Metal fate and partitioning in soils under bark beetle-killed trees

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Abstract

Recent mountain pine beetle infestation in the Rocky Mountains of North America has killed an unprecedented acreage of pine forest, creating an opportunity to observe an active re-equilibration in response to widespread land cover perturbation. This work investigates metal mobility in beetle-impacted forests using parallel rainwater and acid leaches to estimate solid-liquid partitioning coefficients and a complete sequential extraction procedure to determine how metals are fractionated in soils under trees experiencing different phases of mortality. Geochemical model simulations analyzed in consideration with experimental data provide additional insight into the mechanisms controlling metal complexation. Metal and base-cation mobility consistently increased in soils under beetle-attacked trees relative to soil under healthy trees. Mobility increases were more pronounced on south facing slopes and more strongly correlated to pH under attacked trees than under healthy trees. Similarly, soil moisture was significantly higher under dead trees, related to the loss of transpiration and interception. Zinc and cadmium content increased in soils under dead trees relative to living trees. Cadmium increases occurred predominantly in the exchangeable fraction indicating increased mobilization potential. Relative increases of zinc were greatest in the organic fraction, the only fraction where increases in copper were observed. Model results reveal that increased organic complexation, not changes in pH or base cation concentrations, can explain the observed differences in metal partitioning for zinc, nickel, cadmium, and copper. Predicted concentrations would be unlikely to impair human health or plant growth at these sites; however, higher exchangeable metals under beetle-killed trees relative to healthy trees suggest possible decline in riverine ecosystem health and water quality in areas already approaching criteria limits and drinking water standards. Impairment of water quality in important headwater streams from the increased

potential for metal mobilization and storage will continue to change as beetle-killed trees decompose and forests begin to recover.

Keywords: Metal mobility, Bark beetle infestation, Soil chemistry, Forested ecosystem regulation

1. Introduction

Forests are important regulators of ecosystem function controlling water, energy, and nutrient fluxes. Natural and anthropogenic disturbance of forest cover sets off a ripple effect through the biosphere, lithosphere, hydrosphere and atmosphere of forested mountain ecosystems. Mountain pine beetle (MPB; *Dendroctonus ponderosae*) infestation has resulted in devastating tree death in the Rocky Mountains of the western United States and Canada with important ecological and societal implications. Warming climate and drought conditions have led to the unprecedented severity of the current infestation, as the MPB has impacted forests at higher elevations and latitudes, and attacked trees with weakened defenses (Kaiser et al. 2013; Mitton and Ferrenberg 2012). The resulting and extensive land cover change not only impacts the aesthetic and commercial value of affected forests, but also the water and biogeochemical cycles in these important mountain watersheds (Mikkelsen et al. 2013a). Previous biogeochemical research has focused on nutrient cycles in killed stands, and suggests the potential for increased fluxes of dissolved organic carbon (DOC), nitrogen, base cations, and aluminum into soils as trees are killed and decompose (Kaňa et al. 2013; Griffin and Turner 2012; Clow et al. 2011; Griffin et al. 2011; Huber et al. 2004; Tokuchi et al. 2004; Zimmermann

et al. 2000); however results are highly watershed specific (Mikkelsen et al. 2013a). Increased nitrification, base cation release from litter or exchange processes, or changes in organic acid inputs may also alter soil pH and ultimately metal mobility. Decreases in soil pH are possible from increased nitrification (Feller 2005); however, studies of soil chemistry alterations under beetle-killed trees in North America and Europe found increases in soil pH, likely related to exchange-site competition from increased base cation inputs (Kaňa et al. 2013; Xiong et al. 2011). The changing chemistry in soil waters resulting from insect-induced tree death and decomposition may have important implications for trace metal mobility, but has only recently been studied (Mikkelsen et al. 2014). In that study, pine needle leachate mobilized metals from soil columns, which consistently eluted higher concentrations of Zn, Cu, and Al than artificial rainwater. Eluted metal concentrations were found to be most strongly correlated with DOC and Ca ions, suggesting the importance of organic complexation and base cation competition in mobilizing metals in beetle-impacted forests and increased metal inputs from decomposing needles (Mikkelsen et al. 2014).

Metal mobility in soil water is largely controlled by sorption, complexation, and mineral phase reactions, including ion exchange, specific adsorption, organic complexation, and precipitation (Gong and Donahoe 1997). These processes depend on pH and soil composition (i.e. iron and manganese oxide, organic matter (OM), and clay content); however, the relative importance of each process may not be consistent for all trace metals. For example, pH-dependent specific adsorption may be the dominant process for Zn, whereas Cu mobility depends on complexation with organic ligands (Gong and Donahoe 1997; Sauvé et al. 1997; McBride et al. 1997). Metal complexation with OM is likely to increase as forests progress through MPB infestation. Although initial losses of labile carbon in mineral soils were observed as root

exudation ceases (Xiong et al. 2011), prolonged carbon fluxes from organic horizons may occur as tree needles and boles continue to decay (Huber et al. 2004; Kaňa et al. 2013). Soil carbon and pH changes may cause different metal mobilization processes to be important with tree death and subsequent regrowth; however the relative importance of each mechanism is unknown. The long-term effects of widespread MPB-induced tree death on metal mobility are also uncertain. As forests recover from widespread lodgepole pine death, regrowth often contains more subalpine fir, Engelmann spruce, and aspen (Pelz and Smith 2012), which may lead to changes in soil properties with trees species (Andersen et al. 2004), such as greater nutrient availability in new aspen forests (Buck and St Clair 2012). In addition, if organic matter and metals accumulate in the soil from needle leachate and bole decomposition, metals may continue to be leached as the forest recovers (Brun et al. 2008).

To understand the potential for delayed metal release and improve understanding of mobilization mechanisms, it is important to characterize the solid phase metal concentrations in soil, which may be the source of metals, but wherein different mechanisms are responsible for metal release. Resulting distributions provide a better understanding of how metals are mobilized by identifying where and how metals are sequestered in soils, highlighting the potential for release from environmental perturbations from land cover change, such as changes in pH or water and OM fluxes. The relationship between plants and metal partitioning and mobility in forest soils is complex and often complicated by spatial variability in total metal content and soil composition (Nowack et al. 2010). Specific studies concerning the effect of litter-derived organic matter on metal fractionation are limited; however, humic acids applied as soil amendments have been observed to affect mobility differently for different metals (Janoš et al. 2010). Application of solid humic acid redistributed Zn and Pb to a less mobile phase in moistened soils but

increased Cu mobility (Clemente and Bernal 2006). These effects were more pronounced in acidic soils than in calcareous soil, highlighting the relevance for soils typical of pine forests. Increases in Cu mobility may be attributed to complexation to soluble organic matter or colloids (Clemente and Bernal 2006), and may also reflect redistribution from residual to OM-bound fractions in soils (Janoš et al. 2010).

The objective of this study is to identify the potential mechanisms of metal mobilization and fractionation in soils collected beneath trees undergoing different stages of MPB-attack. Our approach uses a two-stage leaching procedure to identify the potential for increased metal mobility under dying trees. After identifying potential changes in metal mobility at the first site, a sequential extraction procedure (SEP) is employed to identify where metals are complexed in pine forest soils from an additional sampling event and how that may change as infestation progresses. Laboratory findings are then enriched through geochemical modeling to further identify controls on metal mobility and soils that may be more susceptible to metal leaching from MPB-induced tree death as well as provide generalizable results that transcend tree-scale soil heterogeneities between field samples. Collectively, this study moves toward a better understanding of metal mobilization mechanisms and changes in complex forest soil impacted by the MPB.

2. Methods

2.1 Sample collection

Two soil sampling events were conducted, one for each leaching procedure. Depending on the site, samples were collected from under green, red and grey phase trees. Green phase trees are alive and transpiring and are considered the control in these experiments. Red phase trees

have been attacked and cease to transpire by the following growing season. During this phase, trees retain the majority of their needles, which have turned red in color. By three to four years after attack, trees enter the grey phase and have lost almost all needles (Wulder et al. 2006).

The first set of samples was collected from Keystone Gulch basin (39.60° N, 105.98° W) near Dillon Reservoir, CO on September 22, 2010. Soils in this area are mapped as Frisco and Peeler sandy loams, which are derived from glacial drift and alluvium and colluvium from granite and granitic gneiss; these soils are composed of approximately 57% sand, 27% clay, and 16% silt (NRCS 2013). The bedrock in the region around this sampling location was hydrothermally altered and may contain higher acid-producing sulfide minerals and possibly trace metals than in non-altered regions of Colorado (Neubert et al. 2011). Samples were taken from beneath three green phase trees and three red phase trees on both north and south facing aspects (12 trees total). Samples were removed from both organic (5 – 20 cm) and mineral horizons (20 – 25 cm) and collected from a single pit one meter downslope from the bole. Ashy E horizons were not sampled as they were intermittently observed under green trees, but generally not under red phase trees. Mean diameters of the trees selected were 18.5 ± 4.0 cm (green pines, $n = 6$) and 21.3 ± 4.0 cm (red pines, $n = 6$).

After the initial site was logged and slash was burned, the second set of samples was collected from Rocky Mountain National Park (RMNP), near Estes Park, CO (40.33° N, 105.60° W) on September 6, 2012. Soils throughout this study area are mapped as Isolation gravelly sandy loam and formed from alluvium, colluvium, or till derived from granite, gneiss, and schist and were observed to be shallow and poorly developed; these soils are composed of approximately 65% sand, 13% clay, and 22% silt (NRCS 2013). Based on understanding gathered from Keystone Gulch soils, samples were collected on a south-facing slope beneath

three of each phase of tree (green, red, grey), resulting in 9 bulk samples for analysis. Three shallow pits located half way between the trunk and drip line were used to collect the samples. Prior to analysis, soil samples from the pits were combined and homogenized creating one sample for each tree. Samples were collected from the most decomposed organic layer represented under each tree, comparable to the organic layer from the first sample set. Mean diameters of the trees selected were 17.0 ± 2.0 cm (green pines, $n = 3$), 21.0 ± 4.0 cm (red pines, $n = 3$) and 23.3 ± 3.5 cm (grey pines, $n = 3$).

2.2 Soil preparation and laboratory procedures

Samples were air-dried and passed through a 2mm sieve after manually removing twigs and roots. Dry soils were stored at $<4^{\circ}\text{C}$ until use (Rubio and Ure 1993). A 5-10 g sub-sample was weighed and dried at 50°C for 24 hours before reweighing to determine moisture content and to minimize volatilization of organics (Nelson and Sommers 1996). Organic matter content was determined by loss on ignition (LOI) at 400°C for a minimum of 8 hours (Nelson and Sommers 1996; Blume et al. 1990). pH was determined by combining a 10g sub-sample of air-dry soil with either 10 mL of 0.01 M CaCl_2 (allowed to stand for 10 minutes after mixing) to measure $\text{pH}_{\text{CaCl}_2}$ (Thomas 1996) or shaken in DI water to determine $\text{pH}_{\text{H}_2\text{O}}$.

For both leaching procedures, all extractants were made with 18 M Ω water and acid washed glass lab equipment. When continuous agitation was required, samples were shaken horizontally on a shaker table at speeds specified in the standard dual leach and SEP protocols (300 and 50 rpm, respectively). All temperature-controlled steps were performed in a water bath with 50 rpm of continuous agitation and occasional manual agitation. The leachate was analyzed with inductively coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer 5300).

Prior to analysis, samples were centrifuged per the appropriate dual leach and SEP protocols (515 g for 30 minutes and 3000 g for 5 minutes, respectively) and all leachates were filtered to 0.45 µm with nylon acetate filters. Soil leaches identified Cd, Cu, Ni and Zn as elements of interest that are also regulated by water quality statutes (U.S. Environmental Protection Agency 2012). The ICP detection limit for Cd was 0.1 µg/l, Cu was 0.9 µg/l, for Ni was 0.8 µg/l and for Zn was 0.3 µg/l.

2.3 Two-phase leaching of Keystone Gulch soils

For green and red phase samples from the Keystone site, 2.0 g (n=12) of soil dried at 50 °C for 24 hours was leached with 40 mL of solution in two parallel leaching procedures, both with solid-liquid ratio of 1:20 (van der Sloot et al. 1996). To gauge the most labile quantity of metals and simulate conditions in soil-water during a moderate rain event, the first leaching phase used an artificial rainwater composed of dilute salts (Table 1) according to Davies et al. (2004). The second leaching phase used nitric acid to determine total recoverable metals (Table 1; U.S. Environmental Protection Agency 1992). From these leaches, the solid-liquid partitioning coefficient was calculated as (Allison and Allison 2005):

$$K_D = \frac{L}{S} * \frac{[M_N] - [M_A]}{[M_A]} \quad (1)$$

where K_D is the solid-liquid partitioning coefficient (L kg⁻¹), L / S is the liquid to solid ratio (20 L kg⁻¹), $[M_N]$ is the concentration of metal “M” determined by nitric acid leaching procedure (mg L⁻¹), and $[M_A]$ is the dissolved concentration of metal “M” analyzed in artificial rainwater leachate (mg L⁻¹). Lower K_D values correspond to increased metal mobility.

2.4 Sequential extractions of soils in Rocky Mountain National Park

Sequential extractions procedures (SEP) were based on Li et al (1995) and define four operational extractable fractions consistent with those in the widely used Tessier SEP (Tessier et al. 1979): exchangeable, carbonate-bound and specifically adsorbed (acid-soluble), iron/manganese oxide-bound (reducible) and organic matter and sulfide bound (oxidizable). Consistent with the SEP procedure (Li et al. 1995), soils were ground using an acid washed mortar and pestle and sieved to <150 μm prior to extractions. Ground and sieved soils (1 g) from each tree were transferred to a 50 ml polypropylene centrifuge tube for extraction with the leachates and procedures described in Table 1. Between leaches, samples were centrifuged and the supernatant was decanted into new polypropylene tubes. Samples were washed with 8 ml of 18 M Ω water, continuously agitated for 5 minutes, and centrifuged again prior to the next step. Final matrix adjustment and acidification with HCl was performed consistent with the SEP procedure for the leachate aliquots from steps 1-4 (4, 1, 15, 15 mL, respectively).

2.5 Geochemical modeling

A three-site model of metal sorption in soils was used to capture important release mechanisms including ion exchange on clay surfaces, surface complexation with iron oxides, and organic complexation. The multi-surface model was populated with metal concentrations based on the results of the sequential extractions (Table S1) and implemented in PHREEQC (Parkhurst and Appelo 2013).

Ion exchange: Metal exchange on clay surfaces was represented using the Gaines-Thomas exchange convention in PHREEQC and associated databases. The exchange sites were initially populated with the combined cation concentration leached during the exchangeable, acid

soluble, and organic matter extractions performed in the SEP. The total number of sites was based on a cation exchange capacity of 10 meq/100 g, consistent with Isolation gravelly sand loam in RMNP (NRCS 2013).

Surface complexation: Metal surface complexation was modeled using a two-site diffuse double layer surface complexation model, parameterized using the surface area, site density and thermodynamic data of Dzombak and Morel (1990) for complexation to strong and weak affinity sites on hydrous ferric oxide (HFO). The iron oxide content was based on the extracted iron from the reducible step of the SEP and assuming 10% was HFO (Appelo and Postma 2005).

Organic complexation: The reactive fraction of soil organic matter (SOM) was assumed to be predominantly humic substances (i.e. humic and fulvic acids) that are well described by a discrete site model. The model was based on the wateq4f.dat database for PHREEQC, with additional constants from the WHAM family of models (Tipping 1998; Tipping and Hurley 1992) to generate the T&H.dat database (available through Appelo and Postma 2005). The WHAM-based model describes proton and metal sorption using eight binding sites of varying strengths, representing carboxylic and phenolic acid groups. The model also includes bidentate complexes, ultimately resulting in 20 total binding sites. Electrostatic effects from cation binding to the surface of organic matter are accounted for using the diffuse-layer functionality of PHREEQC, assuming a constant layer thickness. Tridentate complexes (available in WHAM VI; Tipping 1998) or complex couplings and additional electrostatic effects (available in PHREEQC-Model VI; Marsac et al. 2011) were not used in this model. Complexation to dissolved organic matter was implemented in the model by adding additional surface sites for DOC with the WHAM-based thermodynamic constants for humic substances.

3. Results & Discussion

3.1 Effect of tree death on metal mobility coefficients in Keystone Gulch soils

The mobility of selected metals was quantified using solid-liquid partitioning coefficients (K_D), with lower coefficients indicating greater mobility. Mean K_D values were consistently lower under red trees than under green trees for each metal within both soil horizons analyzed with varying levels of significance (Fig. 1, Table S2). Assuming green trees represent predisturbance soil conditions, lower K_D values under attacked trees indicate increased mobility. This trend was significant for Al, Ba, Cd, Cu, Ni, and Zn ($p < 0.05$) in the organic horizon and in the mineral horizon for Al, Cd, and Fe ($p < 0.05$), with less significant ($p < 0.1$) decreases noted for Ba and Ni. In general, leachates from mineral horizon samples had lower metal mobility (Fig. 1, Table S2) and higher pH (Table 2) than organic soils. Changes in the partitioning coefficients from the green to red phase reflect the combined effect of metals leaching from the solid phase and increasing mobile cation inputs from the litter leachate. Zn, Ni, Cu, and Cd contents determined by the nitric acid leach in this study are 10-35% lower than total metal concentrations from XRD analysis of soils in the Keystone area reported by Mikkelsen et al (2014). Acid-leached Zn concentrations were generally consistent with expected background levels from a previous report; however Cu concentrations were 50-80% lower and Cd concentrations 10-20 times higher than background levels reported nearby (HRC 2001). These differences highlight the spatial heterogeneity of metal contents in these soils.

Base cations also exhibited greater mobility under attacked trees (Fig. 1). Greater K and Mg mobility under red trees was observed in both the organic and mineral soil horizons ($p < 0.1$ and $p < 0.05$), and for Mg on south facing slopes ($p < 0.05$). Analyses of Ca and Na partitioning coefficients indicate the potential for increased mobility; however, only increased Na mobility

with tree death in the organic soil horizon was significant ($p < 0.1$). In general, rainwater leached concentrations of base cations were greater in organic soil horizons under red trees than under green trees. This finding is consistent with literature reports of significantly higher K in both humus and topsoil layers under beetle-killed trees, and higher Ca and Mg concentrations in only the humus layer (Kaňa et al. 2013). In a beetle-impacted spruce forest, earlier release of K was observed, suggesting other cations may become more important as attack and decomposition progress (Kaňa et al. 2013). The loss of plant uptake of base cations may also cause the accumulation of easily exchanged cations in the soil under dead trees, consistent with observations of K accumulation after attack (Xiong et al. 2011).

Fig. 1 Mean solid-liquid partitioning coefficients (K_D) $\pm$ one standard deviation for trace metals of interest and base cations in the organic horizon and mineral horizons under red (squares) and green (circles) phase trees at Keystone Gulch.

No significant changes in pH were observed between tree phases at either site (Table 2), although pH was significantly higher in the mineral than organic horizon at Keystone Gulch ($p < 0.01$). Correlations between pH and $\log K_D$ were consistently stronger under red trees than under green trees for the Keystone Gulch samples binned across aspect and soil horizons. For example, the coefficient of determination (R^2) for the Zn regression increased from $R^2 = 0.0001$ for green trees to $R^2 = 0.84$ for red trees (Fig. 2). Improved explanation of variance (indicated by an increase in R^2 , or $\Delta R^2 > 0.20$) was also found for Ba ($\Delta R^2 = 0.35$), Ni ($\Delta R^2 = 0.37$), and Mn ($\Delta R^2 = 0.68$). Consistent with Fig. 1, the mobility associated with green trees was generally lower than that associated with red trees at a given pH value, particularly at lower pH values.

The higher correlation under red trees suggests that metal mobilization is influenced either directly by differences in pH, or indirectly by processes that are influenced by pH or vary concurrently with pH, such as ion exchange, DOC mobility, and mineral solubility. Ion exchange and DOC related changes may also be related to cation and carbon release as trees are killed and decompose. In green trees, however, the poor correlation indicates more complex or heterogeneous controls on metal mobility, potentially including complexation with DOC, carbonates, or other ligands of interest in forest soils. The more correlated response under red trees may also be attributed to the composition and degree of degradation of the litter, which may be expected to be more uniform under red trees, and is consistent with the smaller variance in K_D under red trees, as represented by the error bars in Fig. 1. By nature of the response to infestation and resulting tree die-back phases, trees in the red phase can be expected to drop more needles over a relatively short time period compared to green trees, which may steadily accumulate a more variable composition of litter that has decomposed and leached over a range of timescales (Klutsch et al. 2009).

Fig. 2 Regressions of Zn mobility (reported as $\log K_D$) versus pH binned across horizons and aspects at Keystone Gulch for soils under red (squares) and green (circles) phase trees.

The magnitude of change in metal partitioning also differs based on aspect (Fig. 3). The increased mobility associated with red trees was observed on both north and south facing slopes; however, this trend was more pronounced on south facing slopes for Zn and Cd (Fig. 3). This difference between north and south facing slopes may be related to inherent differences in soil composition with aspect or the enhancement of these differences as a result of tree death. For example, the greater difference on south facing slopes may be attributed to increased exposure to solar radiation due to canopy loss, which would accelerate the rate of photodegradation (Austin and Vivanco 2006) as a tree progresses through stages of infestation. Increased decomposition of the litter layer releases more soluble metals and base cations as well as leachate DOC. If the increase in DOC and base cations outweighs the increase in soluble metals, total recoverable metals from samples of the underlying soil will decrease. The increased wetting and drying cycles and potential differences in soil moisture on south facing slopes therefore have the

potential to increase the resultant change in partitioning coefficient. In addition to increased exposure, beetles preferentially attack weakened trees in drier topographical locations, such as south-facing slopes, and thus south-facing slopes are often attacked first (Kaiser et al. 2013). As a result, selected trees on south-facing aspects may have been attacked earlier, allowing more time for decomposition and uptake loss. Different predisturbance moisture regimes may also cause distinct soil properties under preferentially attacked trees. At Keystone Gulch, the absence of consistently observed E-horizons under red trees may reflect a drier predisturbance condition consistent with preferential MPB-attack.

Fig. 3 Mean solid-liquid partitioning coefficients (K_D) $\pm$ one standard deviation for select metals in the organic horizon under red (squares) and green (circles) phase trees on south (S) and north (N) facing slopes at Keystone Gulch.

3.2 Soil moisture trends under MPB impacted trees at two sites

In general, MPB-induced tree death altered the soil moisture at the tree scale for both sampling events (Figure 4, Table 2); however, the significance of these trends depended on

aspect and soil horizon. At Keystone Gulch, soil moisture was higher under red trees than under green trees, in both soil horizons (Table 2). Across horizons, soils under red trees on south facing slopes were on average 60% wetter relative to green trees (6.2%, $n=6$ compared to 3.9%, $n=6$; $p<0.11$); however no pronounced differences were observed between green and red phase trees on north facing slopes. Comparing across aspects and soil horizons revealed modest increases in soil moisture under red phase trees compared to living pines (42% increase, $n=12$; $p<0.12$). Trends in organic soils sampled on south facing slopes from RMNP in 2012 were consistent with those from Keystone Gulch in 2010; however, soil moisture increases were only significant under red trees (103% increase; $p<0.01$; Table 2) and not under grey trees.

Soil moisture trends with tree death are consistent with observations and models of transpiration loss and needle fall in MPB-impacted forests (Bearup et al. 2014; Hubbard et al. 2013; Maness et al. 2013; Mikkelsen et al. 2013c). In the initial stages of attack, transpiration ceases, suggesting increases in soil moisture. In the grey phase, after needles fall, increased solar exposure and ground evaporation may cause the soil moisture to decrease from the red to grey phase. This interplay between evaporation and transpiration may also have important implications for wetting-drying cycles and related litter decomposition and nutrient release. Drying cracks observed at Keystone Gulch in the boundary between the litter and organic horizons in red phase trees further suggest intensified wetting–drying cycles, particularly on exposed south-facing slopes.

Fig. 4 Spatial representation of soil moisture and organic content in RMNP soil samples. Marker size represents property values ranging from 5-14% for moisture content and 4-43% for organic content (LOI) in a field plot defined in latitude and longitude. “GN” indicates green, “R” indicates red, and “GY” indicates grey phase trees.

3.3 Effect of tree death on metal fractionation in forest soils from Rocky Mountain National Park

In Keystone Gulch soils, the observed decrease in partitioning coefficients, indicating increased metal mobility under red phase trees, suggests initial losses of total sorbed trace metals from the soil profile, particularly in the event of increased wetting and drying cycles and changes in soil moisture. As tree death progresses, it is also likely that base cations and select trace metals leached from fallen litter will accumulate in the soil profile and result in cation exchange processes or increased metal loading in the organic layer. In general, sequential extraction

results from soils collected in RMNP suggest the accumulation of metals is also an important control on metal mobility (Fig. 5); however, different metals exhibited different solid-phase behavior. Consistent with other studies of trace metals in lodgepole pine trees (Ayer and Kratochvil 1986), small scale soil heterogeneity created substantial variability in this largely qualitative analysis.

Fig. 5 Total metal content (bar height) divided into metal content sequentially extracted from the exchangeable, acid-soluble, reducible, and oxidizable soil fractions (segment tone) for individual trees in each phase of tree death; i.e. unimpacted (green), killed but retaining needles (red), and dead with most needles dropped (grey). Note the Ni, Cu, and Cd content are multiplied by a factor of 3, 4, and 50, respectively.

Cd, Cu, Ni, and Zn were identified as metals of interest in this analysis due to their presence in RMNP soils and trends that were consistent with changes in the organic layer partitioning coefficients in Keystone Gulch discussed above. High concentrations of Cd, Cu, Ni, and Zn in soils may also have eco-toxicological implications for conifer seedlings, including inhibited regeneration and root growth (Kukkola et al. 2000; Kahle 1993) and for aquatic insects, causing substantial mortality at low concentrations (Clements et al. 2013; Schmidt et al. 2013; U.S. Environmental Protection Agency 2002). SEP results for other metals are provided in Fig. S1. Zn, Ni, and Cd were primarily extracted from the reducible fractions (averaging 51%, 41%, and 35% across tree phases, respectively), and Cu exhibited the highest percentage in the oxidizable fraction (averaging 45%, compared to 25% for Zn and 20% for Ni; Table S3). This is consistent with literature that suggests that Cu is readily complexed by soil organic matter, but Zn and Ni are associated with iron and manganese oxides resulting from co-precipitation, or in the case of organic soils from sorption of organic complexes onto oxide surfaces (McKnight et al. 1992). Cu also exhibited a lower percentage in the exchangeable fraction (averaging 4.6% across all tree phases, compared to 14% for Zn and 21% for Ni, Table S3), suggesting that mobilization from mechanisms such as pH change or base cation competition are less likely for Cu. In contrast, 33% of Cd was extracted from the exchangeable fraction.

Differences in metal fractionation between tree phases were not statistically significant, although more than 10 samples per condition might enhance statistical validity (Helsel and Hirsch 2002), which was not feasible at the time of sampling in RMNP due to limitations on suitable red trees for sampling with consistent size, age, and local topographical conditions. Thus, a discussion of the qualitative differences is provided, which lends considerable insight to metal release mechanisms. In contrast to expectations from the partitioning coefficient analysis,

metal leaching from the exchangeable or acid-soluble phases was not generally observed as trees progressed from the green to grey phase (Fig. 5). In contrast, metals, particularly Zn and Cd, accumulated in soils under grey trees, as depicted by the grey bars in Fig. 5 (29% and 33% increase, respectively, $p < 0.25$). This observation may be attributed to metal inputs from increased litterfall (Mikkelsen et al. 2014), loss of uptake as transpiration ceases (Kahle 1993), or higher concentrations in needles with tree death (Ayer and Kratochvil 1986). Zn increases under grey trees are primarily observed in the oxidizable phase, suggesting association with organic matter. One grey-phase tree presented an exception to this observation and had a Zn concentration in the oxidizable phase that was the same or less than the red or green phase trees (Fig. 5). This tree, depicted as tree GY2 in Fig. 4, also had anomalously low total organic matter content (4% compared to 35%, the average of remaining grey trees based on LOI), providing evidence that Zn and organic matter release and accumulation from needle decomposition, might be an important controlling mechanism. The distribution of Cd shifted toward the exchangeable fraction, indicating increased Cd mobility with tree death. The lack of Cd increase in the organic fraction signifies that the mechanisms controlling Cd mobility in soils differ from Zn. Small amounts of Cd were detected in needle leachate (Mikkelsen et al. 2014), thus if Cd is accumulating with needle deposition it is more easily redistributed to more labile soil fractions than Zn. Changes in Ni fractionation were not detectable (Table S3), which is partially consistent with the observations of the partitioning study, wherein no changes in rainwater-leached Ni concentrations were observed. Decreases in the Keystone Gulch Ni partitioning coefficient with tree death can largely be attributed to decreases in total sorbed concentrations. This expected leaching from the solid phase was not observed in the RMNP extractions, and may be a rapid phenomenon that was not captured in the second sampling event. In contrast, total Ni in RMNP

soils increased by 29% from the green to red phase and by 8% from the green to grey phase, although increases were not statistically significant and biased by one red tree with high Ni in the acid soluble phase (Fig. 5). Increases in total extractable metals may reflect loss of uptake by red trees. Unlike Cd, Ni and Zn, total extractable Cu concentrations did not increase in the grey phase; however grey phase trees exhibited a stronger redistribution of Cu to the oxidizable phase from the green and red phase (Fig. 5, Table S3). The difference between Cu, Cd, and Zn behavior suggests that inputs of Zn and Cd from litter leachate may be redistributed to other soil fractions and offset potential metal leaching whereas Cu is only redistributed to the organic fraction.

3.4 Modeling metal-mobilization mechanisms (pH, base cations, and DOC)

A mixture of sorption sites can be used to describe metal complexation in soils (Weng et al. 2001) and to identify how soils respond to biogeochemical and environmental perturbations. Simulations using a three-site hydrochemical model populated with metals from SEP results demonstrated that despite different extraction mechanisms, nearly all of the mobilizable fractions of metals of interest (Zn, Cd, Cu, Ni) are associated with organic matter in shallow RMNP soils. Simulated adsorption of trace metals to clay surfaces was minimal with the highest percentage determined for Zn at only 0.1% of the solid phase. Modeled surface complexation to iron oxides was even less important, with the highest percentage determined for Cu (0.02% of the solid phase). The relative unimportance of surface complexation in RMNP soils is consistent with the low iron oxides content in the Alfic Argiustolls typical of this region (NRCS 2013) and the lack of correlation between Fe content and Zn, Ni, Cd or Cu content in the reducible fraction.

Competing ions (i.e. Na, K, Fe, Al) were modeled only in the clay and iron oxide phases and were not simulated using the organic complexation model due to the lack of humic complexation constants for these cations; however, this omission had little effect on the fraction of Zn, Ni, Cd, or Cu sorbed to clay surfaces (<0.1% increase in trace metals associated with clay exchange sites). The lack of response to decreased concentrations of competing ions suggests that clay exchange-site limitations and major-cation availability are not controlling trace metal mobility in this model. When the modeled soil surface was exposed to increased base cation concentrations typical of pine needle leachate (i.e., increases in Ca, Mg, Na, K by over an order of magnitude from rainwater leachate; Mikkelsen et al. 2014) increases in the modeled leachate concentrations of Zn, Ni, Cd and Cu were small (2.6%, 0.0%, 0.0%, 0.5%) and were associated with modeled Zn and Cu inputs from litter leachate, rather than competition from base cations. Similarly, exposing the surface (initially equilibrated at pH=5.0) to a leachate solution with the original base cation and DOC concentrations but lowering pH to 4.0 had minimal effect (<1%) on metal partitioning. The lack of response to a one pH unit decrease is likely related to the initially low pH used in this model and typical of pine forest soils in RMNP. Soils with higher initial pH values that are closer to the sorption edge or with a lower ratio of organic matter to iron oxides (Appelo and Postma 2005) may be more susceptible to changes in pH.

The largest simulated change in metal partitioning resulted from the addition of DOC as humic substances. The modeled solid-liquid partitioning coefficient calculated for soils leached with pine needle tea was consistently lower than for rainwater leached soils, representing the range of leachates for soils under pines (Fig. 6). This change was only significant with the addition of DOC, and was not observed with increased base cation concentrations. The decrease in partitioning coefficient is qualitatively consistent with the findings of the two-phase

extraction, and suggests that the presence of DOC drives these changes in mobility; however, at higher metal concentrations these differences may be less pronounced (Doig and Liber 2007). The modeled change was subtlest for Cd and greatest for Cu (Fig. 6), representative of the strong affinity of Cu to DOC. Pine needle tea (needles leached in a controlled environment) provides an extreme case of relevant DOC concentrations at up to 100 mg L^{-1} (Mikkelsen et al. 2014). Soil water DOC under red phase trees at Chimney Park, Wyoming averaged 23 mg L^{-1} , compared to 14 mg L^{-1} under green trees in Niwot Ridge, Colorado (Mikkelsen et al. 2014; Biederman et al. *Submitted*). As seen in Fig. 6, substantial differences in metal partitioning may occur with modest increases of DOC that are particularly relevant in the $10\text{-}20 \text{ mg L}^{-1}$ range, which is comparable to the differences in reported soil water concentrations under the red and green phase trees. The high partitioning coefficients modeled for Cu in the absence of humic substances indicate that exchange processes are relatively unimportant to Cu mobility in these soils, consistent with the low percentage of Cu in the SEP exchangeable fraction (Table S3). Common consensus in the literature also specifies organic complexation as the dominant process controlling Cu speciation in surface waters with environmentally relevant DOC concentrations ($2\text{-}7 \text{ mg C L}^{-1}$; McKnight et al. 1983) that are lower than soil water concentrations from under living and infested lodgepole pines (Fig. 6).

Fig. 6 Modeled solid-liquid partitioning coefficients (K_D) influenced by the addition of dissolved organic carbon as humic and fulvic acids. K_D values are calculated from the ratio of modeled sorbed to aqueous concentrations for zinc (circle), nickel (square), copper (triangle), and cadmium (star). Relevant DOC concentrations for rainwater, pine needle tea, and soil water from under green and red phase trees are provided on the top of the graph. Rainwater leached (black) and needle leached (white) K_D values of Zn, Ni, Cu, and Cd are highlighted in the inset.

3.5 Environmental Implications

Of the metals discussed, Zn is the most likely to persist in MPB-impacted soils. Zn from litter leachate accumulates in the solid phase and is primarily associated with the organic fraction of forest soils, although more mobile soil fractions maintained consistent Zn content with tree death. Cd increases in the exchangeable fraction suggest a more immediate impact to soil water quality. Minimal detectable change was observed in Ni fractionation under impacted trees. Cu mobility and fractionation are most strongly controlled by the presence of organic matter. Total

Cu in the solid phase did not increase; however, the percentage associated with the organic phase was generally higher under grey trees than under either red or green. The redistribution of Cu to a more refractory soil fraction in the grey phase suggests that delayed Cu release is less likely; however, mobilization during periods of high DOC inputs in leachate from increased needle fall (i.e. at the start of the grey phase) is still a concern. Despite the potential for increased mobility, total leached metal concentrations of Zn, Ni, and Cu are still below EPA primary and secondary drinking water standards (U.S. Environmental Protection Agency 2012) and human health risk from these metals is expected to be minimal (Fig. 7). Exchangeable Cd levels, however, exceeded the MCL for all tree phases. The higher toxicity of Cd highlights the importance of monitoring changes in small Cd concentrations, particularly in areas with high background levels of Cd. Despite these increases in potential exchangeable metals, it is likely that sequestration prior to reaching nearby streams and in-stream dilution will mitigate some or all of these increases.

Elevated or depleted metal content in soils may also stress remaining and regenerating vegetation. In Scots pine seedlings, amendments of 5 mg Ni kg⁻¹ dry soil resulted in healthy root growth. Higher Ni content (>15 mg Ni kg⁻¹ dry soil) caused injury to pine seedlings including inhibited root growth (Kukkola et al. 2000). In RMNP soils, total Ni content for one red tree exceeded this injury threshold (19.3 mg Ni kg⁻¹ dry soil, Fig. 5) and caused the average Ni content to increase from 10.9 mg Ni kg⁻¹ dry soil under green trees to 14.0 mg Ni kg⁻¹ dry soil under red trees. Although the increases in total Ni may suggest the potential for plant stress, the amount in the exchangeable, and thus bioavailable (Preciado and Li 2006), fraction was low, with only 6% lower exchangeable Ni under healthy trees. The average exchangeable Ni under red trees was 2.7 mg Ni kg⁻¹ dry soil, suggesting small increases in available Ni with tree death

may actually facilitate regeneration and root growth. Similarly, spruce seedlings grown in solutions of 30-60 μM Zn exhibited inhibited root growth, an indicator of the effects of heavy metal toxicity (Kahle 1993). The total solution concentration leached from the four soil fractions in RMNP soils increased from 41.1 μM Zn under green trees to 55.4 μM under grey trees; however, only 14.0 μM Zn was extracted from the exchangeable phase under grey trees. Cd inhibited root growth in spruce seedlings when grown in solutions of 5-60 μM Cd, with complete root growth cessation in solutions with >30 μM Cd (Kahle 1993). Total and exchangeable Cd in the present study were substantially lower than this threshold, even under grey trees (0.61 and 0.37 μM Cd respectively), which exhibited higher Cd contents than under living trees (Fig. 7). Increases in soil metal content accumulated under killed trees are likely a result of additional metal inputs to the system through the decomposition of needle and woody materials with tree death, indicating an open system. Despite increases in total Zn, Ni, and Cd contents that approach critical thresholds, it is unlikely that the increase in the bioavailable fraction is sufficient to stress regenerating vegetation (Fig. 7).

Fig. 7 Exchangeable metals from RMNP soils compared to sensitive aquatic species toxicity limits. Bar color indicates tree phase and lines indicate limits for protection of drinking water (Zn – EPA secondary limit, Cu – MCL) and evergreen seedlings (Zn & Cd - Kahle 1993, Ni - Kukkola et al. 2000), with specific impairment thresholds for each metal indicated by horizontal lines.

4. Conclusions

Impacts to nutrient and biogeochemical cycles from MPB-induced tree death may ultimately lead to changes in metal partitioning in forest soils. The combined experimental and modeling results of this study demonstrate that metal mobility increases in soils under attacked trees and is primarily controlled by increased SOM and DOC fluxes, rather than increased fluxes of base cations into the soil, or changes in soil water pH. Consistent with previous observations (Kaňá et al. 2013; Griffin et al. 2011; Xiong et al. 2011), soil pH at both sites generally increased with tree death; however, differences were not statistically significant. In contrast, soil moisture increased under affected trees and soils exhibited evidence of increased wetting and drying cycles. In addition to accelerated organic matter decomposition that may result from increased soil moisture (Huxman et al. 2004), loss of forest canopy may further affect carbon cycling. Increased solar exposure not only causes warmer soil temperatures (Magid et al. 1999) but also more photodegradation of litter (Austin 2011; Austin and Vivanco 2006). Complex changes to the biogeochemistry of carbon and forest hydrology may result in differences in the character and transport pathways of DOC. The combined effect of these alterations likely explains trends in water treatment plant data which suggest more hydrophobic DOC (i.e. fulvic and humic acids) is exported from infested watersheds in Colorado (Mikkelsen et al. 2013b). These changes in

DOC character and concentration persist through the late summer rather than coinciding solely with snowmelt flushes in the spring (Mikkelsen et al. 2013b). Whereas the relationship between DOC character and metal complexation is inconsistent (Mikkelsen et al. 2014; Croué et al. 2003; Van Schaik et al. 2010; Guggenberger et al. 1994), the persistence of altered DOC transport and quality through the late summer and observations of increased groundwater contributions to streamflow during this period (Bearup et al. 2014) support the potential for altered metal transport during low flow periods when small changes in metal exports may be more significant. The influence of organic carbon on metal mobility identified in this study further emphasizes the importance of understanding changes to the structure and type of organic compounds, including organic acids, in MPB-impacted forests.

Spatial heterogeneity commonly presents a challenge to distinguishing trends in soil properties from individual tree treatments (Nowack et al. 2010; Ayer and Kratochvil 1986) and determines the relative importance of biotic and physical processes (Austin 2011). In this study, the qualitative findings of the sequential extraction procedure, the variable organic matter content in RMNP soils (Fig. 4), and the difference in Ni behavior between the RMNP and Keystone Gulch sites exemplify this challenge across tree to site scales. Adequate understanding of physical variability is needed to appropriately upscale these results in watershed models and to understand long term and regional trends in metal mobility in MPB-infested forests. Ultimately, the degree of impact to the water quality in important headwater streams from the increased potential for metal mobilization and storage will continue to change as beetle-killed forests regenerate and typical forest metal and nutrient cycles are restored.

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Supplemental Information

One supplemental figure (S1) and three tables (S1-S3) with details on mobilization of additional metals and initial modeling conditions.

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