 2005). The selected water content approximately corresponds to summer soil water content 10 cm BSS in our region (Fierer and Schimel 2002; Fierer and others 2005; Steinaker and Wilson 2008b). Soil was equilibrated in the vials for 48 h at 17°C before the addition of plant tissue to eliminate the flush of CO_2 that normally accompanies the rewetting of a soil (Lee and others 2000). Roots and shoots were oven-dried at 60°C to constant mass and ground to approximately 0.5 mm pieces. Grinding to a small, uniform particle size is commonly employed in laboratory incubations to standardize the material and eliminate the confounding effect of different particle sizes (Dalias and others 2001; Fierer and others 2005). In the field, litter size would be reduced by soil organisms; therefore, this study does not examine the complete route that biomass may typically take to form soil C (Johnson and others 2007). A sub-sample (100 mg) of dried ground roots or shoots was added to each of the vials.

The resulting mixtures, which contained 10 mg added plant tissue per gram of soil (Fierer and others 2005), were homogenized by manual shaking and the vials resealed. Each root- and shoot-amended soil was replicated five times yielding a total of 25 incubation vials (that is, 5 replicates × 2 vegetation types × 2 tissues, +5 control vials containing only soil but no other tissue).

Soils were incubated for 4 months to simulate one growing season in the northern Great Plains; that is, the period with mean temperature at least 9°C. Incubation temperature was maintained at 17°C throughout the experiment to mimic soil temperatures at 10 cm BSS during the growing season in our region (Steinaker and Wilson 2008b). Incubation occurred in the dark to exclude photodegradation (Parton and others 2007).

Vial headspaces (that is, between the soil surface and the cap) were sampled 1, 3, 6, 9, 18, 50, and 120 days after the start of the experiment. Gas samples (20 ml) were withdrawn by penetrating the self-resealable cap with a 20-ml gas-tight syringe (Bradford and others 2009) transferred into 12-ml pre-evacuated Exetainer vials (Labco, Credigion, UK) and stored in a cool and dry place until analysis. Carbon dioxide (CO_2) evolution was determined by gas chromatography (using a Varian CP4900 Micro-GC equipped with dual micro-thermal conductivity cells; Varian, Mississauga, Canada) and expressed as parts micrograms (μg) of C per gram dry soil per day (μg C g^{-1} dry soil d^{-1}). Vials were opened for 10 min every third day (after sampling, on sample days) to avoid O_2 depletion. After every opening, vials were weighed and water added as needed to assure that water content remained constant. A preliminary trial to test the system demonstrated that CO_2 concentrations inside the vials did not reach inhibitory levels and that anaerobic respiration did not occur during the incubation.

Tissue Quality

Roots and shoots from *Agropyron* stands and native grassland were analyzed for lignin and N content. Five root and five shoot samples of each vegetation type were taken from untreated control plots. Roots were collected in July 2011, from the top 20 cm of the soil—where the bulk of root length occurs (Steinaker and Wilson 2008a)—and washed. Leaves were collected just after senescence, in September 2012. Root and shoot samples were analyzed for lignin by the acid detergent fiber method and N content by combustion at Agri-Food Laboratories, (Guelph, Canada).

Soil C

Soil cores were collected from untreated control plots in both the *Agropyron* and native grassland stands in May 2011 and separated into three depth classes: 0–10, 10–20, and 20–30 cm BSS. In total, we collected five replicate cores (15 total samples) from each of the two vegetation types. The soils were sieved, air-dried, and analyzed for organic C content (Agri-Food Laboratories, Guelph, ON). These measurements were not corrected for bulk density.

Statistical Analyses

Root length was analyzed using repeated measures multivariate analysis of variance (rmMANOVA) with vegetation type, depth, mowing, and N as main effects. Shoot mass was also analyzed using rmMANOVA with vegetation type, mowing, and N as main effects. Similarly, CO_2 evolution was analyzed using rmMANOVA with vegetation type as a main effect. Controls (vials with soil but no added tissue) were treated as a vegetation type for the CO_2 evolution analysis in order to include them in the rmMANOVA. Soil organic C was analyzed

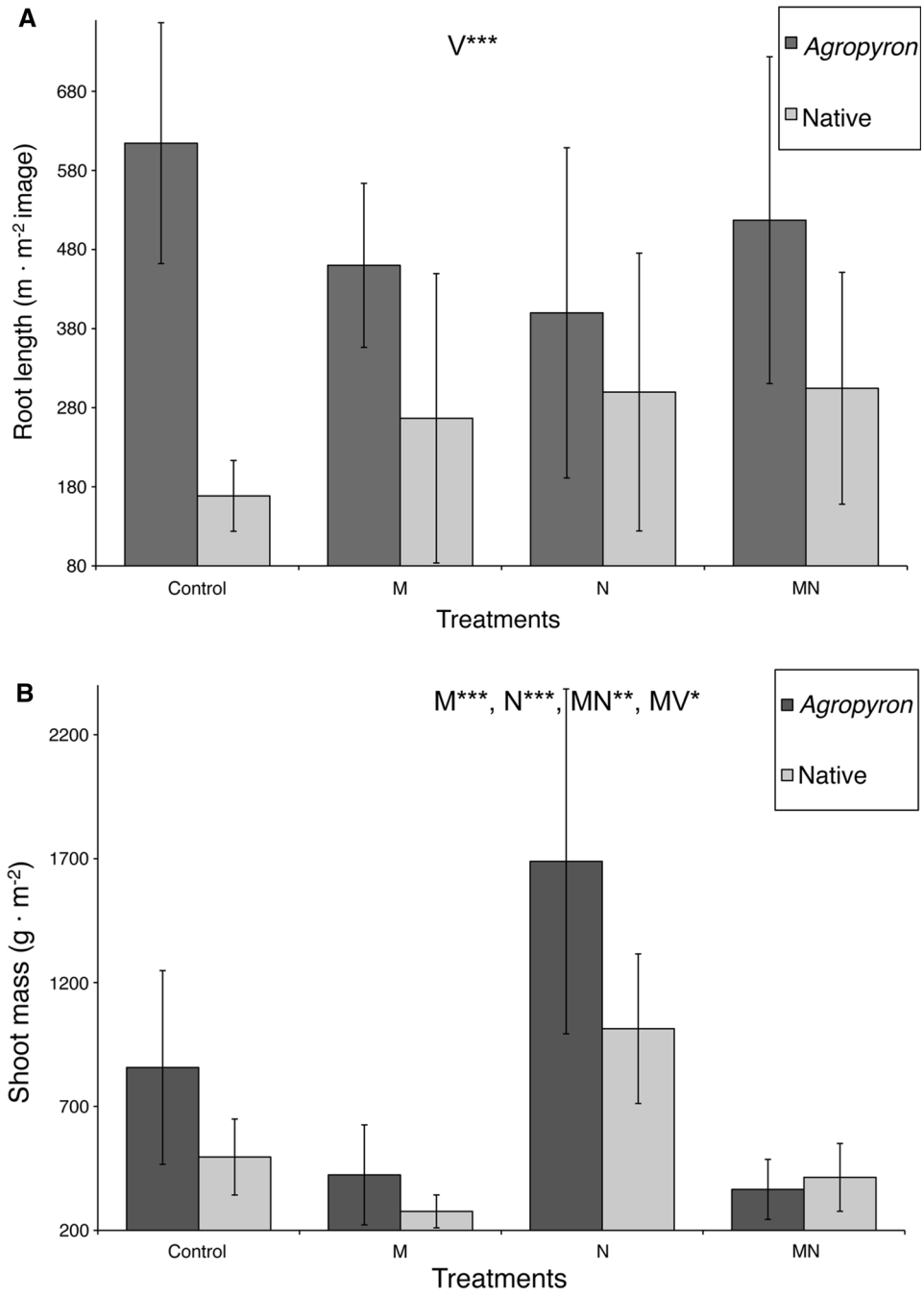

Figure 1. **A** Total root length in June 2013 (mean ± SD), per m^2 of minirhizotron image beneath the invasive grass *Agropyron cristatum* (*dark bars*) and native grassland (*light bars*) (averaged across three depth classes) in four treatments; **B** Shoot mass in June 2013 (mean ± SD) per m^2 of crested wheatgrass and native grassland area in four treatments. ANOVA details in Tables 1 and 2. *Control* untreated plots, *M* plots mowed annually for 7 years, *N* plots receiving nitrogen annually for 7 years, *MN* plots both mowed and receiving nitrogen, *V* vegetation type, *MV* interaction of vegetation type with mowing; *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.0001$.

using a two-way ANOVA with vegetation type and depth as main effects. Differences in tissue nutrient content between the two vegetation types were tested using one-way ANOVA. Statistical analyses were conducted using JMP 10.0 (SAS Institute Inc., Cary, NC). To help stabilize the variance, all data were fourth-root transformed. Fourth-root transformations are an effective and widely used method for normalizing skewed data (Quinn and Keough 2002). Residuals of transformations were distributed normally.

Results

Roots, Shoots, and Soil C

Root length in the final year was significantly greater beneath *Agropyron* than beneath native grassland (Figure 1A; Table 1). Root length did not vary significantly with mowing or N addition, either separately or in combination (Figure 1A; Table 1). Root length increased throughout the period from 2011 to 2013, (that is, 5–7 years after rhizotron installation) (Figure S1; Table S1) though

Table 1. Between-Subjects Effects from Repeated Measures ANOVA for Roots and Shoots with Vegetation Type, Mowing, and Nitrogen (N) as Main Factors and Their Interactions

Source of variation	NumDf	DenDf	*F* value	*P* value
Root				
Vegetation type	1	84	40.0325	<.0001*
Mowing	1	84	0.4053	0.5261
Vegetation type × mowing	1	84	0.3444	0.5589
N	1	84	0.0150	0.9029
Vegetation type × N	1	84	2.9609	0.0890
Mowing × N	1	84	0.5131	0.2521
Vegetation type × mowing × N	1	84	1.8039	0.1378
Shoot				
Vegetation type	1	28	0.0599713	0.2056
Mowing	1	28	4.3201918	<0.0001*
Vegetation type × mowing	1	28	0.2347678	0.0160*
N	1	28	0.7345729	<0.0001*
Vegetation type × N	1	28	0.0012977	0.8502
Mowing × N	1	28	0.3938653	0.0025*
Vegetation type × mowing × N	1	28	0.0002582	0.9328

For within-subject and depth effects and its interactions see S1.
NumDf degrees of freedom in the model, DenDf degrees of freedom associated with the model errors.
** $P < 0.05$.*

no significant time × vegetation type interaction was detected (Table S1).

Shoot mass in control plots during the final year was twofold greater in *Agropyron* stands than in native grassland (Figure 1B), though the main effect was not significant due to interactions with other factors (Table 1). In contrast to root length, shoot mass decreased significantly with mowing and increased significantly with N addition (Figure 1B; Table 1). As well, mowing-induced reductions in shoot mass were greater in the *Agropyron* stands than in the native grassland, resulting in a significant vegetation type × mowing interaction (Figure 1B; Table 1). Likewise, a significant mowing × N interaction occurred because N-induced increases in shoot mass were greater in non-mowed plots than in mowed plots (Figure 1B; Table 1).

Soil organic C content did not differ between the *Agropyron* and native grassland (Figure S2, vegetation effect: $F_{1,47} = 0.94$, $P = 0.34$). However, soil organic C decreased significantly with increasing depth ($F_{2,47} = 42.35$, $P < 0.001$)—with a significant depth × vegetation type interaction ($F_{2,47} = 5.15$, $P = 0.01$). Indeed, the decrease in soil organic C with depth was greater under *Agropyron* than under native grasses (Figure S2).

Decomposition

Carbon dioxide evolution from the root-amended soil was significantly greater (about twofold) for *Agropyron* than the native grasses (Figure 2A; Table 2). Furthermore, root CO_2 evolution in vials containing native grass roots was almost identical to that in vials with no added roots. Additionally, root CO_2 evolution decreased significantly over time, though there was no significant vegetation type × time interaction (Figure 2A; Table 2). In summary, native grass roots made no detectable contribution to rates of CO_2 evolution, whereas *Agropyron* roots doubled the rate of CO_2 evolution.

Carbon dioxide evolution from the shoot-amended soil was significantly greater (63–64%) than that from the controls for both *Agropyron* and the native grasses. Moreover, CO_2 evolution from soil amended with *Agropyron* shoots was greater than from soil amended with native grasses, but only at the start of the incubation (Figure 2B; Table 2). Carbon dioxide evolution from soil amended with the shoots of both vegetation types decreased significantly over time, though the decrease in CO_2 evolution associated with *Agropyron* occurred significantly faster than that of the native grassland (vegetation × time interaction) (Figure 2B; Table 2).

Tissue Quality

Root lignin content was significantly less for *Agropyron* than for native grasses (Figure 3A, $F_{1,16} = 9.63$, $P = 0.01$), but root N content and lignin:N ratio did not differ significantly between the two

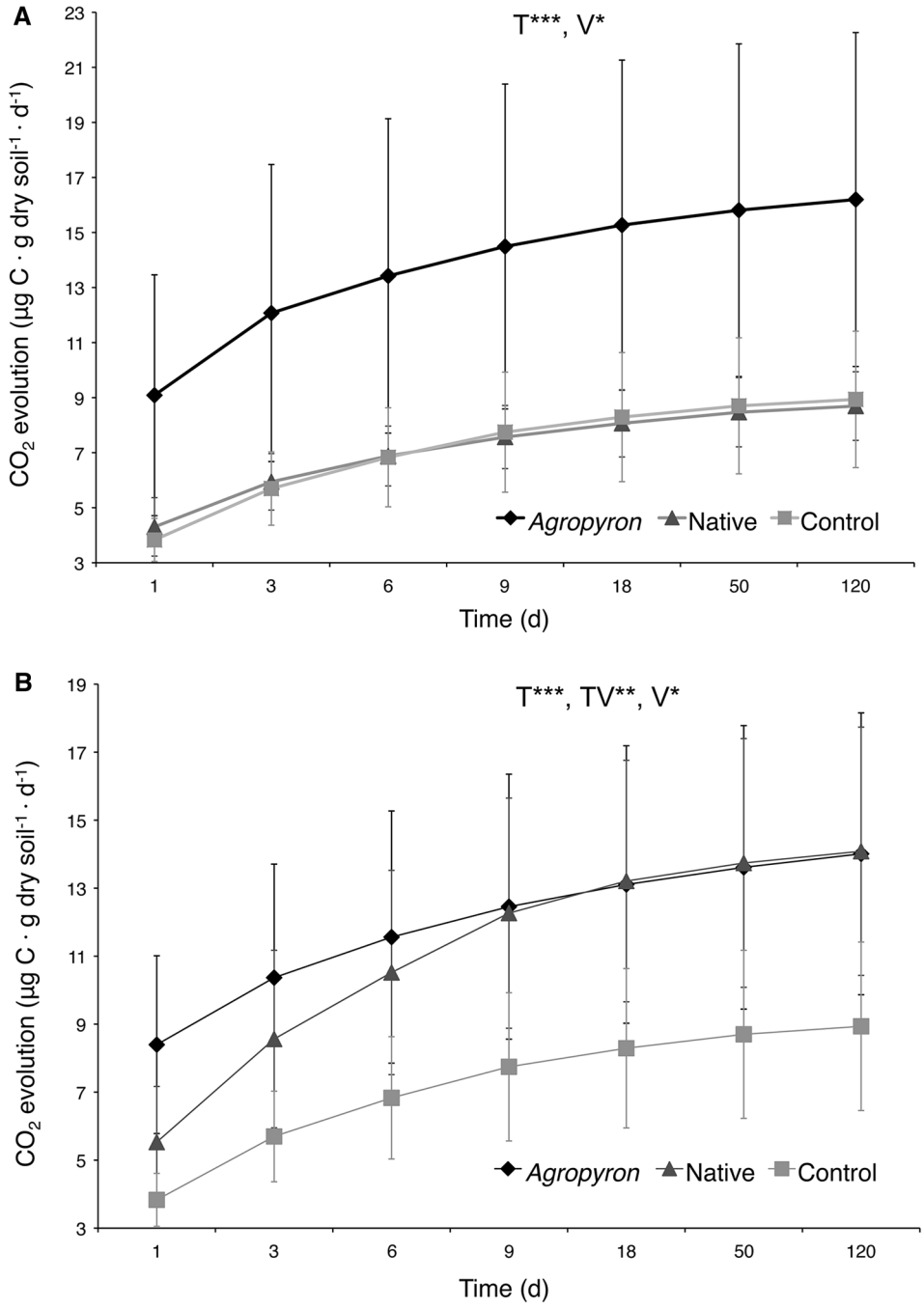

Figure 2. Daily rate of **A** root and **B** shoot CO_2 evolution (mean ± SD) in parts per thousand (ppt) from litter of the invasive grass *Agropyron cristatum* (*diamonds*), native grassland (*triangles*), and in controls (*squares*) over 120 days. *T* time, *V* vegetation type, *TV* interaction; *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.0001$.

vegetation types ($F_{1,16} = 0.21$, $P = 0.65$ and $F_{1,16} = 0.58$, $P = 0.46$, respectively).

Shoot lignin content was significantly greater for *Agropyron* than for native grasses ($F_{1,16} = 25.73$, $P = 0.001$). Similar to roots, shoot N content did not differ between the two vegetation types ($F_{1,16} = 1.93$, $P = 0.18$). However, shoot lignin: N ratio was significantly greater for *Agropyron* than for native grassland (Figure 3B, $F_{1,16} = 9.63$, $P = 0.01$).

Discussion

Roots of the invasive grass *Agropyron* had significantly faster decomposition and lower lignin concentration than did those of native grassland species. These differences may account for the lack of a significant difference in soil C between *Agropyron* stands and native grassland, even though *Agropyron* had a much larger root system (that is, significantly greater root length) than the native grassland.

Table 2. Repeated Measures ANOVA Results for Root and Shoot CO_2 Evolution, with Vegetation Type as a Factor

Source of variation	NumDf	DenDf	*F* value	*P* value
Root				
Between subjects				
Vegetation type	2	12	5.7392	0.0178*
Within subjects				
Time	6	7	48.5709	<0.0001*
Shoot				
Time × vegetation type	12	14	0.7571	0.6821
Between subjects				
Vegetation type	2	12	4.3683	0.0355*
Within subjects				
Time	6	7	205.253	<0.0001*
Time × vegetation type	12	14	5.3923	0.0019*

NumDf degrees of freedom in the model, DenDf degrees of freedom associated with the model errors.
** $P < 0.05$.*

Thus, our results for roots, which account for 50–90% of grassland production (Mokany and others 2006), conform to general results for shoots, which show a 50–120% increase in litter decomposition rates following invasion (Liao and others 2008).

This, to our knowledge, is the first comparison of root decomposition rates between invaded and uninvaded vegetation. Our findings that *Agropyron* roots decompose at a rate twice that of native grass species echo the results of Chen and Stark (2000) who found that rates of C mineralization in soils beneath *Agropyron* were greater, but not significantly so, than those in soils beneath the native shrub *Artemisia tridentata*.

Decomposition rates generally increase with tissue quality (Parton and others 2007) and we found evidence to support this link. Indeed, *Agropyron* roots, which had a significantly lower lignin content than the native grasses, were mineralized more rapidly—and to a greater extent—than the roots of the native grasses. Similarly, roots of *Agropyron* (and another invasive grass, *Bromus tectorum*) had lower lignin:N ratios than the native shrub *Artemisia tridentata* (Hooker and others 2008), and root C:N ratios were significantly lower beneath *Agropyron* than native grassland 250 km NW of our study site (MacDougall and Wilson 2011). In New Zealand, invader root lignin:N ratios were lower in the invasive forb *Hieracium* than in native grasses, but only at less-productive sites (Scott and others 2001). Root lignin:N ratios were generally lower in monoculture plantations of invasive than native grasses (Dijkstra and others 2006). Overall, the trend is towards higher root tissue quality in invaded grasslands.

Shoots differed from roots in that tissue quality was lower in the invasive *Agropyron* than in native grasses both at our study site (Figure 3) and in North Dakota (Hendrickson and others 2001). Similarly, shoot tissue quality is lower in the invasive grass *Bromus tectorum* (Evans and others 2001) and the forb *Hieracium* (Scott and others 2001) than in native grasses. Despite differences in tissue quality between roots and shoots, C sequestration in grasslands may be more affected by roots than by shoots because 80–90% of the biomass in grasslands occurs below ground (Mokany and others 2006). Further, because photodegradation plays an important role in grassland leaf decomposition, the effect of leaf tissue quality on belowground C is likely to be relatively minor (Parton and others 2007).

Greater root length, mass, and number for *Agropyron* compared to native species is well-documented (Richards 1984; Bilbrough and Caldwell 1997; Ivans and others 2003; Peek and others 2005; MacDougall and Wilson 2011; Balogianni and others 2014). Mowing had no significant effect on roots either at our study site or in Kansas (Kitchen and others 2009). Similarly, N addition had no effect on roots, confirming results for *Agropyron* roots in Utah at similarly low rates of N addition (Bilbrough and Caldwell 1995). Thus, environmental variation caused by biomass removal or nutrient availability is unlikely to influence the magnitude of the difference in root length between *Agropyron* stands and native grassland.

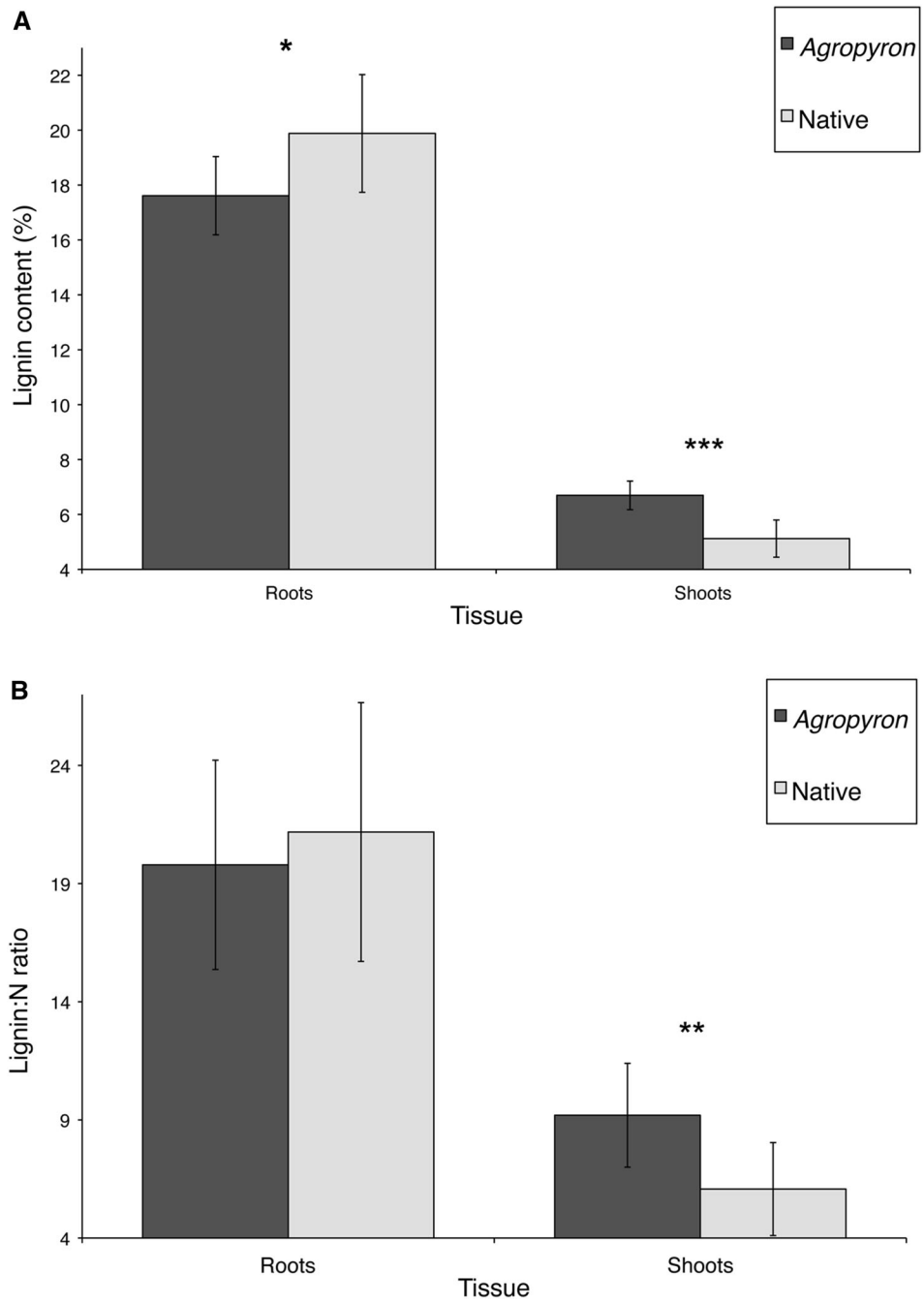

Figure 3. **A** Lignin content (mean ± SD) (%) and **B** lignin:N ratio of plant tissue in root and shoots of the invasive grass *Agropyron cristatum* (*dark bars*) and native grassland (*light bars*). *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$.

In spite of significantly greater root length beneath *Agropyron*, soil C did not differ significantly between the two different vegetation types. Similar results were found in previous studies (Chen and Stark 2000; Krzic and others 2000; Willms and others 2005; Hooker and others 2008; MacDougall and Wilson 2011). Differences in the rates of root decomposition between *Agropyron* and native grasses may explain how the invader can increase root length (and presumably rates of root litter deposition into soil) without increasing soil C.

In addition to litter quality and root length, other factors may influence the proportion of root and shoot litter C that is eventually stabilized in the soil. For example, potential differences in soil parameters (soil as structure and moisture) between the two vegetation types might have modified the litter decomposition and soil C formation in our study sites (Cotrufo and others 2013). The greater *Agropyron* root length had no effect on soil moisture in the present study (data not shown), although it might have had a direct effect on soil structure by

enhancing soil porosity and soil aggregation (Milleret and others 2009). Such an alteration in soil structure would have further increased C soil storage through the incorporation of labile material into soil aggregates (Jastrow and Miller 1997). However, soil C did not increase, either because litter quality was a more important factor than soil structure in C decomposition or because soil structure was not affected by *Agropyron* invasion. Other parameters that affect soil C formation that might have been altered by *Agropyron* invasion include microbial community composition, soil pH and N levels, mycorrhizal associations, and microclimate (Bronick and Lal 2005; Wilson and others 2009; Cotrufo and others 2013).

A retrospective power analysis of soil C data revealed a possibility of committing a Type II error ($\beta = 0.16$). The lack of power to detect significant changes in soil C, given the large soil heterogeneity at fine scales, is a common issue (Throop and Archer 2008), and the ability to detect these changes is largely site-specific (Saby and others 2008). Thus studies conducted at fine spatial and temporal scales consider changes in soil C at $P < 0.10$ as significant (Carney and others 2007; Strickland and others 2010). However, even at the 10% significance level, differences in soil C between our vegetation types were statistically undetectable ($P = 0.34$).

Roots continued to colonize the minirhizotron tube surface 5–7 years after tube installation (Figure S1). Thus, in this study root length may reflect the ability of roots to colonize empty soil following a disturbance (that is, rhizotron tube installation) rather than root length at equilibrium. Nevertheless, comparisons between the invasive and native species should still be valid. Isotope dilution studies in a semiarid Colorado grassland estimated root turnover at 5.4 years (Milchunas 2009). Turnover time may be longer further north, for example, at our study site, or rhizotrons may give different values that other root turnover methods (Strand and others 2008).

Shoot mass was significantly reduced by mowing, more so for *Agropyron* than native species (Figure 1, significant vegetation type × mowing interaction: Table 2). This may reflect the taller stature of *Agropyron* relative to native grassland dominated by the short grass *Bouteloua gracilis* and the spike moss *Selaginella densa* (Wilson and Pärtel 2003). In contrast to the differential impact of mowing on invaded and native grassland, N addition caused shoot mass of both vegetation types to be increased to about the same extent (Figure 1, no vegetation type × N interaction: Table 2). This result is in contrast to observations that N addition favors invasive grassland species (Maron and Jeffries 2001; Seabloom and others 2003; Foster and others 2009). Our results, however, are for separate invaded and native stands, and do not preclude the possibility that high nutrient availability increases invasion rates of *Agropyron* into native vegetation.

One implication of our root results is that the proposed positive feedback by invasive species that both cause and benefit from rapid nutrient cycling (Cornwell and others 2008; Chapin III and others Chapin and others 2009, Strickland and others 2010) also applies to relatively unproductive grassland habitats, and thus possibly also deserts and tundra where production is mostly belowground. Going further, higher nutrient concentration in one invasive species has been shown to accelerate the decomposition of pre-existing soil C in invaded soils, resulting in invasion-driven losses in accumulated soil C (Tamura and Tharayil 2014).

Thus, our results highlight a potential increase in CO_2 evolution following invasion. Exotic plants invade approximately 700,000 ha/year of U.S. wildlife habitat (Pimentel and others 2005). If invaders double CO_2 evolution in general, then a major shift may occur in how wild lands regulate atmospheric C. Invasion may result in less C storage, causing soils to switch their role from C sinks to C sources.

In summary, *Agropyron* increased root length regardless of mowing and N addition treatments. This increase, however, was not associated with an increase soil C. *Agropyron* roots had significantly higher decomposition rates and lower lignin concentrations than roots of native grassland. Together, the results suggest that the increased mass and root length of invasive species have no effect on soil C because the dominant tissue produced, roots, has a higher decomposition rate.

ACKNOWLEDGEMENTS

We thank Medicine Lake National Wildlife Refuge for access and logistical support. We thank anonymous reviewers and S. Hobbie for helpful comments and the Natural Sciences and Engineering Research Council of Canada for funding.

REFERENCES

Balogianni VG, Wilson SD, Vaness BM, MacDougall AS, Pinno BD. 2014. Distinct root and shoot responses to mowing and fertility in native and invaded grassland. Rangel Ecol Manag 67:39–45.

Belnap J, Phillips SL. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecol Appl 11:1261–75.

Bilbrough CJ, Caldwell MM. 1995. The effects of shading and N status on root proliferation in nutrient patches by the perennial grass *Agropyron desertorum* in the field. Oecologia 103:10–16.

Bilbrough CJ, Caldwell MM. 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. Ecology 78:231–43.

Bradford MA, Watts BW, Davies CA. 2009. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. Glob Chang Biol 16:1576–88.

Bronick CJ, Lal R. 2005. Soil structure and management: a review. Geoderma 124:3–22.

Carrillo Y, Dijkstra FA, LeCain D, Morgan JA, Blumenthal D, Waldron S, Pendall E. 2014. Disentangling root responses to climate change in a semiarid grassland. Oecologia 175:699.

Carney KM, Hungate BA, Drake BG, Megonigal JP. 2007. Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proc Natl Acad Sci 104:4990–5.

Castro-Diez P, Godoy O, Alonso A, Gallardo A, Saldaña A. 2014. What explains variation in the impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. Ecol Lett 17:1–12.

Chapin FSIII, Matson PA, Mooney HA. 2002. Principles of terrestrial ecosystem ecology. New York: Springer.

Chapin FSIII, McFarland J, McGuire AD, Euskirchen ES, Ruess RW, Kielland K. 2009. The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. J Ecol 97:840–50.

Chen J, Stark JM. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biol Biochem 32:47–57.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Perez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Diaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065–71.

Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Chang Biol 19:988–95.

Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA. 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Glob Chang Biol 15:2003–19.

Dalias P, Anderson JM, Bottner P, Coûteaux MM. 2001. Temperature responses of carbon mineralization in conifer forest soils from different regional climates incubated under standard laboratory conditions. Glob Chang Biol 7:181–92.

Dìaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jalili A, Montserrat-Marti G, Grime JP, Zarrinkamar F, Asri Y. 2004. The plant traits that drive ecosystems: evidence from three continents. J Veg Sci 15:295–304.

Dijkstra FA, Hobbie SE, Reich PB. 2006. Soil processes affected by sixteen grassland species grown under different environmental conditions. Soil Sci Soc Am J 70:770–7.

Ehrenfeld JG. 2010. Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80.

Ehrenfeld JG. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–23.

Evans RD, Rimer R, Sperry L, Belnap J. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecol Appl 11:1301–10.

Fierer N, Schimel JP. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777–87.

Fierer N, Craine JM, McLauchlan K, Schimel JP. 2005. Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–6.

Foster BL, Kindscher K, Houseman GR, Murphy CA. 2009. Effects of hay management and native species sowing on grassland community structure, biomass, and restoration. Ecol Appl 19:1884–96.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu WD, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao JP, Cornelissen JHC. 2013. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. J Ecol 101:943–52.

Heidinga L, Wilson SD. 2002. The impact of an invading alien grass (*Agropyron cristatum*) on species turnover in native prairie. Divers Distrib 8:249–58.

Henderson DC, Naeth MA. 2005. Multi-scale impacts of crested wheatgrass invasion in mixed-grass prairie. Biol Invasions 7:639–50.

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo DL. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94:40–57.

Hendrickson JR, Wienhold BJ, Berdahl JD. 2001. Decomposition rates of native and improved cultivars of grasses in the Northern Great Plains. Arid Land Res Manag 15:347–57.

Hickman JE, Ashton IW, Howe KM, Lerdau MT. 2013. The native–invasive balance: implications for nutrient cycling in ecosystems. Oecologia 173:319–28.

Hooker TD, Stark JM, Norton U, Leffler AJ, Peek M, Ryel R. 2008. Distribution of ecosystem C and N within contrasting vegetation types in a semiarid rangeland in the Great Basin, USA. Biogeochemistry 90:291–308.

Ivans CY, Leffler AJ, Spaulding U, Stark JM, Ryel RJ, Caldwell MM. 2003. Root responses and nitrogen acquisition by *Artemisia tridentata* and *Agropyron desertorum* following small summer rainfall events. Oecologia 134:317–24.

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411.

Jastrow JD, Miller RM. 1997. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. In: Lal R, Kimble JM, Follett RF, Stewart BA, Eds. Soil processes and the carbon cycle. Boca Raton: CRC Press. p 207–23.

Jobbágy EG, Jackson RB. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–36.

Johnson JMF, Barbour NW, Weyers SL. 2007. Chemical composition of crop biomass impacts its decomposition. Soil Sci Soc Am J 71:155–62.

Kitchen DJ, Blair JM, Callaham MA Jr. 2009. Annual fire and mowing alter biomass, depth distribution, and C and N content of roots and soil in tallgrass prairie. Plant Soil 323:235–47.

Köchy M, Wilson SD. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. J Ecol 89:807–17.

Krzic M, Boersma K, Thompson DJ, Bomke AA. 2000. Soil properties and species diversity of grazed crested wheatgrass and native rangelands. J Range Manag 53:353–8.

Lee JS, Daniels BL, Eberiel DT, Farrell RE. 2000. Polymer mineralization in soils: effects of cold storage on microbial populations and biodegradation potential. J Polym Environ 8:81–9.

Lesica P, DeLuca T. 1996. Long-term harmful effects of crested wheatgrass on Great Plains grassland ecosystems. J Soil Water Conserv 51:408–9.

Liao CZ, Peng RH, Luo YQ, Zhou XH, Wu XW, Fang CM, Chen JK, Li B. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytol 177:706–14.

Loya WM, Johnson LC, Nadelhoffer KJ. 2004. Seasonal dynamics of leaf-and root-derived C in arctic tundra mesocosms. Soil Biol Biochem 36:655–66.

Luyssaert S, Schulze ED, Borner A, Knohl A, Hessenmoller D, Law BE, Ciais P, Grace J. 2008. Old-growth forests as global carbon sinks. Nature 455:213–15.

MacDougall AS, Wilson SD. 2011. The invasive grass *Agropyron cristatum* doubles belowground productivity but not soil carbon. Ecology 92:657–64.

Maron JL, Auge H, Pearson DE, Korell L, Hensen I, Suding KN, Stein C. 2014. Staged invasions across disparate grasslands: effects of seed provenance, consumers and disturbance on productivity and species richness. Ecol Lett 17:499–507.

Maron JL, Jeffries RL. 2001. Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. Ecol Appl 11:1088–100.

Meisner A, Hol WHG, de Boer W, Krumins JA, Wardle DA, van der Putten WH 2014. Plant–soil feedbacks of exotic plant species across life forms: a meta-analysis. Biol Invas 1–11 (in press).

Milchunas DG. 2009. Estimating root production: comparison of 11 methods in shortgrass steppe and review of biases. Ecosystems 12:1381–402.

Milleret R, Le Bayon RC, Gobat JM. 2009. Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. Plant Soil 316:1–12.

Mokany K, Raison RJ, Prokushkin AS. 2006. Critical analysis of root: shoot ratios in terrestrial biomes. Glob Chang Biol 12:84–96.

Nave LE, Swanston CW, Mishra U, Nadelhoffer KJ. 2013. Afforestation effects on soil carbon storage in the United States: a synthesis. Soil Sci Soc Am J 77:1035–47.

Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–4.

Peek MS, Leffler AJ, Ivans CY, Ryel RJ, Caldwell MM. 2005. Fine root distribution and persistence under field conditions of three co-occurring Great Basin species of different life form. New Phytol 165:171–80.

Peltzer DA, Bellingham PJ, Kurokawa H, Walker LR, Wardle DA, Yeates GW. 2009. Punching above their weight: low-biomass non-native plant species alter soil properties during primary succession. Oikos 118:1001–14.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. New York: Cambridge University Press.

Richards JH. 1984. Root growth response to defoliation in two Agropyron bunchgrasses: field observations with an improved root periscope. Oecologia 64:21–5.

Saby NPA, Arrouays D, Antoni V, Lemercier B, Follain S, Walter C, Schvartz C. 2008. Changes in soil organic carbon in a mountainous French region, 1990–2004. Soil Use Manag 24:254–62.

Scott NA, Saggar S, McIntosh PD. 2001. Biogeochemical impact of *Hieracium* invasion in New Zealand's grazed tussock grasslands: sustainability implications. Ecol Appl 11:1311–22.

Seabloom EW, Borer ET, Boucher VL, Burton RS, Cottingham KL, Goldwasser L, Gram WK, Kendall BE, Micheli F. 2003. Competition, seed limitation, disturbance, and reestablishment of California native annual forbs. Ecol Appl 13:575–92.

Shipley B, Lechowicz MJ, Wright I, Reich PB. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87:535–41.

Steinaker DF, Wilson SD. 2008a. Scale and density dependent relationships among roots, mycorrhizal fungi and collembola in grassland and forest. Oikos 117:703–10.

Steinaker DF, Wilson SD. 2008b. Phenology of fine roots and leaves in forest and grassland. J Ecol 96:1222–9.

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R. 2008. Irreconcilable differences: fine-root life spans and soil carbon persistence. Science 319:456–8.

Strickland MS, Devore JL, Maerz JC, Bradford MA. 2010. Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. Glob Chang Biol 16:1338–50.

Tamura M, Tharayil N. 2014. Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. New Phytol 203:110–24.

Taylor BN, Beidler KV, Cooper ER, Strand AE, Pritchard SG. 2013. Sampling volume in root studies: the pitfalls of undersampling exposed using accumulation curves. Ecol Lett 16:862–9.

Throop HL, Archer SR. 2008. Shrub (*Prosopis velutina*) encroachment in a semidesert grassland: spatial–temporal changes in soil organic carbon and nitrogen pools. Glob Chang Biol 14:2420–31.

Vila M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, Pergl J, Schaffner U, Sun Y, Pysek P. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–8.

Weidenhamer JD, Callaway RM. 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 36:59–69.

Willms WD, Ellert BH, Janzen HH, Douwes H. 2005. Evaluation of native and introduced grasses for reclamation and production. Rangel Ecol Manag 58:177–83.

Wilson GW, Rice CW, Rillig MC, Springer A, Hartnett DC. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–61.

Wilson SD, Pärtel M. 2003. Extirpation or coexistence? Management of a persistent introduced grass in a prairie restoration. Restor Ecol 11:410–16.

Zhang L, Wang H, Zou J, Rogers WE, Siemann E. 2014. Non-native plant litter enhances soil carbon dioxide emissions in an invaded annual grassland. Plos One 9:e92301.

Ziter C, MacDougall AS. 2013. Nutrients and defoliation increase soil carbon inputs in grassland. Ecology 94:106–16.