Ecology, 94(1), 2013, pp. 106–116

Nutrients and defoliation increase soil carbon inputs in grassland

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Abstract. Given the regulatory impact of resources and consumers on plant production, decomposition, and soil carbon sequestration, anthropogenic changes to nutrient inputs and grazing have likely transformed how grasslands process atmospheric CO_2. The direction and magnitude of these changes, however, remain unclear in this system, whose soils contain ~20% of the world's carbon pool. Nutrients stimulate production but can also increase tissue palatability and decomposition. Grazing variously affects tissue quality and quantity, decreasing standing biomass, but potentially increasing leaf nutrient concentrations, root production, or investment in tissue defenses that slow litter decay. Here, we quantified individual and interactive impacts of nutrient addition and simulated grazing (mowing) on above- and belowground production, tissue quality, and soil carbon inputs in a western North American grassland with globally distributed agronomic species. Given that nutrients and grazing are often connected with increased root production and higher foliar tissue quality, we hypothesized that these treatments would combine to reduce inputs of recalcitrant-rich litter critical for C storage. This hypothesis was unsupported. Nutrients and defoliation combined to significantly increase belowground production but did not affect root tissue quality. There were no significant interactions between nutrients and defoliation for any measured response. Three years of nutrient addition increased root and shoot biomass by 37% and 23%, respectively, and had no impact on decomposition, resulting in a ~15% increase in soil organic matter and soil carbon. Defoliation triggered a significant burst of short-lived lignin-rich roots, presumably a compensatory response to foliar loss, which increased root litter inputs by 33%. The majority of root and shoot responses were positively correlated, with aboveground biomass a reasonable proxy for whole plant responses. The exceptions were decomposition, with roots six times more decay resistant, and grazing impacts on tissue chemistry, with shoots undergoing significant alterations, while roots were unaffected. Because neither treatment affected concentrations of decay-resistant compounds in roots, the implied net effect is higher soil C inputs with potentially longer residency times. Areas managed with nutrients and moderate grazing in our study system could thus accumulate significantly more soil C than unmanaged areas, with a greater capacity to serve as sinks for atmospheric CO_2.

Key words: consumers; decomposition; grasslands; nutrient limitation; Nutrient Network (Nutnet); resources; soil carbon inputs; Vancouver Island, Canada.

Introduction

There is uncertainty regarding the circumstances whereby terrestrial ecosystems function as sources or as sinks of atmospheric carbon (C). Two factors likely to play an influential role are alterations of global nutrient budgets and changes in the abundance and identity of consumers, two of the most extensive anthropogenic impacts on ecosystems globally (Micheli 1999, Worm et al. 2002). Soil nutrients and consumers strongly affect primary production in terrestrial plant communities, thereby influencing C cycling through their controls on biomass, tissue quality, nutrient availability, and decomposition (Gruner et al. 2008). Studies on the effects of nutrient addition and consumers acknowledge that it is likely neither one nor the other that controls ecosystem function, but rather their interactive effects (Worm et al. 2002, Borer et al. 2006, Hillebrand et al. 2009). However, the nature of this interaction, and how it influences C cycling, remains unclear.

These uncertainties are especially pronounced in grasslands, which cover ~30% of the earth's terrestrial surface and where nutrient and consumer impacts are ubiquitous with grassland diversity, evolution, structure, and function (McNaughton 1984, Axelrod 1985, Scurlock and Hall 1998, Frank et al. 2002). Many of the consequences of anthropogenic changes to nutrients and consumers will unfold belowground, as 50–90% of grassland annual net primary productivity (NPP) occurs in the roots (Bontti et al. 2006, Mokany et al. 2006), and root production, senescence, and decomposition are highly influential processes in ecosystem C and N cycling (Van Der Krift and Berendse 2002). Despite this importance of root dynamics to ecosystem function, it

Manuscript received 18 November 2011; revised 16 August 2012; accepted 16 August 2012. Corresponding Editor: H. A. L. Henry.

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has been estimated that <10% of all studies of grassland C dynamics actually measure belowground responses (Cahill et al. 2009), and many are based on methodologically constrained data (e.g., estimating root NPP based on one-time destructive soil cores; see Milchunas et al. 2005, Bontti et al. 2006, Strand et al. 2008, Milchunas 2009). Estimates of root production based on soil cores, for example, are generally conservative compared to minirhizotron sampling (Milchunas 2009). It is also unclear whether belowground responses to nutrients and defoliation mirror those observed in foliage (Bardgett and Wardle 2003).

Given these methodological challenges, research on nutrient–consumer impacts on belowground processes, including C dynamics, has produced inconsistent generalizations (Bardgett and Wardle 2003, Derner and Schuman 2007, Augustine et al. 2011). Nutrient additions have been associated with both increased and decreased soil C, with the mechanisms explaining these divergences unclear (Hobbie 2008). Nutrient additions may lower soil C by shifting growth from roots to shoots (Christian and Wilson 1999), or by stimulating litter decomposition via decreases in the ratios of C:N or lignin : N in plant tissues. As a result, nutrient-based increases in production may not translate into increases in soil C storage (Heath et al. 2005, Billings et al. 2006, Steinbeiss et al. 2008). Alternatively, given that grasslands are typically nutrient limited, soil nutrient additions may increase soil C by stimulating belowground production (van Groenigen et al. 2006). Nutrient additions can also reduce microbial activity, possibly by intensifying the limiting effects of other resources such as P or soil moisture (Zeglin et al. 2007). This may, in turn, reduce decomposition despite elevated C availability.

Similarly, grazing has been associated with both increased and decreased soil organic matter and soil organic carbon (SOM and SOC, respectively), depending on environmental context including management, species composition, and site productivity (Vandermaarel and Titlyanova 1989, Briske and Richards 1995, Conant et al. 2001, Bardgett and Wardle 2003, Derner and Schuman 2007, Augustine et al. 2011, Bagchi and Ritchie 2011). Grazing may increase soil carbon by stimulating root production and litter inputs (Frank et al. 2002). Grazing can also trigger changes to tissue chemistry that decrease palatability, which, in turn, may reduce rates of decomposition (Frank and McNaughton 1993, Milchunas and Lauenroth 1993, Agrawal 1998, Hafner et al. 2012). Alternatively, persistent grazing can decrease soil C by reducing root productivity (and thus litter inputs; Bagchi and Ritchie 2010*a*, Augustine et al. 2011), by concentrating root production and litter accumulation toward the soil surface where it is more susceptible to decomposition (Milchunas and Lauenroth 1993, Derner et al. 2006, Derner and Schuman 2007, Ingram et al. 2008, Bagchi and Ritchie 2010*b*), or by stimulating microbial biomass and activity by increasing root exudation in defoliated plants (Bardgett and Wardle 2003).

Finally, strong interactions between nutrients and grazing may result in variable effects on soil C dynamics, through their combined influence on species diversity, microbial activity, biomass production, and tissue palatability (Collins et al. 1998, Gough and Grace 1998, Bardgett and Wardle 2003, Lal 2004, Suwa and Maherali 2008, Bagchi and Ritchie 2010*a*, 2011). The effect of grazing on belowground processes, for example, can vary with site productivity, with positive feedbacks sometimes occurring between grazing and belowground processes (e.g., grazing stimulating root production, soil C inputs, and microbial activity) at sites with higher resource availability (Bardgett and Wardle 2003, Gavrichkova et al. 2010, Augustine et al. 2011, Bagchi and Ritchie 2011, Hafner et al. 2012).

The uncertainty surrounding nutrient–defoliation responses makes it difficult to predict whether C storage will be higher in managed vs. unmanaged grasslands (Milchunas and Lauenroth 1993, Bardgett and Wardle 2003), with the effects of management a consequence of the balance between production, which drives soil C inputs, and tissue quality, which influences decomposition. An absence of management may result in less whole-plant production, but higher tissue C:N ratios and slower decomposition rates, theoretically leading to a net increase in soil C storage. Given the frequency of agricultural abandonment in North America since the mid-1900s (Houghton et al. 1999), and the role that unmanaged land plays in soil C storage globally (Wise et al. 2009), the issue of C storage in unmanaged grassland has relevance for modeling C dynamics. Alternatively, the net effect of managing grasslands with fertilization and grazing may be increased belowground production with higher C storage compared to unmanaged areas, despite possible elevations in decomposition as a result of fertilization.

Here, we tested these alternatives by quantifying the effects of soil nutrient addition and defoliation on above- and belowground plant dynamics and soil properties (including C and N cycling) in a western North American grassland system, investigating the impacts on C dynamics compared to unmanaged areas. We integrated continuous minirhizotron sampling of root production and mortality with sampling of aboveground biomass and plant tissue quality, including carbon fractions, to determine how plants respond to factorial combinations of soil nutrient additions and foliage loss. We then quantified litter decomposition rates, soil nitrogen levels, and accumulated soil organic matter and soil organic carbon, thereby determining the mechanistic pathways by which nutrients and defoliation can mediate soil C inputs in grasslands.

Methods

We worked in a 30-ha old-field site on Vancouver Island, Canada (48°48′ N, 123°38′ W). The site is

homogeneous in soil depth (>1 m) and plant cover with clay-silt-loam glaceomarine soils. Plant cover is composed of agronomic pasture species with global distributions, suggesting potential generality to pasture systems in other temperate regions of the world. The dominant species are C_3 agronomic perennial grasses (*Alopecurus pratensis*, *Poa pratensis*, *Festuca rubra*) and broad-leaved N_2-fixers (*Vicia* spp., *Trifolium* spp.). *A. pratensis* currently dominates most areas, including our study plots (cover 85% ± 5.6% [mean ± SE]; $n = 30$ 1-m^2 plots; A. S. MacDougall, *unpublished data*). A dark-brown organic-rich Ah horizon occurs to depths of 19–38 cm from the soil surface, with a mean pH of 5.5 (Maslovat 2002). We focused our belowground sampling within this area of the soil column because root activity was largely restricted to this zone; it also captures the top 10 cm of the soil column where rates of C sequestration are generally the highest (Conant et al. 2001).

The regional climate is Mediterranean, with annual rainfall at ~1100 mm; most precipitation falls from October to April when temperatures average 4°C, with a summer drought period from June to September. As recently as the 1860s, the study area was oak savanna with a species-rich grass–forb understory (MacDougall 2008). Plowing and planting of the agronomic species occurred in the late 1800s and these species have persisted since, with the site being grazed and occasionally hayed until 2001. It is now unmanaged, with black-tailed deer present but preferring forbs in adjacent areas (MacDougall 2008). This experiment is part of the Nutrient Network, a cooperative global experiment testing the effects of nutrients and consumers on grassland diversity and function (information *available online*).[3]

In 2007 we started experimental nutrient additions to 6 of 12 plots (5 × 5 m each) to test the effects of multiple nutrient limitation on productivity and consumers (Firn et al. 2011, Harpole et al. 2011). The remaining 6 plots were maintained as controls. Nutrient addition rates and sources were: 10 g $N \cdot m^{-2} \cdot yr^{-1}$ as time-release urea, 10 g $P \cdot m^{-2} \cdot yr^{-1}$ as triple-super phosphate, 10 g $K \cdot m^{-2} \cdot yr^{-1}$ as potassium sulfate, and 100 $g \cdot m^{-2} \cdot yr^{-1}$ of a micronutrient mix (MicroMax [6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn]; Scotts, Marysville, Ohio, USA). N, P, and K were applied annually; the micronutrient mix was applied only once, at the start of the study, to reduce potential toxicity effects on soil microbial processes.

The simulated grazing trials occurred in randomly located 2.5 × 2.5 m subplots in each of the 12 plots. On-site rotational sheep grazing trials occur at the study area, but these animals could not be used in the current study due to the small plot sizes. Defoliation by sheep in these trials was observed to be nonselective, with all grasses and forbs reduced to a canopy height of ~5 cm within three weeks, after which they were rotated to a different location (stocking rate: 5 animals per ~500 m^2). To simulate these effects, we uniformly mowed the grass throughout the plots to 5 cm at peak growing season in May 2010, and all cut biomass was removed with raking. We were not able to mimic the heterogeneous patterns of waste deposition by the grazers, which can have potentially significant effects on fine-scale production in grasslands (Steinauer and Collins 2001).

We used minirhizotron technology in 2010 to quantify responses of root production, root mortality, and total root biomass to the nutrient and simulated grazing treatments (Bartz Technology Corporation, Santa Barbara, California, USA). Minirhizotron tubes were installed in 2007, allowing three years for the roots to recolonize the areas disturbed by tube insertion (12 plots × 2 subplots [mowed/unmowed] = 24 tubes). The tubes were installed at 45° angles to depths up to 52 cm depending on rock obstruction (range 28–52 cm; mean depth 41 cm). Minirhizotron techniques allow for repeated sampling without additional disturbance, and yield more reliable estimates of root dynamics than harvest-based methods, which tend to underestimate root production (Frank et al. 2002, Steinaker and Wilson 2005, Milchunas 2009, MacDougall and Wilson 2011). Images (18 × 14 mm) were collected at 10-d intervals from May to July, for a total of 8 sampling periods, at 1.38 cm intervals along the entirety of each tube.

Root lengths were measured at every third depth increment in each tube ($n = 2759$ images). As C and N content in roots varies with diameter and color (MacDougall and Wilson 2011), roots were categorized by two color classes (white, brown) and two diameter classes (fine, <0.3 mm; coarse, ≥0.3 mm). Individual roots were tracked through time, allowing for calculations of length changes, timing of root arrival within the image, and time of death. In the initial image in each time sequence, all root measurements were classified as root biomass (i.e., standing crop); in all subsequent images, new roots (not present in the previous image) were recorded as "production," and roots that were black, disintegrated, or no longer visible were recorded as "mortality." Root length is indicative of belowground resource acquisition, as more extensive root networks have more surface area for absorbing moisture and nutrients. It is not, however, necessarily an accurate representation of levels of soil organic matter inputs from root litter, given that networks of fine roots can have far less biomass than networks of coarse roots. To account for this, we converted root length (m of roots/m^2 of image) to root mass (g root/m^2 of image) by calculating "specific root length (SRL; m root/g root) from fresh roots of each diameter and color class collected in July 2010 (Steinaker and Wilson 2005).

We estimated aboveground standing crop in unmowed plots by harvesting all aboveground biomass in

[3] http://nutnet.umn.edu/

two 10 × 100 cm strips per plot, and separating the biomass by living grass foliage, litter, and living forbs. All biomass was dried at 68°C for 48 h prior to weighing to the nearest 0.01 g.

To determine the responses of tissue quality to nutrients and defoliation, fresh root and foliage samples were analyzed for total C and N, as well as C partitioning (fractions of the tissue C pool composed of percentages of lignin, cellulose, and dextrose [simple sugar]) at the Agri-food Laboratory, Guelph, Ontario, Canada. Laboratory gloves were used during sample collection and handling to prevent C contamination. Root and foliage samples were taken from both mowed and unmowed sections of each of the 12 main plots in July 2010, for a total of 24 samples of each tissue type (root or foliage). Root samples were obtained from 10 cm diameter cores, by rinsing and sieving the cores to leave only the roots. Foliage samples came from the aboveground biomass harvests, and were consolidated from randomly selected leaves from different plants to minimize possible among-plant variability in tissue quality. For mowed plots, foliage samples were taken from post-mow regrowth.

To determine the pools sizes of soil nitrogen (N), SOM, and SOC, a 10 cm diameter soil sample to 20 cm deep was collected from each of the 12 main plots. Soil was sieved to remove rocks, separated from roots, and air-dried, with elemental analysis used to quantify total C, organic C, SOM, NH_4^+, and NO_3^- values (University of Guelph Soil Lab, Guelph, Ontario). We also measured soil inorganic carbon, but it was only detected in minute amounts in one sample.

To quantify the effects of nutrient addition on decomposition of roots and foliage, we conducted an 11-month (July 2010–June 2011) litterbag trial using plant tissue collected from random locations within each plot. Root bags were 5 × 5 cm, constructed of 50-micron sterilized polyester monofilament woven mesh (Industrial Netting, Minneapolis, Minnesota, USA). Foliage bags were 8 × 8 cm, and constructed from 1-mm nylon mesh. All tissue was collected from the dominant grass *A. pratensis*, so the treatment effects would not be confounded by species. *A. pratensis* dominated both control plots and nutrient addition plots. Two bags each of roots and foliage were constructed using tissue from each of the 12 main plots ($n = 48$ bags). Root and foliage samples were oven-dried to constant mass at 65°C, following common protocol for decomposition studies (e.g., Hobbie 2008, Hobbie et al. 2010, Goebel et al. 2011). We used fresh (white) roots to ensure that decomposition had not yet begun, as justified by Hobbie (2008). Root bags contained 0.5 + 0.05 g (oven-dried mass), and were placed randomly into 10 cm deep slits in soil not subject to nutrient additions (i.e., tissue was harvested from fertilized and unfertilized plants, but the decomposition trials occurred in unfertilized areas). Foliage was similarly harvested after reproduction but before full senescence, again to ensure that decomposition had not started. Each foliage bag contained 2.0 ± 0.1 g (oven-dried mass), and was installed on the soil surface under the litter layer. After harvest, contents were oven-dried and reweighed.

Statistical analyses

Data analyses were performed in SAS version 9.2 (SAS Institute 2009). The experiment was a completely randomized factorial design, with the minirhizotron component assessed with a split-plot analysis. For the minirhizotron data, we analyzed root production (growth), mortality, and root biomass (standing crop), each of which were recorded in terms of root length (m root/m^2 image), and root mass (g root/m^2 image), for a total of six dependent variables. Given the variability in tube depth, we analyzed root responses to 28 cm (the deepest location in the soil column with data available for all tubes). Extending the analysis to greater depths (i.e., in the 21 of 24 tubes with images deeper than 28 cm) did not change the outcome of any of the analyses, as root production, mortality, and standing crop observed in all of the 24 tubes were mostly concentrated above this depth.

We first determined the background effect of nutrient addition on root dynamics. The data for each of the six dependent variables were run as a repeated-measures mixed model analysis. Color, diameter, date, and nutrient addition were considered fixed effects, and plot considered a random effect. Time was treated as a repeated measures variable, with repeated measures taken on plot × color × diameter interaction. Various covariance structures were tested, and chosen based on fit statistics. If judged necessary according to the residual analysis, data were grouped by independent variables to account for heterogeneity of errors. All decisions were based on F tests at a Type I error rate of 5%, with a Kenward-Roger correction applied to the degrees of freedom (e.g., Bolker et al. 2008). Residual normality was confirmed in order to assess model assumptions, and inference statistics were generated and outliers removed using Lund's test. Linear and quadratic contrasts were generated for trends over time, as were contrasts comparing root dynamics in nutrient-added vs. control plots. Post hoc comparisons were made using Tukey-Kramer HSD tests. To analyze root responses by depth, data were treated similarly, except that responses for each plot were averaged over time (rather than summed over depth) for each color/diameter class, and depth was treated as the repeated measures variable. Linear and quadratic contrasts were generated for depth, as well as contrasts comparing root dynamics in nutrient-added vs. control plots by depth. For the second level of analysis, mowing was added as a split-plot factor (with nutrient addition as the whole plot factor), and the data treated as above.

Content of C and N for roots and foliage was analyzed as a split-plot design in a general linear model. The dependent variables were root and foliage C:N, and

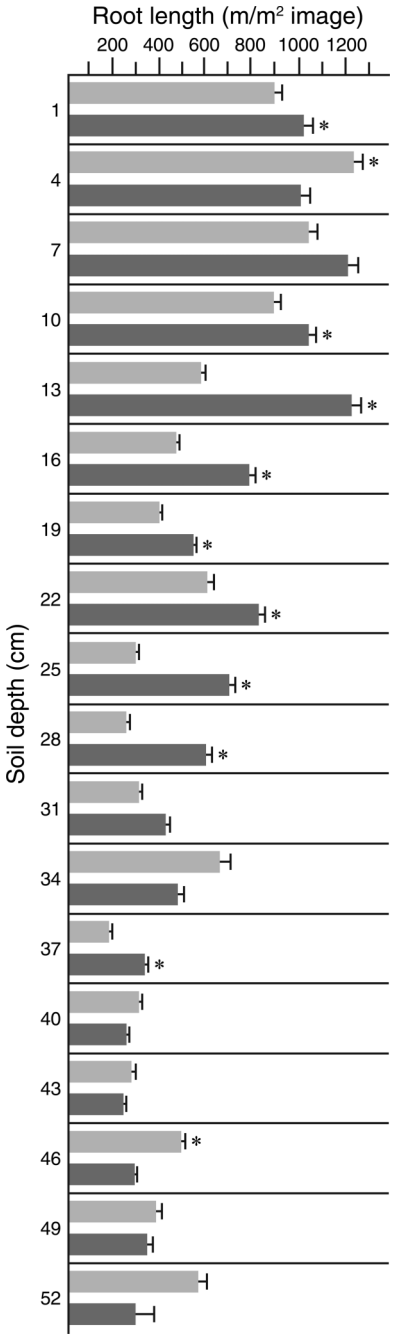

FIG. 1. Root length by soil depth, in fertilized plots (dark-gray bars) and control plots (light-gray bars), averaged over the 2010 growing season (mean + SE) from grassland on southeastern Vancouver Island, Canada. Root responses to the combined effect of fertilization and mowing did not differ significantly from those for fertilization alone, and are not shown. Responses to mowing did not differ significantly from the controls and are also not shown. An asterisk (*) indicates significant ($P < 0.05$) nutrient effects on root length between fertilized vs. control plots at each depth category.

independent variables for each were nutrient addition, mowing, and their interaction. Nutrient addition was treated as the whole-plot factor, with mowing as the split-plot factor. Root and foliage C-partitioning data was also analyzed using a general linear model, with lignin, dextrose, and cellulose as dependent variables, and nutrient addition, mowing, and their interaction as independent variables for each analysis.

Soil and decomposition data were analyzed by ANOVA, with NH_4^+, NO_3^-, total carbon, and soil organic matter as the dependent variables, and nutrient addition as the independent variable. For the decomposition analysis, the dependent variable was change in mass, with nutrient addition as the independent variable.

RESULTS

Although we anticipated interactions between nutrients and defoliation, all responses were either additive (e.g., the treatments combined to increase production) or individual (e.g., defoliation increased root production at similar magnitudes in both control and fertilized plots, nutrient additions similarly increased root production deeper in the soil column in both control and defoliated plots). Given the absence of interactions, we focused our analysis on the main effects of the two treatments.

Nutrient additions

Nutrients increased early-season root growth, for root length (m roots/m^2 of image, $F_{1,95.6} = 15.68$, $P = 0.001$) and root biomass (g roots/m^2 of image, $F_{1,164} = 6.25$, $P = 0.0135$). Nutrients also increased the final total standing crop of roots ($F_{1,7} = 28.1$, $P < 0.0001$), and deepened the zone of concentrated root accumulation (Fig. 1). The total number of dying roots per image per sampling period (for both length and biomass) increased with nutrient addition (m roots/m^2 of image, $F_{1,43.7} = 6.30$, $P = 0.0158$; g roots/m^2 of image, $F_{1,47.3} = 9.50$, $P = 0.0035$).

Nutrients caused no change to root : shoot ratios (nutrient added: 0.79 ± 0.1 [mean ± SE]); control: 0.87 ± 0.36), as increases in root biomass were matched by increases in aboveground production. Tissue quality in roots and shoots also responded similarly to nutrient addition, with significant reductions in C:N ratios with nutrients compared to control plants: Ratios of C:N for foliage decreased from 30.9 ± 1.3 to 27.0 ± 1.0, and ratios for roots decreased from 35.4 ± 3.0 to 30.8 ± 1.8. These reductions in C:N were largely explained by more total N in plant tissue, especially in foliage (a 13% increase; $F_{1,23} = 9.56$, $P = 0.0057$), but also in roots even though the increase in total N was not significant ($F_{1,23} = 4.27$, $P = 0.052$).

Despite the shifts in C:N ratios, nutrients had no significant effect on the percentage of lignin, lignin : N ratios, cellulose, or dextrose in foliage or root tissue (Fig. 2a, b). Nutrient addition increased the percentage of N in leaves ($F_{1,7} = 10.91$, $P = 0.02$), but not in roots.

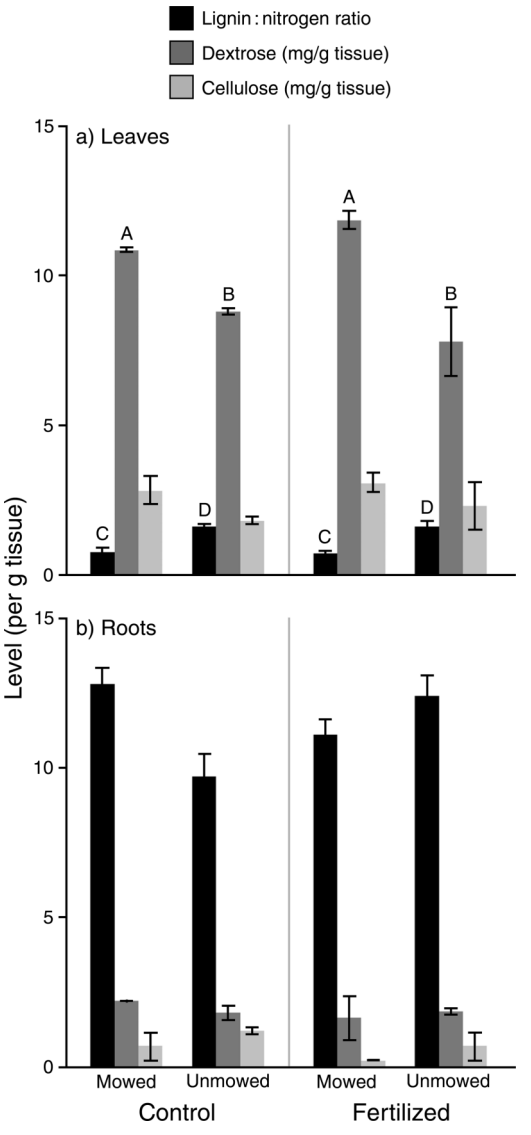

FIG. 2. Responses of lignin : nitrogen ratios, and levels of dextrose and cellulose per gram of tissue in (a) shoots and (b) roots following fertilization and mowing. Values are means ± SE. Letters indicate post hoc *t* test differences within each of the three responses (A, B, dextrose; C, D, lignin : N; cellulose levels were unaffected). There were no significant ($P < 0.05$) root responses or significant interactions between mowing and fertilization for any measure.

Overall, the lignin content of root tissue was ~4× greater than leaves, regardless of nutrient addition (Fig. 2a, b).

There was no difference in litter decomposition with nutrient addition after 11 months for either roots ($F_{1,10} = 0.1354$, $P = 0.7206$) or foliage ($F_{1,10} = 0.8738$, $P = 0.3719$). There were significant differences, however, in decomposition between leaves vs. roots, with mass loss of foliage biomass at 65% ± 0.67% compared to only 11% ± 1.16% for roots.

Three years after the initiation of nutrient addition, soil organic matter and total soil carbon (C) were both elevated compared to controls (OM, $F_{1,11} = 10.72$, $P = 0.008$; C, $F_{1,11} = 9.67$, $P = 0.01$; Fig. 3). Background (i.e., control plots) levels of organic matter were 92 ± 1.13 g/kg of soil, with total C levels of 50.5 ± 0.77 g/kg of soil. Nutrient addition increased these values to 106.5 ± 4.01g/kg and 56.7 ± 2.5 g/kg, respectively. Levels of soil NO_3^- and NH_4^+ were slightly higher in soils subject to nutrient addition, but these differences were insignificant (NO_3^-, $F_{1,11} = 1.47$, $P = 0.25$; NH_4^+, $F_{1,11} = 0.31$, $P = 0.58$), suggesting that added nitrogen had been immobilized, taken up by the plants, or lost through leaching.

Defoliation effects

Loss of foliage resulted in a burst of production of short-lived roots not seen in control plots ($F_{1,196} = 6.29$, $P = 0.0129$). This result was not influenced by nutrient addition (Fig. 4). The largest and only significant increase in root growth occurred immediately after mowing (Tukey's test), but root production in every sampling period for the rest of the summer was greater in the mowed plots. The combined effect by the end of July was a 29% increase in root growth caused by foliar removal earlier in the growing season.

Many of these new roots produced after mowing turned brown by the first sampling period (63% ± 12.5% of new roots in mowed plots; 16% ± 10.1% in unmowed plots). Root browning coincided with significant spikes in root death, immediately after mowing and in the last sampling period of the summer (Tukey's test; Fig. 4).

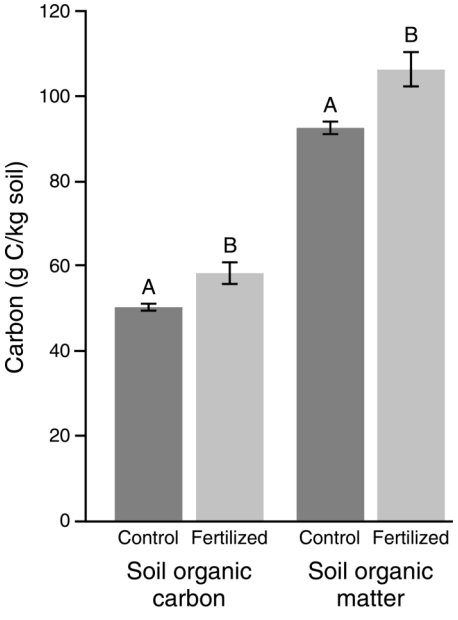

FIG. 3. Differences in soil organic carbon and soil organic matter (mean + SE) after three years of fertilization, from grassland on southeastern Vancouver Island, Canada. Letters indicate significant ($P < 0.05$) treatment differences, using post hoc Tukey tests.

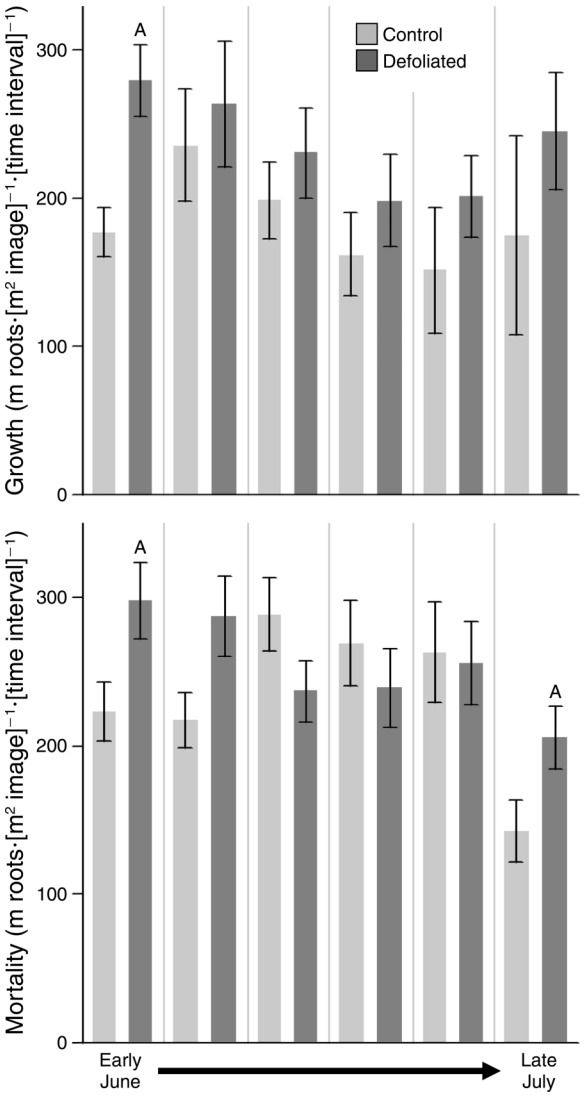

FIG. 4. Responses of root growth and mortality, following mowing in late May prior to the first measurement. Values are means ± SE. Responses to defoliation did not change significantly with fertilization; data are not shown. Both models contrasting mowing vs. controls are significantly different (growth, $F_{1,5} = 10.67$, $P = 0.001$; mortality, $F_{1,5} = 5.45$, $P = 0.001$), with "A" indicating significant mowing effects per time interval based on post hoc Tukey comparisons.

The combined affect by the end of July was a 33% increase in root mortality caused by shoot loss in May.

Combining the post-mow burst of root production with subsequent increases in root mortality, the end result was no net change in root standing crop between the start of the growing season prior to mowing vs. the end of the summer ($F_{1,7} = 1.21$, $P = 0.25$ for root length; $F_{1,7} = 0.20$, $P = 0.94$ for root biomass). Thus, the effects of foliage removal on root standing crop would have not been apparent without continuous sampling, with over one-third the roots observed at the end of the growing season not being present prior to mowing.

Mowing caused a range of responses in the tissue chemistry of foliage that were significantly more pronounced than the effects of nutrient addition on these same measures (Fig. 2a). In contrast, the tissue chemistry of roots showed no response to mowing (Fig. 2b). For leaves, there were reductions in the carbon complexity of newly produced foliage, with increases in simple sugars (dextrose; $F_{1,7} = 23.12$, $P = 0.003$) and declines in lignin ($F_{1,7} = 12.57$, $P = 0.01$). Cellulose levels did not change significantly ($F_{1,7} = 4.3$, $P = 0.08$). In contrast, roots showed no significant shift in lignin, percentage of N, lignin : N ratios, dextrose or cellulose content (Fig. 2b). There was a nonsignificant switch in the effect of defoliation on lignin : N ratios between fertilized and unfertilized plots (Fig. 2b), with the removal of foliage in fertilized plots associated with lower lignin : N ratios in roots than in unmowed plots ($F_{1,7} = 4.9$, $P = 0.08$).

DISCUSSION

We measured above- and belowground responses to nutrient additions and short-term simulated herbivory on agronomic pasture species in a cool and moist temperate grassland, and the effect of these treatments on soil C inputs. Past research indicates the potential for strong interactions between nutrients and grazing (Milchunas and Lauenroth 1993, Bagchi and Ritchie 2011). Nutrient enrichment can reduce root biomass relative to shoot production, while simultaneously increasing tissue quality and decomposition. Grazing can magnify these effects by enhancing leaf nutrient concentrations and levels of root exudation under conditions of high fertility, or possibly offset the effects of nutrient additions by stimulating the production of less decomposable lignin-rich tissue (Bardgett and Wardle 2003). None of these responses were observed. Instead, we found strong additive effects of nutrients and grazing on root biomass, with nutrient additions and mowing increasing production, but not affecting concentrations of recalcitrant carbon in root tissue. For nutrients, this can be explained mechanistically by substantial pretreatment nutrient constraints on above- and belowground production (LeBauer and Treseder 2008). For grazing, foliage removal triggered a significant pulse in the production of short-lived roots, presumably as a compensatory response to foliar loss, thereby increasing root litter deposition (Frank et al. 2002, 2010). Although we could not measure the longer term effects of defoliation on SOM and SOC, the production, mortality, and tissue chemistry data all imply that soil C stocks will increase in the short term. Management with nutrient additions and short-term grazing thus increased carbon stocks compared to unmanaged areas in our study system.

Generalizations about nutrient and consumer effects on grassland soil dynamics have often been based on aboveground responses, despite the fact that roots contribute higher levels of organic matter to soils

(Frank et al. 2002, 2010, Hobbie 2008, Hobbie et al. 2010). Root and shoot responses can be interdependent but not positively correlated, as when root : shoot ratios decrease as nutrient levels rise (Bloom et al. 1985). In the case of nutrient addition, we observed a range of mostly parallel responses between roots and shoots compared to control plots, with nutrients increasing biomass production of both tissue types, increasing production but maintaining root : shoot ratios, and having no affect on levels of lignin, lignin : N ratios, cellulose, or dextrose. For these measures, aboveground biomass served as a reasonable proxy for whole plant responses.

Divergent responses of roots and shoots were noted for two factors: decomposition and tissue quality following defoliation. As is often observed, roots were much more resistant to decomposition than shoots (Gholz et al. 2000, Hafner et al. 2012, but see Adair et al. 2008), a difference that was not altered by nutrient additions. Defoliation caused significant decreases in the lignin content of newly produced leaves, but had no effect on root tissue quality. The short but dramatic burst of root growth caused by defoliation was only evident with continuous monitoring, as many of these roots were short lived, such that root standing crop returned to pretreatment levels by the end of the growing season. A rapid increase in root production following grazing has been reported previously including by minrhizotron studies (Frank et al. 2002), and contradicts the results of short-term pot experiments that typically observe reduced root production with grazing (Frank et al. 2002). Although we did not measure SOM and SOC levels in the defoliated plots, the increase in the production of short-lived roots following defoliation, in both fertilized and control plots, suggests that the deposition of lignin-rich root litter in soils will increase significantly.

Our observed root responses are likely connected to the need to rapidly produce new foliage following defoliation (Detling and Painter 1983). The short pulse of root growth may be critical to rapidly acquire nutrients to support replacement of lost foliage. Post-defoliation leaves contained more nitrogen, a greater percentage of simpler sugars (dextrose), and less complex recalcitrant compounds, especially lignin. Higher leaf nitrogen tends to be an indicator of chlorophyll content and enhanced rates of photosynthesis, while higher sugar content tends to reflect the production of leaves with less metabolically expensive carbon compounds. These changes suggest an oft-observed possible trade-off in response to grazer impacts, where photosynthesis is quickly reinitiated but at the expense of plant defense, with higher foliage palatability in newly produced leaves potentially serving as an attractant to grazers (e.g., McNaughton 1984, Du Toit et al. 1990).

The increases in SOM and SOC following nutrient addition would be transient if coupled with increased rates of decomposition (Oren et al. 2001, Reich et al. 2006). Increased decomposition could occur if biomass production shifts from roots to more readily decomposable shoots, based on the assumption that fewer roots are needed as nutrient availability increases. Nutrient addition can also concentrate roots toward the soil surface, where they may be more prone to decomposition. These responses were not observed. Instead, root and shoot production were tightly coupled. Both roots and shoots had lower C:N ratios, but there was no change in lignin content, and nutrient addition drove root production deeper into the soil column. Given that lignin contributes to the slower decomposing recalcitrant carbon pool, the increase in lignin-rich root litter inputs suggests that pools of SOM and SOC will be more persistent compared to unmanaged areas at our study site (Conant et al. 2001, Adair et al. 2008).

Previous studies have also described a failure of nutrient addition to stimulate decomposition despite elevated production (and higher C availability for microbes), implying that nutrient additions can increase soil C storage despite elevated N levels in plant litter (Hobbie 2008, Fornara and Tilman 2012, Talbot and Treseder 2012). The mechanisms behind this lack of response by decomposers are unclear, possibly relating to shifts in the species composition of the microbial community following nutrient addition or a reaction between microbial by-products and N that results in decay-resistant compounds (Hobbie 2008). Nutrient additions may also intensify the limitations of other resources, which then limits microbes more than plants (Zeglin et al. 2007, Liu and Greaver 2010). Because we added N, P, K, and micronutrients to our nutrient plots, the most obvious limiting factor would be soil moisture, especially given the Mediterranean climate of our system, where soil moisture deficits can occur in the summer and early fall. Microbial activity can be maximized in temperate grasslands when moisture and temperature are at their highest, but most precipitation in mediterranean systems falls in the coldest months of the year. Strong seasonal variation in precipitation that includes dry summers constrains microbial processes, including decomposition in other systems (Gallardo and Schlesinger 1992, Hart et al. 1992, Cusack et al. 2009, Hafner et al. 2012).

Our results confirm that nutrient limitation can significantly restrict soil C inputs in grasslands, primarily through constraining net primary production (NPP; Hungate et al. 2003, Luo et al. 2004, Reich et al. 2006, van Groenigen et al. 2006). A meta-analysis of terrestrial systems demonstrated that nutrient additions stimulated NPP by 29% (LeBauer and Treseder 2008). We observed similar response levels, with nutrient additions elevating foliage biomass by 23% and root biomass by 37%. The high demand for nutrients in our system was further illustrated by the lack of change in the soil N pool despite three years of fertilization. Given that we observed increases in total N in foliar and root tissues, this presumably reflects rapid immobilization of nutri-

ents into living tissue, litter, and decaying OM (Reich et al. 2006). Leaching and mineralization could also explain some N loss (Liu and Greaver 2010), although the relative effect may be small given that seasonal rainfall does not coincide with peak growing season or the timing of nutrient addition.

The high demand on soil N may explain why nutrient additions did not alter belowground responses to defoliation. The effect of foliage loss on belowground processes can switch with resource availability, with limitations in nutrients or rainfall reducing root production and soil C inputs (e.g., Reynolds and Pacala 1993, Bagchi and Ritchie 2010*a*, Augustine et al. 2011, Hafner et al. 2012). We observed no effect of nutrients on the magnitude of root proliferation following defoliation, with root standing crop increasing by 15.1% with nutrients and 22.6% with both treatments combined, compared to control plots. This lack of response could also be influenced by our dominant species: an agronomic C_3 grass of temperate grasslands with high foliage production and a relatively low root : shoot ratio, which is planted for its ability to rapidly recovery following defoliation by grazers and haying. Our minirhizotron analyses show this species to possess a shallow and dense root system, which may assist in rapidly acquiring seasonal pulses in nutrients and moisture.

To conclude, grassland soils in temperate regions have long been associated with deep and carbon-rich organic layers where the residency time of organic matter can exceed several decades (Raich and Schlesinger 1992). In turn, the widespread cultivation of grasslands globally during the 19th and 20th centuries likely created a substantial efflux of CO_2 into the atmosphere (Schlesinger 1984, Davidson and Ackerman 1993, Knops and Tilman 2000). Here, we show the potential for management to increase stores of soil C in historically disturbed grasslands. The possibility of managing grasslands for increased carbon stocks has been described previously (Conant et al. 2001, Hobbie 2008, Fornara and Tilman 2012, Hafner et al. 2012), although the nutrient- and consumer-mediated conditions under which this may occur are unclear given that both factors could potentially stimulate decomposition. We show that nutrients and short-term grazing can increase belowground production, without altering concentrations of recalcitrant carbon compounds in root tissue. Although the net benefits of nutrient addition must be balanced against high N_2O release and high offsite CO_2 emissions associated with industrially manufactured nitrogen (e.g., by using legumes and P addition as an alternative to N addition; see Tilman et al. 2006), our work suggests that managed temperate grasslands could sequester significantly more soil C than unmanaged areas, with a greater capacity to serve as sinks for atmospheric CO_2.

Acknowledgments

Thanks to Rachel Germain, Irvin Banman, Collin Elder, Tim Ennis, Elsa Cleland, Stan Harpole, Lori Biederman, Jennifer Firn, Eric Seabloom, Elizabeth Borer, Sarah Hobbie, and Scott Wilson. Funding to C. Ziter was provided by a NSERC undergraduate fellowship. Funding to A. S. MacDougall was provided by NSERC, CFI-MRI, and the Nature Conservancy of Canada.

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