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# Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska

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**ABSTRACT**

Long-term fertilization of acidic tussock tundra has led to changes in plant species composition, increases in aboveground production and biomass and substantial losses of soil organic carbon (SOC). Root litter is an important input to SOC pools, though little is known about fine root demography in tussock tundra. In this study, we examined the response of fine root production and live standing fine root biomass to short- and long-term fertilization, as changes in fine root demography may contribute to observed declines in SOC. Live standing fine root biomass increased with long-term fertilization, while fine root production declined, reflecting replacement of the annual fine root system of *Eriophorum vaginatum*, with the long-lived fine roots of *Betula nana*. Fine root production increased in fertilized plots during an unusually warm growing season, but remained unchanged in control plots, consistent with observations that *B. nana* shows a positive response to climate warming. Calculations based on a few simple assumptions suggest changes in fine root demography with long-term fertilization and species replacement could account for between 20 and 39% of observed declines in SOC stocks.

**KEY WORDS:** *Betula nana*, *Eriophorum vaginatum*, fertilization, fine roots, ingrowth cores, minirhizotrons, soil organic carbon, tussock tundra

## INTRODUCTION

Aboveground primary production in acidic tussock tundra is most strongly constrained by nitrogen (N) and phosphorus (P) availability (e.g., Shaver and Chapin 1980, Chapin and Shaver 1985, Shaver and Chapin 1995, Shaver et al. 2001). Long-term N and P supplements have increased aboveground production and biomass, while changing species composition from a mixture of graminoids, evergreen shrubs and deciduous shrubs, to dominance by deciduous shrubs, particularly *Betula nana* (e.g., Chapin et al. 1995, Chapin and Shaver 1996, McKane et al. 1997, Shaver et al., 2001).

Belowground responses to long-term fertilization are less well known. Tussock tundra holds between 7 and 27 $kg/m^2$ of soil organic carbon (SOC) (Miller et al. 1984, Giblin et al. 1991, Mack et al. 2004, Clemmensen et al. 2006). Introduction of an isotopic label revealed that root litter dominates C inputs and SOC storage in tussock tundra (Loya et al. 2004). Recent results suggest that long-term N and P supplements may lead to substantial losses of SOC from deep soil horizons (Mack et al. 2004), reflecting increases in C efflux and or declines in C input to SOC pools. The mechanisms responsible for observed declines in SOC, however, remain unclear.

Root litter is an important input to SOC pools (Loya et al. 2004), yet little is known about the controls on fine root production, fine root biomass and fine root death in tussock tundra. Fine root demography may change with long-term fertilization, as a result of changes in biomass allocation and or changes in plant species composition. Investigators have long suggested that plants will allocate biomass such that no single resource exerts an overwhelming limitation on production (Brouwer 1962, Davidson 1969, Lambers 1983, Poorter and Nagel 2000). Plants are expected to allocate relatively more biomass to leaves, for instance, with substantial increases in

nutrient availability. A reduced flow of C to fine roots could lead to substantial loss of SOC over the long-term. Fine root demography may also change with long-term fertilization as a result of plant species replacement. In tussock tundra, the dominant graminoid, *Eriophorum vaginatum*, has been largely replaced by the deciduous shrub, *Betula nana*, with long-term fertilization (e.g., Chapin et al. 1995, Chapin and Shaver 1996, McKane et al. 1997, Shaver et al. 2001). Fine root architecture and demography of *E. vaginatum* and *B. nana* differ sharply. *B. nana* maintains a highly-branched, long-lived network of fine roots that is generally restricted to near surface organic horizons. In contrast, *E. vaginatum* exhibits an unbranched fine root system that reaches the maximum depth of thaw, dies back in the fall and is re-produced the following growing season (Chapin et al. 1979, Shaver and Cutler 1979, Sullivan and Welker 2005).

In this study, we asked the following question: has long-term fertilization led to changes in fine root demography and, if so, could these changes contribute to observed declines in the globally-important SOC stocks of tussock tundra? To answer this question, we used traditional harvest methods to estimate fine root biomass (e.g., Shaver et al. 2001), ingrowth cores (e.g., Neill 1992, Nadelhoffer et al. 2002) and minirhizotrons (e.g., Hendrick and Pregitzer 1996, Sullivan and Welker 2005) to estimate fine root production and a simple mathematical exercise to estimate SOC losses that could be attributable to declines in C inputs, as a result of changes in fine root demography with chronic fertilization.

## MATERIALS AND METHODS

### Site and Treatment Descriptions

The study was carried out in moist acidic tussock tundra (Bliss and Matveyeva, 1992) at the Long Term Ecological Research (LTER) site near Toolik Lake in the northern foothills of the Brooks Range, AK (68º 38’ N, 149 º 34’ W, 760 m asl). Aboveground vegetation biomass is

nearly evenly distributed among graminoids, deciduous shrubs, evergreen shrubs and mosses (Shaver and Chapin 1991). The soil is a Rustic Histic Aquiturbel with a 20-30 cm organic horizon (Schimel et al. 2004). Growing season air temperatures at the study site averaged 9.1ºC, soil temperatures at 20 cm averaged 3.9 ºC and precipitation averaged 19.0 cm between 1998 and 2005 (Table 1).

In 1989, four replicate blocks were established in relatively homogenous acidic tussock tundra on a 5% north-facing slope. Fertilizer treatments were assigned to 5 x 20 m plots within each block as part of a larger multi-factorial randomized complete block experiment. Plots were fertilized annually in late May or early June, with N applied as granular $NH_4NO_3$ at a rate of 10 g N $m^{-2}$ $yr^{-1}$ and P as triple super phosphate at a rate of 5 g P $m^{-2}$ $yr^{-1}$. In 1996, a second set of fertilizer plots was established and treated in the same manner as the earlier set. Data reported in the present study were collected during 2003 and 2004, when the long term plots had received nutrient amendments for 15 and 16 years, while the short term plots had received amendments for 7 and 8 years, respectively.

Air temperatures at 5 m were monitored using a CS500 air temperature and relative humidity probe (Campbell Scientific, Logan, UT) and precipitation was measured using a tipping bucket rain gauge (Texas Electronics, Inc., Dallas, TX) at the Toolik Main climate station, where data were logged to a CR21x datalogger (Campbell Scientific, Logan, UT). Soil temperatures at 10 cm in the experimental plots were measured using copper/copper-nickel thermocouple wires and logged to a CR21x datalogger. Maximum soil thaw depth was measured using a stainless steel thaw probe in late August of 1999-2005. Thaw depth measurements for 2004 were not included, as the measurements were taken in late July, rather than late August.

Ingrowth Cores

Angular soil cores (4.9 cm I.D.) were taken at 70° to the soil surface between July 26 and July 29, 2002, such that they sampled the upper 25 cm of the soil profile. Five replicate cores were taken from each treatment in each of the four blocks using a stratified random sampling protocol. Fine root biomass (<2.0 mm dia.) was removed from each core, while care was taken preserve soil horizons. Root-free soil was placed in a nylon mesh cylinder (2.0 mm mesh), such that bulk density of each horizon was near to natural conditions. Mesh cylinders were secured at both ends and returned to their respective core holes within 24 hours of harvest.

Ingrowth cores were harvested between July 19 and 25, 2003. Fine roots were removed from core, washed free of soil in a series of five de-ionized water baths and weighed. *E. vaginatum* fine roots are easily distinguished from those of other species and were, therefore, treated separately. Washed roots were dried for 24 hours at 60°C, ground to a fine powder and analyzed for C and N concentrations with a CHN analyzer (Leco Corporation, St. Joseph, MI).

Fine root biomass harvested from the soil cores and the subsequent ingrowth cores were scaled to the square meter using a volumetric approach. For instance, a 566 $cm^3$ core taken to a vertical depth of 25 cm was assumed to represent approximately 1/442 of a 25 cm deep square meter of soil. The use of angular ingrowth cores and the volumetric scaling approach has two theoretical advantages over traditional vertical cores. First, angular cores should disrupt the vegetation immediately above the ingrowth cores to a lesser degree than vertical cores. This may be particularly advantageous when the vegetation is dominated by plant species with roots that grow predominantly downward, such as *Eriophorum vaginatum* in tussock tundra. Second, angular cores and volumetric scaling facilitate comparison with angular minirhizotrons, which can be scaled using the same volumetric approach (e.g., Norby et al. 2004).

Minirhizotrons

Cellulose acetyl butyrate minirhizotrons were etched with a continuous 0.8 x 1.3 cm grid and sealed at the lower end with a rubber stopper. Angular soil cores (45°) were extracted with a slide hammer to approximately 10 cm beneath the frozen soil interface and minirhizotrons were installed in their place in late August of 2002. Minirhizotrons were filled with removable foam pipe insulation and aboveground lengths were painted white to maintain near natural thermal conditions along their length.

Minirhizotron images (~45/minirhizotron) were collected using a BTC-2 minirhizotron camera system and ICAP software (Bartz Technology, Santa Barbara, California) in mid-July and early September of 2003 and 2004. Images were analyzed for projected root area (length x width) produced during the current year using the MSU ROOTS software package (Michigan State University, East Lansing, MI). Mid-July minirhizotron images were used to estimate fine root production between snowmelt and mid-summer, while early September images were used to account for fine root production between mid-summer and early fall. The mid-summer sampling date reduced the likelihood of fine root production and death between sampling dates.

Root dimensions measured with minirhizotrons are often scaled to the square meter by assuming an image depth of field and scaling the data volumetrically (e.g., Norby et al., 2004). For instance, a minirhizotron with a 0.3 cm depth of field, installed at 45° to the soil surface to a vertical depth of 40 cm, is assumed to sample approximately 13.6 $cm^3$ of soil. Sullivan and Welker (2005) found that use of a 0.3 cm depth of field gave annual root production estimates consistent with calculations based on values in the literature for *E. vaginatum* (Chapin et al. 1988). Therefore, a 0.3 cm depth of field was used to scale minirhizotron data in the present study. Minirhizotron estimates of projected root area were scaled to biomass using a linear regression ($r^2$= 0.95, $P$<0.01).

Estimates of SOC Loss with Long-term Fertilization

Estimates of fine root production and live standing fine root biomass were used to estimate SOC losses that could be attributable to declines in C inputs, as a result of changes in fine root demography with chronic fertilization. For control plots, we assumed a near steady-state condition, where live standing fine root biomass is constant across years and, therefore, fine root production is equal in magnitude to fine root death. For fertilized plots, we incorporated the apparent linear decline in fine root production, the apparent linear increase in live standing fine root biomass and the apparent linear increase in fine root C concentration. This exercise necessitated interpolation between years 0 and 15 and extrapolation between years 15 and 20, the length of the fertilization experiment described in Mack et al. (2004). Fine root death in fertilized plots was calculated on annual time-steps by adding an annualized reduction in fine root production to annual fine root production in control plots and correcting for an annualized increase in fine root C concentration. The annualized decline in contributions to SOC attributable to declines in fine root death with fertilization was calculated by subtracting our estimate of fine root death in control plots from our estimate of fine root death in fertilized plots. The decline in SOC that could be attributed to declines in fine root C inputs over the course of a 20 year fertilization experiment ($\Delta SOC_{fr}$) was calculated as the sum of the annualized reductions in SOC contributions, using fine root production and fine root nutrient data from a relatively cold (2003) and an exceptionally warm growing season (2004) as follows:

$$\Delta\, SOC_{fr} = \sum_{i=1}^{20} ((FRP_{ctl} + FRP_{sl} * i - FRB_{sl}) * (FRC_{ctl} + FRC_{sl} * i) - FRP_{ctl} * FRC_{ctl}), \quad \text{eq. 1}$$

where,

$FRP_{ctl}$= fine root production in control plots (2003: 158.5 g biomass $m^{-2}$; 2004: 144.9 g biomass $m^{-2}$),

$FRP_{sl}$= slope of the decline in fine root production with fertilization (2003: -7.8 g biomass $m^{-2}$ $yr^{-1}$; 2004: -3.4 g biomass $m^{-2}$ $yr^{-1}$),

$FRB_{sl}$= slope of the increase in live standing fine root biomass with fertilization (13.7 g $m^{-2}$ $yr^{-1}$),

$FRC_{ctl}$= C concentration of fine roots in control plots (0.417 g C/g biomass),

$FRC_{sl}$= slope of the increase in fine root C concentration over time with fertilization (0.004 g C g $biomass^{-1}$ $yr^{-1}$).

Slope estimates were generated through linear regressions of fine root production, biomass and nutrient concentrations on the duration of fertilization (i.e., n= 3). Estimates of SOC losses depend upon the slope estimates and should, therefore, be treated cautiously, as rough estimates of SOC losses that could be attributed to declines in fine root production, fine root death and increases in live standing fine root biomass. Estimates of live standing fine root biomass in the present study are to 25 cm depth, rather than 40 cm, which Sullivan and Welker (2005) identified as the approximate maximum depth of fine root production in control plots of nearby tussock tundra. The fine root production: biomass ratio was assumed to be invariant with depth, for the purpose of this exercise. Given estimates of both fine root production and live standing biomass for 0-25 cm, live standing biomass was scaled to 0-40 cm using minirhizotron fine root production estimates for 0-40 cm.

Statistical Analyses

The correlation between projected root area and root biomass was examined using the regression (REG) procedure in SAS 9.1 (SAS Institute, Cary, NC). The effects of short- and long-term fertilization on metrics of fine root biomass, production and nutrient concentrations were tested with one-way Analyses of Variance (ANOVAs) using the General Linear Model

(GLM) procedure in SAS 9.1. Data were $\log_{10}$ transformed prior to analysis, when there was evidence of heteroscedasticity. Comparisons of interest were made using Tukey's Honest Significant Difference (HSD) and differences were considered significant at $\alpha$=0.10. The effects of short- and long-term fertilization on $\log_{10}$-transformed minirhizotron estimates of annual fine root production over the two years of observation were analyzed with a two-way restricted maximum likelihood repeated measures ANOVA using the Mixed Model procedure in SAS 9.1. Again, comparisons of interest were made using Tukey's HSD and differences were considered significant at $\alpha$=0.10.

**RESULTS**

Climate

Growing season air temperatures averaged 9.1°C and precipitation averaged 19.0 cm, over the course of the long-term fertilizer experiment (Table 1). Growing season air temperatures in 2003 were 14% below average and precipitation was 46% above average, while in 2004, growing season air temperatures were 29% above average and precipitation was 19% above average, relative to the 17 year means. Growing season soil temperatures at 10 cm in the long-term fertilizer plots were approximately 2°C cooler and the maximum depth of soil thaw was approximately 2 cm shallower than control plots between 1998 and 2005.

Fine Root Biomass 0-25 cm Depth

Standing stocks of live fine root biomass in the upper 25 cm increased with fertilization in soil cores collected on July 26, 2002 ($F$= 3.3, $P$= 0.05) (Figure 1a). In long-term fertilizer plots, live standing fine root biomass was almost double that of control plots ($P$= 0.03), while in short-term fertilizer plots, live standing fine root biomass was intermediate between control and long-term fertilizer plots. Separation of fine roots by species revealed that live standing fine root

biomass of *E. vaginatum* declined with fertilization ($F$= 2.7, $P$= 0.07), from 27% of total fine root biomass in control plots, to 13% in short-term fertilizer plots, to 3% of total live fine root biomass in long-term fertilizer plots (Figure 1b). Live standing *E. vagniatum* fine root biomass in long-term fertilizer plots had declined to less than 25% of the biomass found in control plots ($P$= 0.07).

Ingrowth Cores 0-25 cm Depth

Ingrowth cores, installed at 70° to the soil surface, sampled the upper 25 cm of the soil profile. Root ingrowth in the upper 25 cm between late July of 2002 and late July of 2003 declined with fertilization ($F$= 5.2, $P$< 0.01) (Figure 2a). Fine root production in the long-term fertilizer plots was less than 20% of fine root production in control plots ($P$< 0.01), while fine root production in the short-term fertilizer plots was intermediate. Separation of fine roots by species revealed a decline in *E. vaginatum* fine root production with fertilization ($F$= 4.9, $P$= 0.01), from 73% of fine root ingrowth in control plots, to 48% of total ingrowth in the short-term fertilizer plots, to 17% of total ingrowth biomass in the long-term fertilizer plots (Figure 2b).

Minirhizotrons: 0-25 cm Depth

Minirhizotron images collected between 0 and 25 cm depth in mid-July 2003 showed a trend of declining fine root production with duration of fertilization (Figure 2a). Variability was high within treatments and there were no significant differences across treatments. Comparison of mid-July minirhizotron data with corresponding data from ingrowth cores revealed strong correspondence with respect to the magnitudes of the estimates and the trends across treatments. Minirhizotron images collected between 0 and 25 cm depth in early September 2003 continued to show a trend of declining fine root production with duration of fertilization. Again, variability

within treatments was high and there were no significant differences across treatments.

Minirhizotrons 0-40 cm Depth

Analysis of annual fine root production estimates (0-40 cm) across treatments and the two years of observation revealed an overall treatment effect ($F$= 2.9, $P$= 0.06) and an effect of year ($F$= 4.0, $P$= 0.05). Several comparisons within and across years are noteworthy. In the comparatively colder 2003 growing season, fine root production declined from control plots (159 g/m$^2$, SE= 67, n= 12), to short-term fertilizer plots (102 g/m$^2$, SE= 42, n= 12), to long-term fertilizer plots (42 g/m$^2$, SE= 12, n= 12) (Figure 3). There were, however, no statistically significant differences across treatments, owing to relatively high variability in the control and short-term fertilizer plots. In the exceptionally warm 2004 growing season, fine root production in the control plots (145 g/m$^2$, SE= 32, n= 12) was greater than observed in long-term fertilizer plots (91 g/m$^2$, SE= 40, n= 12) ($t$= 2.4, $P$=0.02). The highest fine root production in 2004 was observed in the short-term fertilizer plots (217 g/m$^2$, SE= 54, n= 12), where production was higher than observed in the same plots during 2003 ($t$= 2.2, $P$=0.03) and higher than observed in the long-term fertilizer plots during 2004 ($t$= 2.8, $P$<0.01). The test for an interaction between treatment and year was, however, not statistically significant ($F$= 1.7, $P$= 0.18), owing to consistently low fine root production in the long-term fertilizer plots.

During the cool 2003 growing season, 24% of fine root production in control plots occurred between depths of 20 and 40 cm. This figure declined to 11% in short-term fertilizer plots and to 2% in long-term fertilizer plots. During the warm 2004 growing season, only 6% of fine root production in control plots occurred between depths of 20 and 40 cm. There was no evidence of fine root production below 20 cm in short- or long-term fertilizer plots during the warm 2004 growing season.

Estimates of SOC Loss with Long-term Fertilization

Application of equation 1, using minirhizotron estimates of fine root production (0-40 cm) during the relatively cold growing season of 2003, suggests that a decline in SOC of 774 $g/m^2$ could be attributed to declines in fine root production, declines in fine root death and increases in live standing fine root biomass. Minirhizotron estimates of fine root production (0-40 cm) during the exceptionally warm growing season of 2004, suggest that a decline in SOC of 408 $g/m^2$ could be attributed to declines in fine root production, declines in fine root death and increases in live standing fine root biomass.

Fine Root C and N Concentrations

Fine root C concentration increased with fertilization ($F$= 3.1, $P$= 0.07), such that root C was higher in the long-term fertilizer plots than in control plots ($P$= 0.06) (Table 2). Root N concentration also increased with fertilization ($F$= 6.5, $P$< 0.01), such that root N was higher in the long-term fertilizer plots than control plots ($P$<0.01). Increases in root N (83%) were proportionately greater than increases in root C (13%) with long-term fertilization. Consequently, there was a decline in root C: N with fertilization ($F$= 2.7, $P$= 0.09).

**DISCUSSION**

Fine Root Biomass 0-25 cm Depth

There was an increase in live standing fine root biomass with duration of fertilization, such that fine root biomass was significantly higher in the long-term fertilizer plots than in control plots. Increases in live standing fine root biomass closely mirror increases in live aboveground plant biomass (Shaver et al. 2001) and soil fungal biomass (Clemmensen et al. 2006) with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. Furthermore, the amount of live standing fine root biomass (0-25 cm) and the response to long-

term fertilization in the present study are consistent with results in nearby moist non-acidic tussock tundra (Van Wijk et al. 2003), despite dramatic differences in species composition and species-level responses to fertilization between tundra-types (Hobbie et al. 2005).

There was a decline in live standing *E. vaginatum* fine root biomass with duration of fertilization, such that *E. vaginatum* accounted for less of total live standing fine root biomass with long-term fertilization. This observation mirrors the decline in *E. vaginatum* biomass and the corresponding rise in *B. nana* biomass, both above- (Shaver et al. 2001) and belowground (Clemmensen et al. 2006) with long-term fertilization in acidic tussock tundra.

Ingrowth Cores: 0-25 cm Depth

Investigators have long suggested that ingrowth cores may overestimate fine root production, as a result of disturbance-related fine root proliferation and lower competition in the root-free soil (Vogt et al. 1998, Fahey et al. 1999, Lauenroth 2000). Field tests of the method have, however, generally shown that ingrowth cores tend to underestimate fine root production (Hansson et al. 1995, Steele et al. 1997, Hendricks et al. 2006). Ingrowth cores are thought to underestimate fine root production because individual fine roots may be produced, die and begin to decompose between core installation and recovery.

In our study, ingrowth cores showed a decline in fine root production from 88 $g/m^2$ (S.E.= 28) in control plots to 14 $g/m^2$ (S.E.= 4) in long-term fertilizer plots. This trend is consistent with our minirhizotron observations, but contrasts with the results of a previous study over the same depth interval in acidic tussock tundra (Nadelhoffer et al. 2002). There are two key methodological differences between our ingrowth core study and the Nadelhoffer et al. (2002) study: the orientation of the ingrowth cores and the incubation period of the cores in the field. Nadelhoffer et al. (2002) installed ingrowth cores vertically, while we installed ingrowth

cores at an angle. Theoretically, angular core installation should reduce disturbance to vegetation directly above the ingrowth core and should effectively sample roots that grow both downward and laterally. In contrast, cores installed vertically necessarily disturb vegetation directly above the ingrowth core and may under-sample roots that grow vertically, such as those of *E. vaginatum*, a dominant member of tussock tundra control plots. On this basis, we suggest that Nadelhoffer et al. (2002) may have underestimated fine root production in control plots of tussock tundra, while accurately estimating fine root production in fertilized plots, where roots grow more horizontally. Future studies should consider using angular ingrowth cores to effectively sample fine roots that grow both vertically and horizontally.

Nadelhoffer et al. (2002) employed an incubation period of late August to late August and reported fine root ingrowth of 67 $g/m^2$ (S.E.= 10) in plots fertilized for 8 years, while we incubated cores in the field from mid-July to mid-July and found fine root ingrowth of 31 $g/m^2$ (S.E.= 8) in plots fertilized for 6 years. Our ingrowth core and minirhizotron estimates were of similar magnitude, but only when ingrowth core estimates were compared with minirhizotron estimates for the first half of the growing season, suggesting that our ingrowth cores did not effectively sample the later half of the 2002 growing season. Studies in tussock tundra have demonstrated that nearly all losses of leaf litter mass occur during winter (Hobbie and Chapin 1996) and that winter $CO_2$ efflux is an important component of the annual C budget (Oechel et al. 1997, Fahnestock et al., 1998, 1999, Grogan and Chapin 1999, Jones et al. 1999, Welker et al. 2000, Schimel et al. 2006). It is possible that our ingrowth cores underestimated fine root production, as a result of root litter mass loss over winter. On this basis, we suggest that future root ingrowth studies in tussock tundra follow the method used by Nadelhoffer et al. (2002),

where cores were installed late in the growing season and harvested late in the subsequent growing season, following plant senescence.

Minirhizotrons 0-40 cm Depth

Annual fine root production in control plots was estimated at 159 g/m$^2$ in 2003 and 145 g/m$^2$ in 2004. Few studies have measured annual fine root production over the full soil profile in tussock tundra. Literature production data and statistical relationships allow for an independent estimate of expected fine root production. Shaver et al. (2001) presented aboveground apical production for tussock tundra control plots during the 1995 growing season. We used apical production as a proxy for litterfall and calculated expected total root C allocation (TRA) during 1995 using a linear regression model developed across a wide range of forest ecosystems (Raich and Nadelhoffer 1989). This exercise led to a TRA estimate of 253 g C/m$^2$ for 1995. We assumed that fine root production accounts for approximately 1/3 of TRA (Nadelhoffer and Raich 1992), yielding a fine root production estimate of 84 g C/m$^2$ or 189 g/m$^2$, broadly consistent with our minirhizotron estimates of fine root production.

Ingrowth cores revealed that *E. vaginatum* constituted 73% of fine root production in control plots. Sullivan and Welker (2005) collected minirhizotron images on 14 dates during the 2002 growing season and presented a fine root production estimate for *E. vaginatum* of 119 g/m$^2$ in a nearby acidic tussock tundra site. If *E. vaginatum* constitutes the same proportion of total fine root production at this nearby site, ecosystem-scale fine root production would have been approximately 163 g/m$^2$. This estimate is similar to our observations during 2003 and 2004 in control plots of the present study, suggesting that two sampling dates were sufficient to provide reasonable estimates of annual fine root production.

In 2003, there was a near linear decline in annual fine root production (0-40 cm depth) with duration of fertilization, such that fine root production in the long-term fertilizer plots was lower than observed in control plots. Fine root production in control plots was very similar in 2003 and 2004. In contrast with control plots, fine root production was significantly higher in 2004 than 2003 in the short-term fertilizer plots and there was a trend toward higher production in 2004 than 2003 in the long-term fertilizer plots. We suggest the divergent responses of fine root production in control and fertilized plots to an unusually warm year are consistent with observed changes in the species composition of tussock tundra with climate warming. *B. nana*, which rises to dominance with long-term fertilization in tussock tundra, has also shown strong positive responses to both experimental warming (Chapin et al. 1995, Hobbie and Chapin 1998, Bret-Harte et al. 2001) and recent ambient climate warming (Wahren et al. 2005). We suggest greater fine root production in short- and long-term fertilizer plots during an unusually warm growing season reflects the ability of *B. nana* to capitalize upon release from the constraints of cold temperatures. Fertilized plots, dominated by *B. nana*, may be more responsive to an unusually warm year because *B. nana* is more responsive to temperature or because increases in leaf area in the fertilized plots shade the soil surface and hold soil temperatures below a threshold for fine root production during relatively cool growing seasons.

Clemmensen et al. (2006) found an increase in ectomycorrhizal (EM) fungal production and soil fungal biomass with long-term fertilization in the same plots of acidic tussock tundra. EM production was low relative to soil fungal biomass and little mass loss was observed over winter, suggesting that EM fungi may be very long-lived. These results, taken with our observation of declines in fine root production, commensurate with increases in live standing fine root biomass, suggest that long-term fertilization has shifted the belowground ecology of

tussock tundra from a system dominated by highly productive, short-lived fine roots with limited mycorrhizal association (e.g., *E. vaginatum*) to a system dominated by less productive, long-lived fine roots and their long-lived mycorrhizal symbionts (e.g., *B. nana*).

Estimates of SOC Loss with Long-term Fertilization

Mack et al. (2004) revealed a 2 $kg/m^2$ loss of SOC with long-term N and P fertilization in acidic tussock tundra. SOC losses were concentrated in the deep organic and mineral soil layers. Fine root death is an important input to SOC pools in tussock tundra (Loya et al. 2004). In 2003, there was a steep decline in annual fine root production (0-40 cm) with duration of fertilization from 158 $g/m^2$ in control plots, to 102 $g/m^2$ in short-term fertilizer plots, to 42 $g/m^2$ in long-term fertilizer plots. Declines in fine root production were even greater when deep soil layers (20-40 cm depth) were examined. Over the course of a 20 year experiment, declines in fine root production of the magnitude observed during 2003 could account for a 774 $g/m^2$ loss of SOC, after accounting for increases in live standing fine root biomass. In 2004, fine root production in the fertilizer plots was higher than in 2003, while fine root production in the control plots was consistent across years. Nevertheless, there was a trend toward lower fine root production in the long-term fertilizer plots (91 $g/m^2$) than in the control plots (145 $g/m^2$) and linear regression of fine root production on duration of fertilization generated a negative slope (-3.4 $g\ m^{-2}\ yr^{-1}$). It is worth noting that fine root production in deep soil layers (20-40 cm depth) of fertilized plots declined to zero in 2004, despite unusually warm conditions. Over the course of a 20 year experiment, declines in fine root production of the magnitude observed during 2004 could account for a 408 $g/m^2$ loss of SOC, after accounting for increases in live standing fine root biomass. These calculations constitute a rough approximation and suggest that between 20 and

39% of the SOC declines observed with chronic fertilization in acidic tussock tundra (Mack et al. 2004) could be attributed to declines in fine root C input to SOC pools.

Fine Root C and N Concentrations

Root C and N concentrations increased with duration of fertilization, but increases in root N were proportionately greater. Consequently, there was a decline in root C: N with long-term fertilization. The observed 33% decline in fine root C: N can be explained by changes in plant species composition with long term fertilization, as *B. nana* fine roots have a C: N that is approximately 30% lower than fine roots of *E. vaginatum* (Hobbie 1996).

Recent studies suggest microbial respiration of SOC is strongly N-limited in tussock tundra (Weintraub and Schimel 2003, Mack et al. 2004, but see Hobbie, 1996). If higher N concentration in *B. nana* fine root litter lead to more rapid mineralization of SOC, increases in *B. nana* fine root death, as a proportion of total fine root death, could feedback to stimulate losses of SOC with long-term fertilization. *B. nana* fine root production is generally limited to near surface soils (0-20 cm) where conditions are warmer and drier. The upward shift of fine root C inputs to SOC pools may further accelerate SOC losses with long-term fertilization.

Conclusions

Long-term fertilization of acidic tussock tundra has led to dramatic changes in aboveground production, biomass and species composition, giving rise to profound changes in the structure and function of the aboveground community (e.g., Chapin et al. 1995, Chapin and Shaver 1996, McKane et al. 1997, Shaver et al. 2001). Our study and that of Mack et al. (2004) demonstrate that changes belowground have been no less dramatic. Live fine root biomass has almost doubled, fine root production has declined over the full soil profile and nearly ceased at depths greater than 20 cm. Changes in the distribution and demography of fine roots with long-

term fertilization reflect the replacement of *E. vaginatum* with *B. nana*. *E. vaginatum* maintains a highly productive annual fine root system that extends to the maximum depth of thaw. *B. nana* exhibits an unproductive, long-lived, network of fine roots that are confined to near surface soils.

Fine root production increased in fertilizer plots during an unusually warm growing season, but remained relatively constant across years in control plots. We suggest the divergent response of control and fertilizer plots reflects a positive response in *B. nana* to warmer conditions, consistent with observations that *B. nana* shows a positive aboveground response to both experimental and observed climate warming.

Long-term fertilization of acidic tussock tundra has led to substantial losses of SOC, particularly at depth (Mack et al. 2004). Data from the present study argue that between 20 and 39% of observed declines in SOC could be attributed to replacement of the *E. vaginatum* fine root system with that of *B. nana*. The disappearance of fine root production at depth (>20 cm) with long-term fertilization is coincident with the observation that SOC losses have been greatest at depth, providing further evidence that changes in fine root demography have contributed to observed declines in SOC. The upward shift in *B. nana* fine root inputs and their higher N concentration and lower C: N could lead to more rapid mineralization of SOC, increasing the proportion of SOC losses that could be attributed to replacement of *E. vaginatum* with *B. nana*.

Our observations, coupled with those of Mack et al. (2004), challenge the notion that woody shrub encroachment into arctic tundra with a changing climate (Sturm et al. 2001, Tape et al. 2006) will increase ecosystem C storage (Hobbie 1996, McKane et al. 1997). Instead, if increased competition with woody shrub encroachment leads to a loss of deeply-rooted sedge species, ecosystem C storage may decline.

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**Table 1.** Comparison of select climate variables during June, July and August of 2003, 2004 and 1988-2005. Air temperature and precipitation data are from the Toolik main climate station. Soil temperature and thaw data are from the Toolik tussock climate station, located within the experimental site.

| Variable | 2003 | 2004 | 1988-2005 |
|---|---|---|---|
| Air temperature at 5 m (°C) | 7.8 | 11.7 | 9.1 |
| Daily maximum air temperature at 5 m (°C) | 12.0 | 16.5 | 13.6 |
| Daily minimum air temperature at 5 m (°C) | 2.8 | 5.9 | 3.8 |
| Control soil temperature at 10 cm (°C) | 2.4 | 5.9 | 4.0* |
| NP long-term soil temperature at 10 cm (°C) | 1.4 | 2.5 | 2.2* |
| Control soil thaw depth (cm) | 41.8 | . | 42.3* |
| NP long-term soil thaw depth (cm) | 40.0 | . | 40.5* |
| Growing season precipitation (cm) | 27.7 | 22.6 | 19.0 |

***** 1999-2005

**Table 2.** Fine root carbon (C) and nitrogen (N) concentrations, along with C: N, in control plots and those subjected to short- and long-term fertilization with nitrogen and phosphorus. Fine roots used for analysis were those collected from ingrowth cores in July 2003. Significant differences across treatments are identified by different superscripts ($\alpha$= 0.10). Standard errors appear within parentheses.

| Treatment | Fine Root C (mg/g) | Fine Root N (mg/g) | Fine Root C: N |
|---|---|---|---|
| Control | 417.4 (50.3)[a] | 12.7 (2.6)[a] | 33.8 (7.4)[a] |
| Short-term NP | 445.7 (23.5)[ab] | 17.2 (5.2)[ab] | 28.8 (11.5)[ab] |
| Long-term NP | 473.0 (41.2)[b] | 23.3 (9.1)[b] | 22.5 (7.5)[b] |

**Figure 1.** Total live standing fine root biomass (*a*) and live standing *E. vaginatum* fine root biomass (*b*) ($g/m^2$, 0-25 cm) in control plots and those subjected to short- and long-term fertilization with nitrogen and phosphorus. Significant differences across treatments are identified by different letters ($\alpha = 0.10$). Bars are 1.0 SE.

**Figure 2.** Total fine root production estimated by ingrowth cores and minirhizotrons (*a*) ($g/m^2$, 0-25 cm) and *Eriophorum vaginatum* fine root production estimated by ingrowth cores (*b*) ($g/m^2$, 0-25 cm) in control plots and those subjected to short- and long-term fertilization with nitrogen and phosphorus. Ingrowth cores were installed in July of 2002 and harvested in July of 2003, while minirhizotron data reflect fine root production between snow melt and image collection in mid-July of 2003. Statistical comparisons are across treatments, but within each method, with significant differences identified by different letters ($\alpha = 0.10$). Bars are 1.0 SE.

**Figure 3.** Fine root production ($g/m^2$, 0-40 cm) estimated using minirhizotrons in 2003 and 2004 in control plots and those subjected to short- and long-term fertilization with nitrogen and phosphorus. Hatched regions represent fine root production for 0-20 cm. Bars are 1.0 SE.

**Figure 1.**

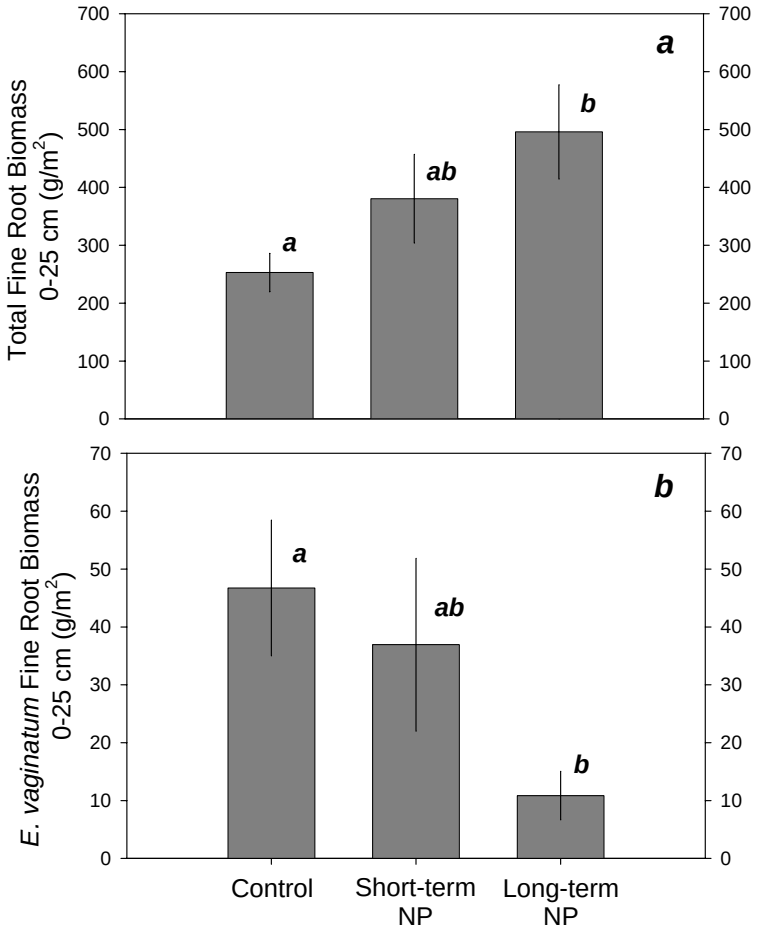

Total Fine Root Biomass
0-25 cm (g/m2)
a
b
ab
a
E. vaginatum Fine Root Biomass
0-25 cm (g/m2)
b
a
ab
b
Control
Short-term NP
Long-term NP

**Figure 2.**

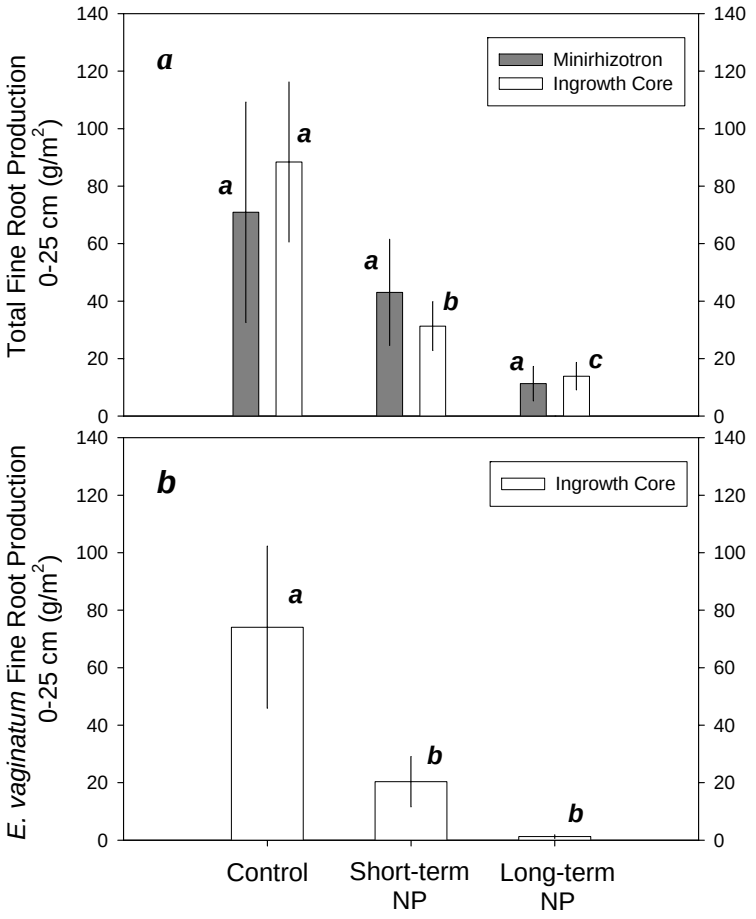
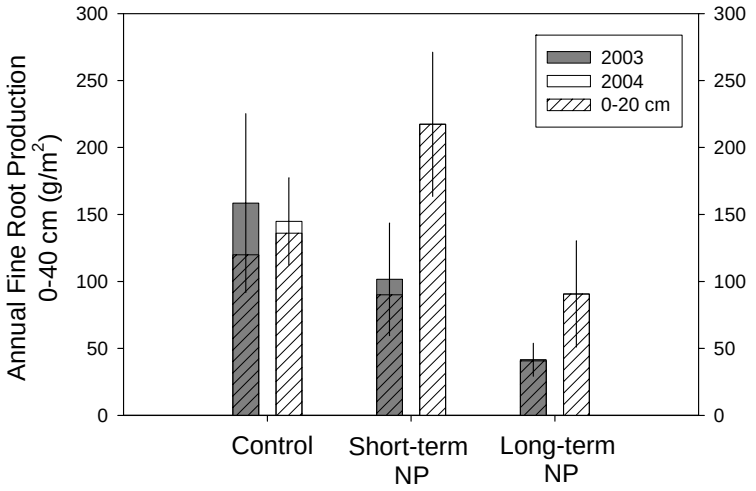

a
Minirhizotron
Ingrowth Core
Total Fine Root Production
0-25 cm (g/m2)
a
a
a
b
a
c
b
Ingrowth Core
E. vaginatum Fine Root Production
0-25 cm (g/m2)
a
b
b
0
20
40
60
80
100
120
140
Control
Short-term
NP
Long-term
NP

**Figure 3.**


Annual Fine Root Production
0-40 cm (g/m2)
300
250
200
150
100
50
0
2003
2004
0-20 cm
Control
Short-term NP
Long-term NP