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SPECIAL TOPIC: COORDINATED APPROACHES TO GLOBAL CHANGE RESEARCH

Changes in soil biogeochemistry following disturbance by girdling and mountain pine beetles in subalpine forests

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Abstract A recent unprecedented epidemic of beetle-induced tree mortality has occurred in the lodgepole pine forests of Western North America. Here, we present the results of studies in two subalpine forests in the Rocky Mountains, one that experienced natural pine beetle disturbance and one that experienced simulated disturbance imposed through bole girdling. We assessed changes to soil microclimate and biogeochemical pools in plots representing different post-disturbance chronosequences. High plot tree mortality, whether due to girdling or beetle infestation, caused similar alterations in soil nutrient pools. During the first 4 years after disturbance, sharp declines were observed in the soil dissolved organic carbon (DOC) concentration (45–51 %), microbial biomass carbon concentration (33–39 %), dissolved organic nitrogen (DON) concentration (31–42 %), and inorganic phosphorus (PO_4^{3-}) concentration (53–55 %). Five to six years after disturbance, concentrations of DOC, DON, and PO_4^{3-} recovered to 71–140 % of those measured in undisturbed plots. Recovery was coincident with observed increases in litter depth and the sublitter, soil O-horizon. During the 4 years following disturbance, soil ammonium, but not nitrate, increased to 2–3 times the levels measured in undisturbed plots. Microbial biomass N increased in plots where increased ammonium was available. Our results show that previously observed declines in soil respiration following beetle-induced disturbance are accompanied by losses in key soil nutrients. Recovery of the soil nutrient pool occurs only after several years following disturbance, and is correlated with progressive mineralization of dead tree litter.

Keywords Nutrient cycling · Tree mortality · Girdling · Lodgepole pine · Bark beetle

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Introduction

Forests and woodlands provide important economic and ecosystem services, including carbon storage and nutrient cycling (Bonan 2008; Anderegg et al. 2013). These services are compromised during periods of widespread forest mortality, linked in recent decades to chronic drought and epidemic insect outbreaks (Breshears et al. 2005; van Mantgem et al. 2009; Allen et al. 2010). Insects and pathogens are the most pervasive cause of disturbance in North American forests. These biotic agents affect significantly more area than fire, and have an economic impact that is nearly five times as great (Dale et al. 2001). In the Western United States, forest mortality from insects and disease is now more than 50,000 km^2, with some watersheds experiencing almost complete canopy mortality (Raffa et al. 2008; Man 2012). This forest die-off has been driven primarily by an unprecedented epidemic of the mountain pine beetle (*Dendroctonos ponderosae* Hopkins; MPB) in coniferous subalpine and montane forests (Hicke et al. 2012; Man 2012).

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In contrast to forest disturbance from harvesting and fire, bark beetle infestation does not immediately alter the leaf area and stem density of the affected forest stand (Edburg et al. 2012). In addition, bark beetles are selective mortality agents, preferentially targeting larger conifer host trees with thicker phloem when available (Amman 1972). Female MPBs coordinate mass attacks on the selected host through the release of an aggregation pheromone (Raffa and Berryman 1983), which often results in clusters of dead trees surrounded by unaffected trees. Upon the successful infestation of a host tree, MPB larvae consume phloem tissue, boring galleries that sever the connections between the needles and the roots. At the same time, they inoculate the xylem tissue with species of mutualistic blue-stain fungi which colonize and occlude the xylem elements, reducing hydraulic conductance and transpiration (Yamaoka et al. 1990; Hubbard et al. 2013). Tree mortality follows within the growing season.

With tree mortality, biogeophysical and biogeochemical processes are altered, interacting in coupled ways dependent on the time since disturbance (Edburg et al. 2012; Mikkelson et al. 2013). At the tree scale, MPB-induced mortality may affect soil nutrient balances both directly by altering the availability of substrate for decomposition and indirectly through biogeophysical impacts to soil microclimate (Edburg et al. 2012; Bright et al. 2013). Initially, stand structure is not impacted, as needles turn red but remain on the tree for several years, dependent on the degree of crown exposure (Klutsch et al. 2009). With tree death, plant C uptake ceases, and soil respiration and labile C pools decrease with loss of root respiration and exudation (Xiong et al. 2011; Moore et al. 2013). Transpiration also ceases, increasing soil water content and potentially decreasing soil and litter temperature (Morehouse et al. 2008; Clow et al. 2011; Griffin et al. 2011; Hubbard et al. 2013; Keville et al. 2013). Soil N pools can increase with diminished tree uptake, and greater soil moisture availability may contribute to increases in N and C mineralization rates (Griffin et al. 2011). Where studied, increases in inorganic dissolved N have been consistently observed with bark beetle disturbance, but with large differences in the reported magnitude of NO_3^- and NH_4^+, changes in net mineralization and nitrification rates and the total N exported to the watershed (Huber 2005; Morehouse et al. 2008; Clow et al. 2011; Griffin et al. 2011; Griffin and Turner 2012; Keville et al. 2013; Rhoades et al. 2013).

Over approximately 3–5 years after beetle infestation, progressive canopy loss results in standing gray snags. At the stand scale, decreasing leaf area index reduces canopy drag, changing fluxes of surface energy and increasing solar radiation and precipitation to the forest floor. This in turn potentially increases evaporation, changes snow accumulation and ablation, and increases both the spatial and temporal variability of soil moisture and temperature (see Edburg et al. 2012). Effects on the soil microclimate at the tree scale depend on the interaction of these processes and are likely site-specific. For example, reported temperature effects after bark beetle disturbance have been variable, with observations of no change, increases attributed to greater solar radiation reaching the forest floor, and reported temperature decreases with greater moisture and insulation from increased litter fall (Morehouse et al. 2008; Griffin et al. 2011; Griffin and Turner 2012).

With canopy loss, greater inputs of relatively low C:N needle litter and fine branches as well as the continuing decomposition of roots are expected to enhance decomposition, soil nutrient pools (including inorganic P), and soil respiration (Morehouse et al. 2008; Griffin et al. 2011; Edburg et al. 2012; Kaňa et al. 2012). These carbon inputs may result in nitrogen immobilization by microbes and uptake of N by surviving vegetation with microbial biomass turnover. In turn, enhanced nitrification (Morehouse et al. 2008) and greater water yields (Pugh and Gordon 2013) may leach particulates, NO_3^-, and DOC to streams (Huber 2005), with important implications for water quality (see Mikkelson et al. 2013). Over subsequent decades, nutrient balances will be determined by snag fall rates and the slower decomposition of recalcitrant bole wood (Fahey 1983; Bigler and Veblen 2011), the regrowth of surviving vegetation and changes in forest community composition (Collins et al. 2010; Griffin and Turner 2012). How much these changes are reflected on the ecosystem scale will depend on the severity of infestation and understory vegetation characteristics, as well as pre- and post-disturbance stand structure (Edburg et al. 2012).

The mechanisms by which MPB outbreaks impact forest biogeochemistry—mortality effects on substrate supply and changes in stand structure—are therefore heavily dependent on time since disturbance. Our knowledge of the directions in which changes in soil nutrient concentrations occur, as well as the timing and causes of such changes following disturbance, is hindered by a lack of longer-term observations. To investigate these questions, we compared two parallel disturbance chronosequences in two stands of Colorado subalpine forest. One chronosequence of tree mortality was caused by mechanical girdling and spanned 8 years, and the other was caused by natural MPB kill and spanned 5 years. Girdling simulates the effect of bark beetle damage to the phloem tissues of infected trees by severing the connection between the needles and the roots, and immediately eliminates the deposition of newly fixed phloem sugars and amino acids to the soil; tree mortality follows girdling within 1–3 years. To assess the impacts of tree mortality on soil microclimate and biogeochemical pools, we measured changes in light, soil temperature, moisture, pH, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), microbial biomass C and N, and inorganic pools of nitrogen and phosphorus. With both disturbances, we hypothesized an initial decline in soil DOC and dissolved organic nitrogen (DON) as well as microbial biomass C and N due to the loss of root exudation. In recent tree

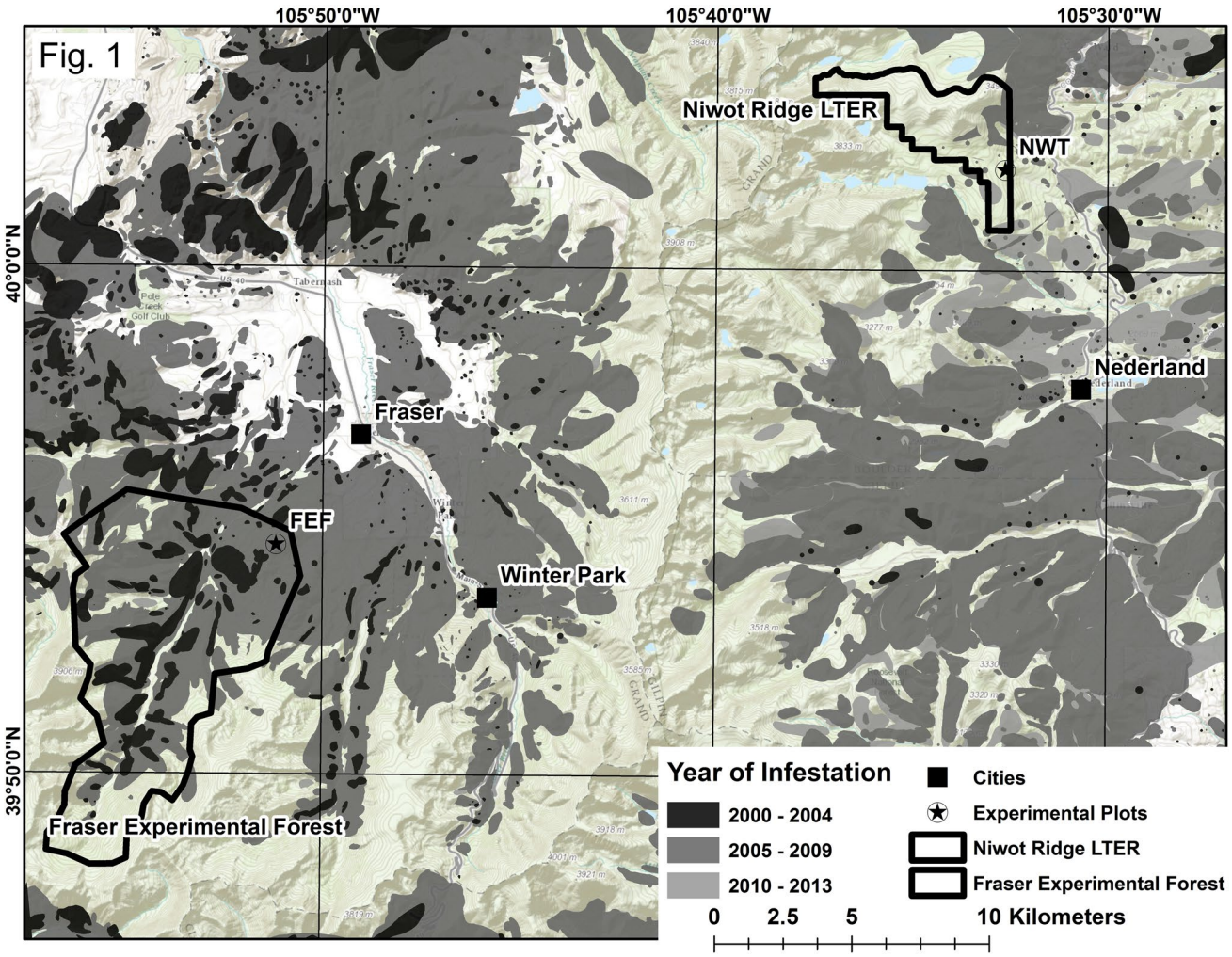

Fig. 1 Map showing the location of the Fraser Experimental Forest (FEF) and the Niwot Ridge (NWT) long-term ecological research sites in Colorado, USA, encompassing the tree mortality chronosequences. Mountain pine beetle infestation from 2000 to 2013 was mapped from USFS aerial damage surveys. The total area infested within the *polygon* denoting the Niwot Ridge region was altered to reflect ground survey observations of ~6 % active infestation area in 2009

mortality plots, we predicted reduced plant uptake of both water and soil nutrients, leading to increases in soil moisture and inorganic pools of N and P, with the effect lagging in the girdled plots compared to the beetle-killed plots. In plots with older tree mortality, we predicted increases in soil pools of DOC, DON, inorganic P and N, and microbial biomass C and N with greater needle inputs and stimulated decomposition in wetter soils.

Methods

Study site descriptions

The Fraser Experimental Forest consists of 93 km^2 maintained by the US Department of Agriculture located within the Arapaho National Forest, Colorado, USA (Fig. 1). The plots are located at 39°54′27″N 105°51′10″W at 2,913 m in elevation. The dominant tree species is lodgepole pine (*Pinus contorta* Dougl. ex Loud.), which composes about half of the tree cover in the district. Engelmann spruce [*Picea engelmannii* (Parry)] and subalpine fir [*Abies lasiocarpa* (Hook.) Nutt. var. lasiocarpa] make up an additional ~25 % of the tree cover, predominantly at higher elevations, on north slopes, and along stream channels. Most of the forest was established during secondary succession following a stand-replacing fire in 1685, and low-elevation portions, including our site, were logged in the early 1900s. The climate at the site consists of long, cold winters and short, cool summers, and the average annual temperature is 0.5 °C. Annual precipitation averages 737 mm, with approximately 60 % falling as snow from October to

May. Soils are derived from gneiss and schist with angular gravel, stone, very little silt and clay, and a thin organic-rich mineral horizon of ~3–12 cm in undisturbed stands. More information on site characteristics can be found at http://www.fs.fed.us/rm/fraser/. The Fraser Experimental Forest has been heavily impacted by the mountain pine beetle outbreak beginning in 2002, with tree mortality approaching 70 % throughout the valley (Fig. 1) (Wilkes 2009).

The Niwot Ridge long-term ecological research (LTER) site is located at 3,050 m elevation in a subalpine forest just below the Continental Divide near Nederland, Colorado (40°1′58″N; 105°32′47″W; Fig. 1). The girdling chronosequence plots are located within the lodgepole pine dominated portion of the site. The Niwot LTER forest has a similar stand size and structure to the Fraser site, and is also in recovery from logging in the early 1900s. Annual precipitation for the site averages 800 mm (approximately 65 % falling as snow), and the mean annual temperature is 1.5 °C. Soils at the site are sandy inceptisols derived from granitic moraine with a mineral texture of sandy loam and a thin organic rich mineral horizon ranging from ~3 to 9 cm. For previous studies involving soil respiration and tree girdling, see Moore et al. (2013), Scott-Denton et al. (2003, 2006), and Weintraub et al. (2007). In contrast to the Fraser Experimental Forest, the Niwot Ridge LTER forest has not been significantly affected by the regional mountain pine beetle outbreak, though there are areas of localized infestation (Fig. 1) (Wilkes 2009).

Establishment of disturbance chronosequences

In collaboration with Dr. Jose Negron, a USFS research entomologist specializing in work on Western US bark beetles, we selected clusters of *P. contorta* on the same hillslope in the Fraser Experimental Forest that had experienced high levels of infestation by mountain pine beetles. Plot selection was based on our aim to minimize chronosequence spatial, age, and parent substrate variability. The plots were contained within an approximately 25-ha area, with plot elevations ranging from 2,884 to 2,909 m, slopes from 3° to 13°, and aspects from 15° to 351° (spanning approximately 30°; referenced to N). Originally, trees were aged for the time since beetle infestation based on the degradation status of the crown (Klutsch et al. 2009). Plots of approximately 9–15 m^2 were established, containing 3–5 pine trees either exclusively alive or beetle-killed as the focal point of the plot center and subsequent measurements. All samples and measurements were taken in areas excluding live or dead trees respectively to at least 1 m. We set up a total of 18 plots: three per infestation age class and six control plots consisting of live trees that were equivalent in bole size. Beyond the focal cluster of trees at the plot center where measurements and sampling occurred, the outer plot area consisted of a mosaic of live and dead trees. What we refer to as "beetle-killed plots" averaged 83.4 % tree mortality; our so-called "control plots" averaged 33.9 % mortality. The control plots were geographically distributed throughout the site, in pockets of the forest stand where the beetle infestation had been less severe. There were no obvious differences in plot tree structure or age, or in soil or aspect characteristics that were correlated with the locations of surviving pockets of trees.

Subsequent to the establishment of the chronosequence, we employed a novel dendrochronological technique that allowed us to more accurately determine year of tree death within our plots. In mid-August 2012, we revisited the site and took two cores parallel to the slope at 1.3 m from the ground of all trees within the plots measuring greater than 10 cm in dbh. The live tree core data were compiled into a site master chronology with known outside ring dates that allowed the dead trees to be cross-dated. Details on the dendrochronological method used to determine plot tree year of death can be found in Online Resource 1 of the Electronic supplementary material (ESM). All results and analysis included in this paper reflect the dendrochronologically determined year of mortality for time since disturbance.

Previously, researchers investigating tree rhizodeposition and soil respiration at the Niwot Ridge site (Scott-Denton et al. 2006; Weintraub et al. 2007) created plots of *P. contorta* that were girdled in 2002, 2003, and 2004. We also girdled trees in 2008, 2009, and 2010 or left trees alive as controls. Plot trees were girdled by using a saw to make two parallel incisions approximately 15 cm apart around the circumference of the tree at breast height, and employing the sharp edge of a hatchet to scrape away the bark, phloem, and cambial layers between the incisions. Any regrowth of cambial tissue was removed by scraping 2 weeks later. Previous work by Scott-Denton et al. (2003) at the Niwot site showed the preponderance of fine roots to be in shallow surface layers, and girdled plots were trenched or re-trenched around the perimeter from 15–30 cm in depth to help minimize rhizospheric inputs from trees outside the plot. Sampling was randomized in the plot center to avoid edge effects. This work allowed the creation of an 8-year girdling disturbance chronosequence. Three ~20-m^2 forest plots containing 3–12 medium-dbh-class lodgepole pines were established, for a total of 24 plots. Plots spanned elevations of 3,016–3,028 m, slopes of 4.7–6.7°, and aspects of 78–94° over an approximate area of 5.5 ha.

Plot microclimate and characteristics

Concurrent with soil sampling for chemical analyses, we recorded one set of air and soil temperature measurements

at four plot locations between the hours of 10:00 and 16:00. Air temperature was measured between these times at 10 cm above the ground surface with a linearized thermistor in the chamber of an LI-6400 gas exchange system (LI-COR Inc., Lincoln, NE, USA). Soil temperature was measured over an integrated soil depth range of 0–15 cm using a soil temperature probe (Omega Engineering Inc., Stamford, CT, USA) and the LI-6400. Soil moisture was determined gravimetrically by weighing samples after collecting 5 g of wet soil from the organic-rich mineral horizon and then drying it for a minimum of 48 h at 60 °C. For the Fraser Experimental Forest sites, we identified all live and dead standing trees with respect to species and measured each tree for diameter at breast height (dbh). Plot tree density was determined by summing the basal area of living or dead trees in the greater plot area. Percent ground cover of coarse woody debris, defined as non-beetle-killed logs, stumps, and snags ≥10 cm in size (Harmon et al. 1986), as well as understory vegetation cover including shrubs and seedlings, were visually estimated to the nearest 5 %. Plot scale plant area index (PAI) and canopy openness was calculated in plots for the Fraser Experimental Forest chronosequence using hemispherical photos and Gap Light Analyzer software (Frazer et al. 1999) for image processing. We modeled the percent transmitted light flux through the canopy from July 1 to July 31, 2011, a time representative of forest growing-season photosynthetic activity. For pertinent details, see Online Resource 2 of the ESM. Litter depth and depth of the organic rich mineral soil horizon were measured at four central locations randomized within each plot.

Soil sampling, extraction, and chemical analyses

Plot soils were sampled repeatedly throughout the 2010 growing season for a total of six times at the Fraser site and seven times at the Niwot Ridge site at approximately two-week intervals from June 21st, 2010 to September 29th, 2010. We collected approximately 100 g of soil from the organic-rich mineral layer (3–12 cm in depth) from a random location within the plot and stored the sample on ice until processed. Within 12 h, we sieved and homogenized the soils, removing coarse plant litter, debris, and larger (>1 mm) roots. Soil samples were then immediately extracted to evaluate the DOC, DON, DIN, and dissolved inorganic phosphorus following the procedures of Weintraub et al. (2007). The extraction solution used, 0.5 M K_2SO_4, collected nutrients from the labile, non-occluded soil pools. This included readily soluble and desorbable P, primarily in the form of orthophosphate. Such phosphate is quickly cycled and readily available for plant uptake but does not represent all potentially available plant P, especially over time. Briefly, 25 mL of 0.5 M potassium sulfate (K_2SO_4) were added to 5 g of homogenized sample; this was then agitated on an orbital shaker for 1 h at 120 rpm and vacuum-filtered through glass fiber filters. Concurrently, an additional 5 g of each sample were fumigated with 2 mL of ethanol-free chloroform for 24 h, vented for 1 h, and then extracted as above to determine the microbial biomass C and N pools. All extracts were frozen at −20 °C until analysis.

Dissolved organic carbon and total dissolved nitrogen in the K_2SO_4 extracts and the fumigated soil K_2SO_4 extracts were quantified using the non-purgeable organic C protocol on a total organic carbon analyzer equipped with a total dissolved nitrogen module (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). Soil microbial biomass C and N were estimated by subtracting the concentrations of DOC and DON in the unfumigated samples and the soil-free controls from those in the fumigated samples (Brookes et al. 1985; Vance et al. 1987). We analyzed dissolved inorganic N and P in the K_2SO_4 extracts spectrophotometrically on a microplate reader (Bio-Tek Inc., Winooski, VT, USA) using vanadium chloride/sulfanilamide reduction chemistry and colorimetric determination of nitrite for nitrate (Doane and Horwath 2003), a modified Berlethot reaction for ammonium (Rhine et al. 1998), and the malachite green colorimetric procedure for dissolved inorganic phosphorus (D'Angelo et al. 2001).

In addition to biogeochemical pools, we assessed pH and bulk density using a digital pH meter (Oakton Instruments, Vernon Hills, IL, USA) and a standard soil corer with a known radius, respectively. Texture (in g L^{-1}) was determined by the Soil, Water and Plant Testing Laboratory at Colorado State University (Fort Collins, CO, USA) using a hydrometer.

Statistical analyses

Differences in soil biogeochemical pools and plot characteristics were evaluated for significance across time since disturbance by repeated-measures mixed-model ANOVA (SAS proc mixed; The SAS Institute, Cary, NC, USA), with random variation at the plot level. Litter and organic horizon depths, pH, and bulk density were evaluated by mixed-model ANOVA (SAS proc mixed), with random variation at the plot level. Where time since disturbance was significant, Tukey's HSD ($\alpha = 0.05$ unless specified) was used to determine differences among the means. Relationships among chronosequence variables were also examined using Pearson's correlation analysis (SAS proc corr; $\alpha = 0.05$) and ordinary least squares linear regression analysis (SAS proc reg; $\alpha = 0.05$). Statistical comparisons were performed on log-transformed variables where necessary due to heteroscedasticity.

Table 1 Characteristics of the Fraser tree mortality chronosequence plots for 2010–2011. Values are the mean ± SE; sample sizes vary: undisturbed plot class (year 0, live trees) $n = 6$, beetle-kill YSD 4 plot class $n = 5$, YSD 5 plot class $n = 5$, YSD 6 plot class $n = 2$

Variable	Year since disturbance (YSD) classes for measurements performed in 2011				
	0	4	5	6	*F*, *p*
Total *P. contorta* basal area (m^2 ha^{-1})	40.1 ± 2.9	37.3 ± 5.9	39.1 ± 8.5	29.2 ± 0.2	0.37, ns
Dead *P. contorta* (%)	34.0 ± 8.0[a]	71.0 ± 9.4[b]	82.3 ± 7.3[b]	96.8 ± 0.7[b]	9.29, 0.0012
Plant area index	1.2 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	0.9 ± 0.1	0.41, ns
Canopy density (%)	70.4 ± 2.8	67.0 ± 3.7	67.8 ± 4.3	62.8 ± 1.6	0.49, ns
Percent canopy density per total basal area	1.8 ± 0.1	2.0 ± 0.3	2.1 ± 0.4	2.1 ± 0.1	0.23, ns
Total transmitted light (%)	43.3 ± 2.2	48.8 ± 6.2	50.6 ± 5.1	50.9 ± 3.5	0.61, ns
Percent total light per total basal area	1.1 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	0.47, ns
Variable	YSD classes for measurements performed in 2010				
	0	3	4	5	*F*, *p*
Understory vegetation cover (%)	45.8 ± 4.1	38.5 ± 10.4	54.8 ± 8.2	65.6 ± 6.9	1.51, ns
Litter depth (cm)	1.7 ± 0[a]	1.8 ± 0.2[a]	1.7 ± 0.1[a]	3.0 ± 0.3[b]	4.81, 0.0166
Soil organic layer depth (cm)	3.2 ± 0.3[a]	3.6 ± 0.3[a]	3.6 ± 0.3[a]	6.2 ± 1.2[b]	7.12, 0.0003

Results of ANOVA main effect of time since disturbance are given for each variable*; ns* nonsignificant, *different superscript letters* next to values indicate significant differences among classes (Tukey's HSD, $\alpha = 0.05$)

Results

Fraser beetle-kill chronosequence plot and vegetation characteristics

Trees of *P. contorta* composed over 98 % of the basal area in the selected plots, and there was no significant difference in tree density across the chronosequence (Table 1). Effective plant area index (PAI) and percent canopy density did not significantly decrease with time since disturbance over the chronosequence, but rather reflected overall variations in plot tree density (Table 1). Similarly, we observed no significant differences in the percentage of light reaching the forest floor in beetle-killed and live tree plots; light transmission was better correlated with overall plot tree density ($R^2 = 0.47$, $P = 0.0018$; Table 1) than time since disturbance. Vegetation ground cover was not significantly different between disturbed and undisturbed plots (Table 1). Both plot litter depth and soil organic horizon were significantly greater in the oldest disturbed plots, approximately doubling compared to the live tree plots 5-years post-beetle-kill (Table 1).

Fraser beetle-kill and Niwot girdling chronosequence soil microclimate, properties, and biogeochemical pools

Mean growing-season soil temperature integrated over depths of 0–15 cm and soil moisture content did not significantly differ among disturbed and control plots in the Fraser Experimental Forest chronosequence. In the Fraser plots, soil temperature was significantly though weakly correlated with total transmitted light ($R^2 = 0.23$, $P = 0.045$). In the Niwot Ridge chronosequence plots, mean soil temperature and moisture were variable, where the 2-year post-girdling plots were the coolest (8.0 ± 0.1 °C) and wettest (45.7 ± 4.0 %) and the 8-year post-girdling plots (the oldest) were the warmest (10.0 ± 0.1 °C) and driest (30.8 ± 4.4 %; Table 2). Air temperature was significantly elevated in plots 6 years post-girdling compared to intact tree plots and plots that were 1 or 2 and 7 years post-girdling (Table 2). At both sites, soil temperature had a significant negative linear relationship with mean growing-season gravimetric soil moisture (FEF: $R^2 = 0.24$, $P = 0.037$; NWT: $R^2 = 0.48$, $P < 0.0001$), but was decoupled from air temperature in the Fraser Experimental Forest plots ($R^2 = 0.075$, $P = 0.27$) and only weakly correlated in the Niwot Ridge plots ($R^2 = 0.25$, $P = 0.013$). While the Niwot Ridge plots were generally more acidic than the Fraser plots, soil pH did not change over either disturbance chronosequence (Table 2). Soil bulk density in the organic layer was low at both sites and did not vary significantly across the chronosequences (Table 2).

During the first 4 years following tree mortality, soil dissolved organic carbon (DOC) decreased significantly with time since disturbance in chronosequences from both forest sites. Losses at the Fraser Experimental Forest site averaged 50.6 % at 3 and 4 years after mortality, while losses at the Niwot Ridge site averaged 46.3 % (Fig. 2a; FEF $F = 3.28$, $P = 0.0525$; NWT $F = 5.07$, $P = 0.0038$). Beyond 5 years after disturbance, soil C pools recovered to 94.6 % at the Fraser Experimental Forest site and 78.2 % at the Niwot Ridge site compared to control plots. Mean growing season

Table 2 Growing season (June to September 2010) soil organic layer microclimate and properties for the Fraser (FEF) beetle-kill and Niwot (NWT) girdling tree mortality chronosequences

Variable	Site	Years since disturbance (YSD) classes								
		0	0.5	1	2	3	4	5	6	7	8	*F*, *p*
Air temperature (°C)	FEF	22.9 ± 0.2				22.9 ± 0.21	23.2 ± 0.24	23.5 ± 0.40				0.33, ns
	NWT	21.6 ± 0.2^{a}	22.0 ± 0.2^{ab}	20.1 ± 0.3^{a}	20.9 ± 0.3^{a}				23.5 ± 0.2^{b}	21.3 ± 0.3^{a}	22.1 ± 0.3^{ab}	4.47, 0.0068
Soil temperature (°C)	FEF	9.9 ± 0.1				9.6 ± 0.7	9.4 ± 0.6	9.2 ± 0.1				0.99, n.s
	NWT	8.9 ± 0.1^{abc}	8.9 ± 0.1^{abc}	8.7 ± 0.1^{abc}	8.0 ± 0.1^{c}				9.5 ± 0.1^{ab}	8.4 ± 0.1^{ac}	10.0 ± 0.1^{b}	5.02, 0.004
Gravimetric moisture (%)	FEF	26.4 ± 2.6				30.0 ± 2.6	30.9 ± 2.5	28.7 ± 3.2				0.97, n.s
	NWT	32.6 ± 2.7^{a}	32.2 ± 3.6^{ab}	39.6 ± 4.0^{ab}	45.7 ± 4.0^{b}				30.4 ± 3.6^{a}	41.3 ± 4.0^{ab}	30.8 ± 4.4^{a}	3.29, 0.025
Bulk density (g cm^{-3})	FEF	0.8 ± 0.3				0.4 ± 0.1	0.7 ± 0.2	–				0.74, ns
	NWT	0.3 ± 0.1	0.5 ± 0.1	1.0 ± 0.4	0.3 ± 0.1				0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.01	1.73, ns
pH [−log(H^{+})]	FEF	5.1 ± 0.7				5.2 ± 0.1	5.0 ± 0.1	5.2 ± 0.3				0.85, ns
	NWT	4.6 ± 0.13	4.7 ± 0.23	4.9 ± 0.09	4.7 ± 0.48				4.8 ± 0.09	4.3 ± 0.21	4.5 ± 1.2	0.86, ns

Values are the mean ± SE. Sample sizes vary: at Fraser, undisturbed plot class (year 0; live trees) $n = 6$, beetle-kill YSD 4 plot class $n = 5$, YSD 5 plot class $n = 5$, YSD 6 plot class $n = 2$; at Niwot, undisturbed plot class (year 0; live trees) $n = 6$, girdled plot class $n = 3$. Results of ANOVA main effect of time since disturbance are given for each variable. *ns* nonsignificant, *superscript letters* next to values indicate significant differences among classes (Tukey's HSD, $\alpha = 0.05$). Soil temperature was measured and integrated over depths of 0–15 cm and bulk density and pH were measured over a single sampling date

K_2SO_4 extractable total dissolved nitrogen (TDN) showed no significant trend across the Niwot Ridge mechanical girdling chronosequence (Fig. 2b; $F = 1.8$, ns). In the Fraser Experimental Forest plots, however, from 3–4 years following tree mortality onwards, TDN declined 27.5 % compared to undisturbed plots, decreasing from 52.5 ± 5.3 to 38.0 ± 6.2 μg N g dry soil^{-1}. Five years after beetle kill, TDN increased in the Fraser Experimental Forest plots to 82.7 ± 26.9 μg N g dry soil^{-1}, which exceeded the levels seen in undisturbed plots (Fig. 2b; $F = 3.15$, $P = 0.0587$). Clear temporal changes in dissolved organic nitrogen (DON) were observed at the Fraser site (Fig. 2c; $F = 3.94$, $P = 0.0312$) but not the Niwot Ridge site ($F = 2.19$, ns). Across the disturbance chronosequences, dynamics in soil TDN pools were largely determined by changes in mean growing-season K_2SO_4-extractable dissolved organic nitrogen (DON) (FEF: $R^2 = 0.88$, $P < 0.0001$; NWT: $R^2 = 0.82$, $P < 0.0001$). Differences among plots in soil DON were correlated with differences in DOC in both chronosequences (FEF: $R^2 = 0.84$, $P < 0.0001$; NWT: $R^2 = 0.85$, $P < 0.0001$).

Mean growing-season K_2SO_4-extractable soil ammonium remained equivalent to the amount in live tree plots or increased over time in both chronosequences. In the Fraser Experimental Forest disturbed plots, we observed no significant trend in soil NH_4^+ (Fig. 2d; $F = 1.23$, ns). In the Niwot Ridge chronosequence, soil NH_4^+ increased immediately after girdling, peaking in plots that had been girdled two years previously at 22.2 ± 3.0 μg N g dry soil^{-1}, more than double the NH_4^+ measured in non-girdled plots (9.9 ± 0.8 μg N g dry soil^{-1}; $F = 5.05$, $P = 0.0038$). Unlike the Fraser Experimental Forest chronosequence, soil NH_4^+ declined in the 6–8 year Niwot Ridge post-girdling plots to a level similar to that observed in non-girdled plots (Fig. 2d). Additionally, soil NH_4^+ concentrations were significantly predicted by the presence of soil moisture in both disturbance chronosequences (Fig. 3). Significant changes in mean growing-season K_2SO_4-extractable soil nitrate pools were not seen at either site, with NO_3^- concentrations remaining near the detection limit in all plots (Fig. 2e; FEF $F = 1.11$, ns; NWT $F = 1.0$, ns).

Clear temporal changes in mean 2010-growing-season K_2SO_4-extractable soil inorganic phosphorus were statistically resolvable at Fraser ($F = 7.29$, $P = 0.003$) but not resolvable at Niwot ($F = 2.14$, $P =$ ns). However, the overall temporal pattern is compelling (Fig. 2f; $F = 2.49$, $P = 0.0291$). The changes in inorganic phosphorus exhibited a pattern similar to that observed in soil carbon pools at both sites (FEF: $R^2 = 0.78$, $P < 0.0001$; NWT: $R^2 = 0.54$, $P < 0.0001$). In plots that were measured 3–4 years following tree mortality in the Fraser Experimental Forest plots and 2 years following tree girdling in the Niwot Ridge plots, soil PO_4^{3-} concentrations declined compared to

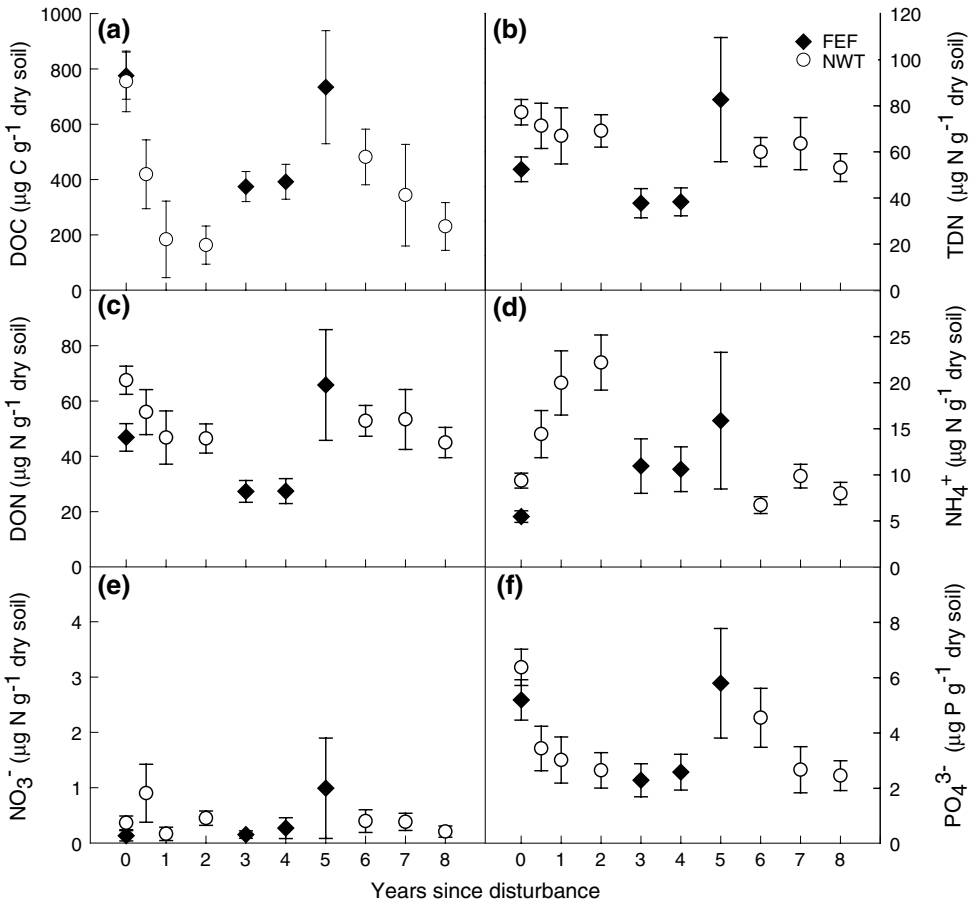

Fig. 2 Variation in soil nutrients in relation to years since beetle kill (FEF, *closed diamonds*) or girdling (NWT, *open circles*). Values are the mean ± SE determined over the growing season in 2010 from K_2SO_4 extractions: **a** total dissolved organic carbon; **b** total dissolved nitrogen; **c** dissolved organic nitrogen; **d** ammonium; **e** nitrate; **f** inorganic phosphorus. At FEF, undisturbed (year 0) plot class $n = 6$, beetle-kill year 3 class $n = 5$, beetle-kill year 4 class $n = 5$, beetle-kill year 5 class $n = 2$. At NWT, undisturbed plot class (year 0) $n = 6$, girdled plot class $n = 3$

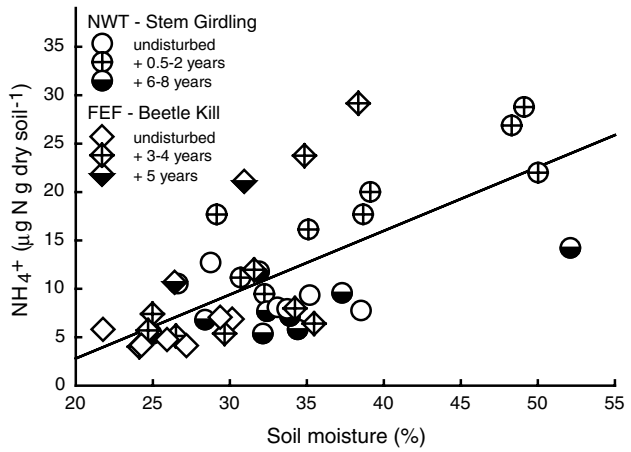

Fig. 3 The *line* represents the relationship between mean 2010-growing-season soil K_2SO_4-extractable ammonium and percent soil gravimetric moisture in the Fraser beetle-kill (FEF) and Niwot girdling (NWT) tree mortality chronosequences. Ordinary least squares linear regression $r^2 = 0.4552$, $P < 0.0001$

undisturbed plots (53.1 % FEF; 55.5 % NWT). Soil PO_4^{3-} pools had recovered by 5 years after disturbance in the Fraser Experimental Forest plots to 111.5 % of the value seen for undisturbed plots, while soil PO_4^{3-} increased in the Niwot Ridge girdled plots to 71.4 % of that observed in non-girdled plots 6 years following girdling, before declining again (Fig. 2f).

Microbial biomass and bulk soil C and N ratios

Mean microbial biomass carbon (MBC) for the 2010 growing season significantly decreased with tree mortality in both the Niwot Ridge and Fraser Experimental Forest chronosequences (Fig. 4a; NWT: $F = 2.77$, $P = 0.0459$; FEF: $F = 5.54$, $P = 0.0102$). In the Fraser Experimental Forest disturbed plots, MBC declined by an average of 38.9 % compared to control plots. In the Niwot Ridge chronosequence, MBC declined an average of 23.2 % in plots with girdled trees compared to non-girdled plots,

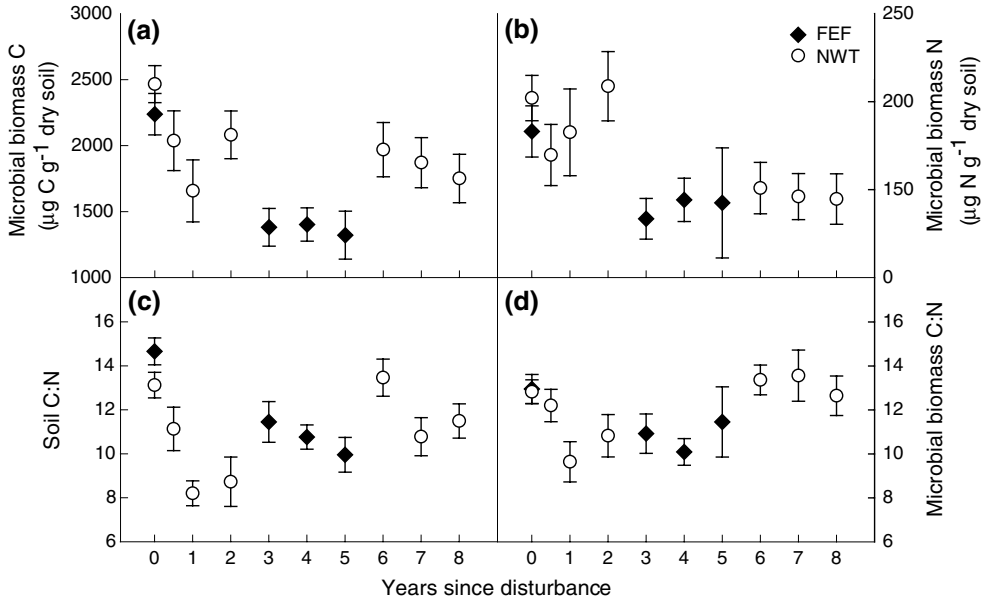

Fig. 4 Variation in soil nutrients and C:N across the beetle-kill (FEF, *closed diamonds*) and girdling (NWT, *open circles*) chronosequences. Values are the mean ± SE determined over the 2010 growing season from K_2SO_4 extractions: **a** microbial biomass carbon; **b** microbial biomass nitrogen; **c** soil dissolved organic carbon to dissolved organic nitrogen (DOC:DON); **d** microbial biomass C:N. At FEF, undisturbed plot class (year 0) $n = 6$, beetle-kill year 3 plot class $n = 5$, beetle-kill year 4 plot class $n = 5$, beetle-kill year 5 plot class $n = 2$; at NWT, undisturbed plot class (year 0) $n = 6$, girdled plot class $n = 3$

Table 3 Pearson's correlation coefficients (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between microbial biomass (MB) C and N pools and soil and microbial biomass dissolved organic C to total dissolved N ratios at the Fraser (FEF) and Niwot (NWT) chronosequence plots

Site	Variable	DOC	DON	NH_4^+	MBC	MBN
FEF	MBC	0.822***	0.615**	−0.186	–	
	MBN	0.749***	0.633**	0.137	0.917***	–
	Soil C:N	0.576*	0.277	−0.667**	0.693**	0.388
	MB C:N	0.461	0.273	−0.543*	0.50*	0.195
NWT	MBC	0.695***	0.647***	0.030	–	
	MBN	0.309	0.429*	0.591**	0.762***	–
	Soil C:N	0.670***	0.404*	−0.771***	0.341	−0.256
	MB C:N	0.477*	0.276	−0.805***	0.219	−0.435*

decreasing from 2,466 ± 140 to 1,895 ± 235 µg C g^{-1} dry soil. In both chronosequences, changes in MBC were significantly correlated with soil DOC pools (Table 3). In the Fraser Experimental Forest chronosequence, mean growing-season K_2SO_4-extractable microbial biomass nitrogen (MBN) decreased in a pattern similar to that observed for MBC (Table 3), though the overall decline was not significant (Fig. 4b; $F = 2.06$, ns). MBN in the Niwot Ridge plots exhibited no significant change as a function of time since disturbance (Fig. 4b; $F = 2.25$; ns), though it was correlated among plots with MBC, soil NH_4^+, and soil DON (Table 3).

The mean growing-season K_2SO_4-extractable soil C:N ratio declined over the Fraser Experimental Forest chronosequence, becoming significantly different from undisturbed plots by 4–5 years after tree mortality (Fig. 4c; $F = 5.54$, $P = 0.0102$). This decline was correlated with increases in NH_4^+ and DOC, but not with DON (Table 3). In the girdled plots, soil C:N decreased significantly 1–2 years after girdling (average 8.5 ± 0.8 C:N) compared to non-girdled plots (13.1 ± 0.6 C:N), before increasing again in the older plots along the chronosequence (average 11.9 ± 0.8 C:N; Fig. 4c; $F = 2.77$, $P = 0.0459$). Consistent with the results from plots in the Fraser Experimental Forest, declines in soil C:N were predicted more robustly by increases in NH_4^+ than changes in DOC or DON (Table 3). Compared to undisturbed plots, mean growing-season microbial biomass C:N decreased significantly in the early years following beetle kill in the Fraser Experimental Forest chronosequence (Fig. 4d; $F = 5.00$, $P = 0.0146$). This decrease was significantly correlated with increases in NH_4^+ and changes in MBC, but

not MBN, soil DON, or soil DOC (Table 3). Within one year after girdling, mean growing-season microbial biomass C:N decreased to values that were lower than those observed in non-girdled plots, before increasing again to a ratio comparable to non-girdled plots (Fig. 4d; $F = 6.81$, $P = 0.0008$). In the Niwot Ridge plots, microbial biomass C:N was robustly correlated with soil NH_4^+, weakly with soil DOC and MBN, but not with soil DON or MBC (Table 3).

Discussion

Early disturbance-induced changes in soil biogeochemical pools and microbial biomass in the Fraser beetle-killed plots

Over the first 4 years following MPB infestation, tree mortality led to significant decreases in soil K_2SO_4-extractable DOC, DON, inorganic P (Fig. 2a, c, f,), and microbial biomass C and N (Fig. 4a, b), presumably due to loss of root exudation. From the observed decreases in soil DOC and DON, we conclude that recently fixed labile carbon exerts a primary control over the cycling of soil C, N, and P following disturbance in these forests (see Högberg and Read 2006). In beetle-kill plots of the Fraser Experimental Forest, soil DOC was reduced 50.6 % at 3–4 years after infestation, similar to the 49 % decreases observed by Xiong et al. (2011), which were also recorded in plots of lodgepole pine trees recently killed (1–3 years ago) by MPBs. As no significant increases (Clow et al. 2011) to modest increases (Mikkelson et al. 2013) in stream water DOC export have been found in forest basins in Colorado that are heavily impacted by mountain pine beetle disturbance, the DOC loss we observed may have been due to heterotrophic respiration. The actual fate of the lost DOC is a matter warranting further investigation, as observations of processes to date cannot clearly account for the losses.

Soil TDN levels were primarily determined by changes in DON (Fig. 2b, c). More than one process may explain the reduction in DON after recent infestation that we observed. Lower DON pools following tree mortality may reflect the loss of root nitrogen inputs (Farrar et al. 2003), but also disruption to the seasonal and trophic dynamics of the rhizosphere. Carbon-rich root exudates first stimulate the microbial immobilization of N, whereafter seasonal or other induced reductions in rhizodeposition lead to C starvation, reduced microbial N demand, and increased mineralization (Weintraub et al. 2007; Kaiser et al. 2011). In a past study of plots with beetle-killed lodgepole trees near the Niwot Ridge plots, Xiong et al. (2011) found a soil food-web shift from fungal- to bacterial-feeding nematodes, with concomitantly lower C:N excretions that may enhance mineralization and accelerate microbial turnover (Moore et al. 2003).

Contrary to our predictions, inorganic P declined immediately following beetle-induced tree mortality (Fig. 2f). This result contrasts with studies of P loading after forest stand clear-cutting (Piirainen et al. 2007) and reports of P increases in the upper soil horizons following beetle disturbance (Kaňa et al. 2012). In our study, loss of root exudates and decreases in overall microbial biomass with disturbance, especially with regard to the mycorrhizal community and associated exoenzymes (Högberg and Högberg 2002), may also have decreased phosphorus mineralization (Dakora and Phillips 2002; Marschner et al. 2011).

In contrast, soil NH_4^+ in the Fraser plots demonstrated a nonsignificant increasing trend, with mean observed values doubling in plots 3–4 years after beetle mortality (Fig. 2d). Levels of soil NH_4^+ following beetle disturbance were of a similar magnitude to those reported in other studies of pine beetle impacts (Griffin et al. 2011; Griffin and Turner 2012; Keville et al. 2013). The observed increases in soil NH_4^+ are likely a product of several different processes. With the death of the tree, inorganic N uptake is eliminated. In addition, although pool sizes do not necessarily reflect or predict mineralization rates (Schimel and Bennett 2004), several lines of reasoning suggest that the increase in ammonium does indeed derive from an increased rate of nitrogen mineralization. First, increases in N mineralization have been reported for beetle-kill chronosequences in *P. contorta* and *P. menziesii*, which were correlated with decreased soil temperatures and increased litter quality (Griffin et al. 2011; Griffin and Turner 2012; but see Keville et al. 2013). Soil NH_4^+ pools in both disturbance chronosequences in our study were significantly correlated with increasing soil moisture and decreasing soil temperature (Fig. 3). Second, reduced rhizodeposition may induce C substrate limitation and reduce microbial demand for N, leading to greater N mineralization rates (Kaiser et al. 2011).

Although transpiration within the plots diminished with the loss of live trees, observed increases in soil moisture and decreases in temperature in recently disturbed plots were minimal and not statistically different in the Fraser beetle-kill chronosequence (Table 2). One possible explanation for the lack of significant observed changes in soil microclimate with mortality is that the smaller study plots are embedded in a matrix of live and dead trees. As such, the forest stand surrounding the plot may influence plot radiation balance, turbulent transfers of water and energy, and the interception and partitioning of precipitation, all of which may confound a mortality microclimate effect on a smaller tree-level scale.

Early disturbance-induced changes in soil biogeochemical pools and microbial biomass in the Niwot girdled plots

Losses of soil K_2SO_4-extractable DOC, DON, inorganic P, and MBC in the first 2 years that followed plot girdling were similar in magnitude to losses observed in beetle-killed plots (Fig. 2a, c, f). The large reduction in soil DOC and DON in disturbed plots was also roughly equivalent to the maximum observed differences (~50 and ~30 %, respectively) between plots with girdled and non-girdled trees in previous experiments in the Niwot Ridge forest (Scott-Denton et al. 2006; Weintraub et al. 2007), as well as other temperate forest girdling studies (Zeller et al. 2008; Kaiser et al. 2011). The gradual decline in these pools over the first several chronosequence years most likely represents the immediate loss of photosynthetic allocation to the roots, along with more gradually diminishing root exudates and rhizosphere biomass, as well as the functional decline of the root system. Högberg et al. (2001) showed that girdling reduced soil respiration by 54 % within 1–2 months compared to non-girdled control plots, even while accelerating the use of root carbohydrate reserves.

Differences in girdling and beetle-kill disturbances are also apparent in the soil moisture dynamics within the chronosequences. Mechanical girdling does not immediately compromise the function of xylem tissues, whereas transpiration rates have been observed to decline within 10–13 days of beetle infestation (Hubbard et al. 2013) or inoculation with beetle-associated blue-stain fungi (Yamaoka et al. 1990). Girdling reduces carbohydrate allocation to roots and causes a feedback to stomatal conductance, which eventually reduces transpiration and limits carbon uptake (Domec and Pruyn 2008; De Schepper et al. 2010). Ultimately, carbon starvation leads to hydraulic failure (McDowell et al. 2011). In the Niwot Ridge forest, soil moisture was not different between undisturbed plots and plots that were mechanically girdled in 2010, but increased in the first 2 years after girdling by 28.7 %, corresponding with the time of maximum tree mortality (Table 2). Soil NH_4^+ also increased in the Niwot Ridge chronosequence plots and peaked within 2 years after girdling (Fig. 2d). With girdling, carbon-starved roots increasingly lack the energetic resources needed for the uptake and assimilation of inorganic nitrogen (Kaiser et al. 2010).

Later disturbance-induced changes in soil biogeochemical pools and microbial biomass in both disturbance chronosequences

After the initial decline in both disturbance chronosequence plots, soil DOC, DON, and PO_4^{3-} pools rebounded in plots 5–8 years after disturbance, in some cases exceeding the concentration observed in undisturbed plots (Fig. 2a, c, f). In the Fraser Experimental Forest chronosequence, these increases corresponded with significant increases in litter and soil organic horizon depth (Table 1). The increase in these soil nutrients supports the hypothesis that increased litter inputs, and possibly root turnover, provides an increase in available substrate that stimulates heterotrophic decomposition and mineralization. Mean growing-season soil respiration fluxes along this chronosequence also followed the observed decline and recovery pattern in soil nutrient pools (Moore et al. 2013).

The increases in the litter and nutrient pools occur prior to significant seedling re-establishment in beetle-killed plots and in the absence of significant changes in understory vegetation cover (Table 1). Although our observations 5 years post-beetle-kill are limited by sample size, these plots show good agreement in their litter and understory vegetation dynamics with regional surveys in the same area. In an extensive survey of 221 0.02-ha plots in the Arapaho National Forest in Colorado, Klutsch et al. (2009) reported a significant increase in litter depth but no change in percent vegetation cover in plots 4 years and older after mountain pine beetle infestation. Furthermore, we observed that the organic-rich mineral layer depth was nearly doubled in the 5-year post-beetle-kill plots compared to control plots (Table 1), likely representing the decay of increased litter inputs. In the girdled plots, the mean soil organic-rich mineral layer in plots 6 years after mechanical girdling was 32–45 % deeper than the organic layers in all other post-girdling time classes (data not shown), and corresponded with greater concentrations of DOC. However, previous experiments in the Niwot plots involving small areas of selective litter removal and understory vegetation clipping undoubtedly impacted litter and decomposition dynamics to a degree, and any thus any conclusions drawn from this must necessarily be tentative.

Soil NH_4^+ pools in the oldest Fraser beetle-killed plots remained elevated when compared to plots with live trees, while significantly decreasing in plots girdled 6–8 years prior (Fig. 2d). In both cases, soil NH_4^+ concentrations significantly correlated with increasing soil moisture and decreasing soil temperature in older disturbed plots (Fig. 3). The decrease in soil NH_4^+ pools observed at Niwot 6–8 years after girdling likely reflects some of these microclimate influences on N mineralization, and illustrates the potential for confounding issues in the space-for-time chronosequence approach.

In accordance with most other studies of MPB impacts on biogeochemistry in Western US forests, increases in soil NH_4^+ were not accompanied by increases in soil NO_3^- (Morehouse et al. 2008; Griffin et al. 2011; Xiong et al. 2011; Keville et al. 2013); nor did a previous girdling study in the Niwot Ridge forest report an increase in soil NO_3^- (Weintraub et al. 2007). While increased

rates of nitrification have been observed with bark beetle disturbance (Morehouse et al. 2008; Griffin et al. 2011), as well as increases in soil water NO_3^- and leaching with European bark beetle mortality (Huber 2005), Knight et al. (1991) showed that levels of tree mortality in Western US lodgepole pine ecosystems must be severe before significant watershed export of nitrate occurs. These low-N-deposition coniferous forest ecosystems exhibit tight recycling of nitrogen (Fahey and Knight 1986; Turner et al. 2007) with high potential for microbial immobilization of NO_3^- (Stark and Hart 1997). In spite of extensive high-elevation regional forest mortality in Colorado, and reports of elevated soil NH_4^+ (Xiong et al. 2011), changes in stream export of NO_3^- have not been detected (Clow et al. 2011; Rhoades et al. 2013). Instead, inorganic nitrogen appears to be immobilized in the microbial biomass pool (see below), and assimilated by surviving trees (Knight et al. 1991) and the understory vegetation (Griffin et al. 2011; Rhoades et al. 2013).

In agreement with several past studies, we found no significant changes in pH in the organic-rich mineral horizon in plots that had been disturbed by natural beetle infection or girdling (Morehouse et al. 2008; Griffin and Turner 2012; Keville et al. 2013; Table 2). Xiong et al. (2011) and Kaňa et al. (2012), however, have reported increases in soil pH with beetle-killed trees, possibly due to elevated base cation availability in the soil solution from increased litter inputs displacing H^+ and Al_3^+ in the soil sorption complex. Additionally, increases in NO_3^- are associated with decreases in pH with bark beetle disturbance in Norway Spruce in Germany (Huber 2005). While the more mobile ions may have been dissolved and exported, our observations indicate that in the persisting soil biogeochemical pools, potential changes in base cations and NO_3^- were not large enough to impact the bulk soil organic horizon pH in affected Colorado forest stands.

Microbial biomass C and N pools in the Fraser Experimental Forest plot soils remained low in older beetle-killed plots despite recoveries in DOC and DON pools (Figs. 2a, b, 4a, b) and soil respiration rates (Moore et al. 2013), as well as increased litter input (Table 1). This implies a possible shift in soil microbial community composition with changes in substrate availability, and warrants further investigation. In the Niwot Ridge plots, microbial biomass C initially decreased after disturbance and remained lower along the chronosequence then in plots with live trees (Fig. 4a). Reductions in microbial biomass C have been consistently reported in pine forest plots following tree girdling, with ectomycorrhizal fungi composing the most significant portions of those losses (Högberg and Högberg 2002; Weintraub et al. 2007). Microbial biomass N pools were maintained and even increased after girdling; a pattern that was similar to that exhibited in soil NH_4^+ levels (Figs. 2d, 4b).

Soil DOC: DON and microbial biomass C:N ratios decreased significantly in disturbed plots in both chronosequences when compared to plots comprising live trees (Fig. 4c, d). As the loss of DOC and DON with recent tree mortality was severe, it is likely that the increases in soil NH_4^+ concentrations increased the amount of N relative to C in bulk soil and microbial pools. The C:N ratio in soil and microbial biomass in the oldest girdling plots recovered to levels equivalent to those seen in undisturbed plots, coincident with the decline in soil NH_4^+ concentration (Figs. 4d, 2d). In addition to the increased concentration of NH_4^+ in the soil, the decomposition of high C:N lodgepole pine litter may help drive the microbial immobilization of N and P (Fahey 1983), which may also in part explain the continuing lower microbial C:N ratios throughout the Fraser Experimental Forest chronosequence (Fig. 4d). Together, these results lead us to conclude that NH_4^+ immobilization by microbial biomass after tree mortality may retain some significant fraction of total soil N, contributing to the low observed levels of watershed export following mountain pine beetle outbreaks (Clow et al. 2011; Rhoades et al. 2013). Increased soil NH_4^+ may also be assimilated to some degree by the existing microbial biomass before uptake by nitrifiers (Turner et al. 2007), which may already be in relatively low abundance in this N limited system (Vitousek et al. 1979).

Conclusions

Tree mortality caused by the mountain pine beetle or mechanical girdling led to similar changes to soil biogeochemical pools but different soil moisture responses which were dependent on the time elapsed since disturbance. For up to 5 years after disturbance, tree mortality led to large short-term losses in soil organic matter in the absence of tree rhizosphere inputs. However, these pools increased 5 years or more after disturbance, coincident with canopy loss and increases in aboveground litter inputs. Soil moisture and ammonium increased with the loss of plant uptake, while wetter soils likely contributed to increased rates of nitrogen mineralization. Increases in soil NH_4^+ co-occurred with decreases in soil and microbial biomass C:N. Microbial immobilization of N combined with other evidence of N uptake by surviving vegetation and lack of significant N export into watersheds suggest the potential for high retention of those nutrients that remain in the soil immediately after disturbance, albeit at lower concentrations. Retention of nutrients as soil C and N cycling recovers following increased rates of litter deposition is also likely, encouraging the regeneration and competitive release of surviving vegetation.

Collectively, these results indicate that within N-limited Western US high-elevation lodgepole pine forests, nutrient retention may contribute to forest resilience after even severe levels of tree mortality caused by the mountain pine beetle.

Author contribution statement NAT, DJPM, and RKM conceived and designed the experiments. NAT, ELD, and EP carried out field and laboratory work. NAT analyzed the data and wrote the manuscript. Other authors provided editorial advice.

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