THESIS

RESPONSE OF STREAMFLOW AND STREAM CHEMISTRY TO PINE BEETLE INDUCED TREE MORTALITY ACROSS NORTHERN COLORADO

Submitted by

Ashley Lynn Menger

Department of Ecosystem Science and Sustainability

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Master's Committee:

Advisor: John D. Stednick

Steven R. Fassnacht
Michael C. Ronayne

ABSTRACT

RESPONSE OF STREAMFLOW AND STREAM CHEMISTRY

TO PINE BEETLE INDUCED TREE MORTALITY IN NORTHERN COLORADO

The lodgepole pine (*Pinus contorta)* forests of western North America recently endured the most severe insect-induced mortality in recorded history. The hydrological and biogeochemical impacts of mountain pine beetle (*Dendroctonous ponderosae*) (MPB) induced die-off are uncertain even with recent conceptual and physical research. The purpose of this study is to provide insight into changes in annual water yield, streamflow generation mechanisms and stream water nutrient concentrations due to the recent MPB epidemic. To evaluate the possible impact, watersheds with varying amounts of MPB induced tree mortality in the north-central Colorado Rocky Mountains are examined. It was hypothesized that the canopy loss associated with the MPB epidemic has led to significant changes in annual water yield, streamflow generation mechanisms and stream water total nitrogen, nitrate, and total organic carbon (TOC) concentrations.

Data stationarity analysis using the Mann-Kendall test showed no significant trend in annual water yield from 1991-2013 with increasing beetle-killed area. Annual mean isotopic signature (^{18}O and ^{2}H) analysis of rain, snow, soil water and stream water showed snow (44%) to be the largest contributor to annual streamflow followed by soil water (38%) and rain (14%). No correlation was found between any mean annual source water and percent beetle-killed area. Isotopic analysis of peak streamflow showed soil water (43%) and snow (42%) to be the largest contributors to peak flow. Snow's streamflow contribution was negatively correlated (p = 0.02) to percent beetle-killed indicating that snow as a source for streamflow decreased as a watershed had a higher proportion of MPB-killed trees. No correlation was found between rain or soil water as source waters to peak streamflow and percent beetle-killed.

Stream water total nitrogen, nitrate and TOC concentrations and fluxes were not significantly changed by the MPB epidemic. There was no correlation between stream water total nitrogen, nitrate or TOC concentrations or flux and percentage of beetle-killed area.

Even though Colorado's forests have been significantly impacted by MPB induced tree mortality, this study suggests that percentage of beetle-killed watershed area has had little impact on annual water yield and stream water

nutrient levels. Source water contribution to streamflow is impacted as a result of MPB induced tree mortality as the fraction of peak streamflow from snow decreased with increasing percentage of beetle-killed area.

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INTRODUCTION

The lodgepole pine (*Pinus contorta)* forests of western North America recently endured the most severe insect-induced mortality in recorded history (Raffa et al. 2008; CSFS 2013). Since 1996, the epidemic in southern Wyoming and northern Colorado caused by the mountain pine beetle (MPB), (*Dendroctonous ponderosae*), has led to the death of more than 1.6 million hectares of lodgepole pine and some Ponderosa pine (*Pinus ponderosa*) and limber pine (*Pinus flexilis*) (USDA 2011). MPB introduce blue stain fungi (*Grosmannia claviger*) into the tree xylem which blocks the transport of water (Edburg et al. 2012). An infected tree dies due to limited water uptake or carbon exchange failure as a result of decreased stomatal conductance (Adams et al. 2009; Sala and Hotch 2009; McDowell et al. 2011).

During the first two years following infestation, pine needles turn from green to red and begin to drop and add to the soil litter layer. Within three years of the initial attack, trees enter the gray phase where all needles have been dropped and twigs and branches are starting to be sloughed (Wulder et al. 2006). Boles begin to fall between 5 and 14 years after needle drop (Schmid et al. 1985; Mitchell and Preisler 1998), depending on soil type, temperature, and moisture conditions (Lewis and Hartley 2005). Whereas pine needles and twigs contain relatively high amounts of nitrogen and decay relatively quickly, boles have low amounts of nitrogen, abundant carbon, and decay more slowly (Pearson et al. 1987). The multi-year transition from infestation to decay causes nitrogen and carbon to be released from the biomass over time.

In order to gain insight into the potential hydrological effects of MPB induced tree mortality, timber harvesting can be used as an analogy. Both types of forest change result in opening the canopy, leading to decreases in both interception and evapotranspiration. Net precipitation, defined as precipitation minus evaporation and transpiration, increases under harvested and dead (MPB) trees while evapotranspiration decreases due to vegetative loss (Adams et al. 2012). These mechanisms lead to decreases in soil moisture depletion and changes in streamflow timing (Troendle and Leaf 1981).

Nitrogen and carbon released from the decaying material will accumulate in the soil either to be used by new vegetative growth or leached into soil or groundwater (Boyer et al. 1997). Hydrologic flushing of catchment soils and biomass due to snowmelt runoff and rainfall events can mobilize nitrogen and carbon (Boyer et al. 1997;

Jones 1999). Nitrogen and carbon additions to streams caused by MPB induced mortality have the potential to cause significant issues for water quality managers along the Colorado Front Range (Mikkelson et al. 2013a).

Although similarities exist between timber harvest and MPB induced tree mortality, differences in the canopy loss mechanisms may result in differing hydrological and biochemical responses. Timber harvesting occurs over a single season with immediate canopy removal. Conversely, forest die-back due to the MPB occurs over multiple years, both in the sense of tree death to needle drop and occurrence of MPB infected trees in a watershed. Only selective trees are suitable hosts for the MPB, resulting in a non-comprehensive, heterogeneous removal in time and space of the forest canopy. Both of these mechanisms may lead to a potential buffering of the increased water and nutrient availability associated with loss of vegetative area.

The hydrological and geochemical impacts of MPB induced die-off remain uncertain even with recent conceptual and physical research (Mikkelson et al. 2013b; Pugh and Gordon 2013). The effect of MPB induced tree mortality on annual water yield, streamflow generation mechanisms and nutrient export in the northern Colorado Rocky Mountains will aid in addressing this knowledge gap.

Annual Water Yield

The primary source of drinking water for communities along the Colorado Front Range originates in forested and snowmelt-dominated watersheds. Extensive tree loss from these watersheds may affect downstream water availability by changing streamflow timing. Decreased canopy cover resulting in decreased snowfall interception will lead to increases in snow accumulation (Troendle and Kaufmann 1985; Troendle 1987; Troendle and Reuss 1997; Pugh and Small 2013). Coupled with decreased evapotranspiration due to canopy loss, soil moisture depletion will decrease (Troendle and Leaf 1981). As snowmelt progresses, excess melt water will enter streams earlier as less water is needed for soil moisture recharge (Troendle and Leaf 1981).

Timber Harvest

Annual water yield increases after timber harvest as a result of decreased evapotranspiration and decreased interception (Troendle and Leaf 1980; Troendle and King 1985; Troendle and Reuss 1997). An increase in annual water yield is typically observed within the first year after harvest and decreases as vegetation recovers (Troendle and King 1985; 1987; Troendle and Kaufmann 1987). A minimum canopy removal of 20% has been suggested for the Rocky Mountain region before the water yield increase is detectable (Stednick 1996). This threshold may be dependent upon the type of harvest and the total annual precipitation following harvest. After compiling timber harvest studies in the region, a linear relation between annual water yield and percent of the watershed harvested suggests that on average, for every 10% of the catchment harvested, annual water yield will increase 9 mm (Stednick 1996). While this suggests there is some relation between the percent of trees harvested and the resulting water yield increase, the proportional increase is not great enough to strongly influence harvesting decisions. Additionally, the range in the annual water yield increase at differing amounts of harvest can be large, e.g. between 25 and 250 mm for a 50% clearcut and 0 to 350 mm for a 100% clearcut (Stednick 1996). The effects of harvest on water yield changes via clearcutting versus thinning, which can be more closely related to the heterogeneous nature of MPB impacted trees, is not inherently clear.

Water yield increases post-harvest have been observed to be the largest in the wettest years while in dry years, the yield increase is not as significant due to a higher proportion of precipitation being required to recharge soil moisture lost to evaporation (Troendle and King 1985, 1987; MacDonald and Stednick 2003; Hubbart et al. 2006). During wet years, soil water depletion after thinning and clearcutting is reduced and water available for streamflow increases in direct proportion to basal area removed, however this effect is not detectable in thinned forests during dry years (Troendle 1987).

Similarly, tree removal via thinning or partial cuts, in contrast to clearcuts, can result in smaller water yield increases because retained vegetation and the vegetation in surrounding areas utilize the increase in available soil moisture (Hibbert 1967). Timber harvest via thinning or partial cuts might require a greater area to be harvested than clearcutting for water yield increases to be measured.

Two subalpine drainages within Deadhorse Creek, CO were used to investigate the annual water yield impact of clearcutting (36% of the North Fork subdrainage land area removed from 1977-1978) and partial cutting (40% of Unit 8 removed from 1980-1981). It was hypothesized that partial cutting would have a significantly smaller effect on annual water yield than clearcutting because the residual stand would utilize any transpirational savings. Water yield analysis of post-treatment years indicated that annual water yield from the clearcut watershed increased significantly. Annual water yield from the partial cut watershed had an apparent increase compared to pre-harvest annual water yield, but was not statistically significant. Additionally, the average annual precipitation during the partial cut post-treatment years was above average, leading water yield increases to be larger than expected (Troendle and King 1987).

Mountain Pine Beetle

In contrast to timber harvesting, water yield effects due to insect induced tree mortality within the Rocky Mountain region are poorly understood. Few studies exist examining the impact of the MPB die-off on streamflow in the region. After a spruce beetle outbreak in the White River and Yampa River watersheds in Colorado from 1941-1946, a retrospective paired watershed study using streamflow records revealed annual water yield increases of 31.8 - 37.9 mm (12-15%) in the White River basin and 23.6 – 35.2 mm (11-16%) in the Yampa River basin compared to the undisturbed Elk River (Bethlahmy 1974, 1975). Water yield increases from these watersheds were reported to be the smallest during the first five years post infestation with the largest yield increases observed after 15 years. Additionally, similar to the effects of timber harvest, the impacts of the beetle kill on water yield were higher during wet years and lower during dry years (Bethlahmy 1974, 1975).

The data interpretation from the White River and Yampa River watersheds are debated however due to the possibly inappropriate use of the analysis of variance and analysis of covariance statistical techniques to compare control watersheds (Alila et al. 2009) and the inconsistent methods used to quantify the level of mortality and differing mountain terrain when pairing control and treated watersheds (Faria et al. 2000).

A recent study examining the current MPB outbreak in the northern Colorado Rockies showed no evidence of annual water yield increases 10 years since the regional peak of the infestation in 2002 using the Mann-Kendall trend test (Maggart 2014). This work included data stationarity analyses of 20 watersheds of varying size from 16 to 539 km^2 with the degree of MPB kill ranging from 5 to 82% of the watershed area. These results may be attributed to the heterogeneous advancement of a stand from red to gray, i.e. the threshold of watershed canopy loss to detect a water yield increase had not been met. The degree of canopy loss in red stands will lead to insignificant changes in snow accumulation compared to live stands, as most needles are still retained (Pugh and Small 2011). This will generate negligible changes in soil moisture depletion and therefore negligible snowmelt excess. As canopy cover decreases with the stand transition from red to gray, hydrologic process should shift towards decreased soil depletion and increased melt water available for streamflow.

Stable Isotope Analysis to Characterize Streamflow Generation Mechanisms

Isotopy

The application of isotopic tracers in catchment hydrology can be a useful tool in identifying the mechanisms responsible for streamflow generation (Kendall and Caldwell 1998). ^{18}O and ^{2}H (deuterium) are natural isotopes in meteoric and groundwaters and are considered ideal hydrologic tracers because they are conservative and source waters (rain, snow and groundwater) retain their distinctive isotopic compositions upon mixing (Kendall and Caldwell 1998).

Two major partitioning factors control the isotopic composition of meteoric water at a location: temperature and distance inland from the source (the ocean) of the water vapor (Kendall and Caldwell 1998). Water that condenses at cooler temperatures is increasingly depleted of heavy isotopes (^{18}O and ^{2}H), i.e., precipitation at cooler temperatures is more composed of relatively less heavy isotopes and more light (^{16}O and ^{1}H) isotopes. Conversely, as an air mass travels inland from the ocean, the isotopic composition of the condensing precipitation is enriched with heavy isotopes compared to the remaining vapor (Dansgaard 1964; Kendall and Caldwell 1998; Gupta 2010). This continental effect continues to progressively remove heavy isotopes as the air mass moves further inland and subsequent precipitation events further deplete the air mass of heavy isotopes.

The combination of temperature differences and the continental effect yields varying isotopic compositions, or signatures for precipitation at different locations. Meteoric water samples from non-marine environments show that ^{18}O and ^{2}H values are linearly correlated due to the fractionation of isotopes during evaporation-condensation processes (Gourcy et al. 2005) The Global Meteoric Water Line (GMWL) described by Equation 1a gives the isotopic signature, i.e. relative contributions of $\delta^{18}O$ and $\delta^{2}H$ (Eqn 1b), in global precipitation (Craig 1961). A Local Meteoric Water Line (LMWL) can also be developed based on local meteorology and may have a different slope and intercept than the GMWL (Ingraham 1998).

$$\delta^{2}H = 8 \times \delta^{18}O + 10 \qquad \text{(Eqn 1a)}$$

$$\delta \text{ (in ‰)} = \left(R_{Sample} \div R_{Standard} - 1\right) \times 1000 \qquad \text{(Eqn 1b)}$$

where *R* denotes the ratio of heavy to light isotopes and standards are reported relative to the Standard Mean Ocean Water (SMOW) (Craig 1961) or the equivalent Vienna-SMOW standard.

In addition to describing isotopic differences in waters geographically, temperature effects at a single location (region) lead to differences in the isotopic composition of snow and rain falling within a region. Vapor that condenses at cooler temperatures (snow) is composed of relatively more light isotopes than rain, and appear on the left-hand side of the LMWL.

Additionally, the isotopic signature of soil water and groundwater is a function of the composition of the infiltrating water. Infiltrating water is subject to evaporation on the soil surface and near surface leading to enrichment in heavy isotopes of the subsurface waters (Gonfiantini et al. 1998). As the effects of evaporation on soil waters are depth dependent, enrichment due to evaporation decreases as soil depth increases (Gat 2010).

The two processes, temperature (Eqn 2a) and evaporation (Eqn 2b), differing the isotopic composition of snow, rain and groundwater are isolated in stream water as each source retains its distinctive signature (Kendall and Caldwell 1998), and the sum of the relative contribution of each determines the signature of streamflow (Phillips and Gregg 2003).

$$\delta_{Q} = f_S \, \delta_S + f_R \, \delta_R + f_G \, \delta_G \qquad \text{(Eqn 2a)}$$

$$1 = f_S + f_R + f_G \qquad \text{(Eqn 2b)}$$

where δ_i is the isotopic composition of stream water (Q), snow (S), rain (R) and groundwater (G) and f_i is the proportional contribution

Timber Harvest

Research into the effects of timber harvest on streamflow generation mechanisms is limited (Zegre 2011). Isotopic investigations into snowpack signature changes under differing timber harvest schemes have found that snow from partial-cut and undisturbed forests were enriched with heavy isotopes relative to snow from clearcut forests (Koeniger et al. 2008). The enrichment was attributed to sublimation from snow intercepted by the canopy with more enrichment in denser canopies (Koengier et al. 2008). Isotopy has not been used to determine changes in the relative contribution of snow, rain and groundwater to streamflow after timber harvest within the Rocky Mountain Region.

Mountain Pine Beetle

The mechanisms responsible for water yield changes in MPB die-back affected watersheds are still largely unknown. The groundwater contribution to streamflow for late summer low flow was investigated in Rocky Mountain National Park, Colorado to determine the potential change in groundwater contribution inputs after the recent MPB epidemic (Bearup et al. 2014). Isotopic and chemical hydrograph separation techniques using ^{18}O and electrical conductivity for samples taken in 2012 were implemented for two watersheds with different levels of killed area. Active MPB infestation within the Big Thompson watershed occurred from 2006-2011 and spanned 32.3% of the watershed area. The North Inlet watershed experienced active MPB infestation from 2004-2009 with 23.7% of the watershed killed. The study concluded that late summer groundwater contributions to streamflow from the more highly impacted Big Thompson were 30$\pm$15% greater when compared to the North Inlet watershed. The increase in groundwater contribution to streamflow was attributed to the evapotranspiration savings resulting from the dead trees (Bearup et al. 2014).

Through the use of isotopic tracers ^{2}H and ^{18}O, a recent study of 20 northern Colorado watersheds showed no significant change in streamflow generation mechanisms, i.e. the expected increase in groundwater contribution to streamflow and a decrease in the contribution from precipitation was not found (Maggart 2014). The contribution to streamflow from snow and rain averaged 80% while groundwater averaged 20% (Maggart and Stednick, in prep). Isotopic analysis of soil, snow and rain waters within each watershed found no significant difference in the isotopic partitioning of stream water in relation to the amount of MPB-killed area (Maggart 2014).

Nutrient Export

Investigations into the effects of forest management on water quality are not new. The chemistry of water can change as it flows through the canopy, soil and subsurface and eventually into streams. Nutrient cycles within the soil and litter layer are dependent upon canopy structure changes that occur when trees are removed (harvest) or lose their needles (MPB). Changes in canopy cover can lead to changes in precipitation, throughfall and evapotranspiration. Tree removal or mortality results in a decline of nutrient uptake however nutrient pools will increase only if nutrient supply from the decreased uptake and mineralization of new organic matter inputs exceed leaching and uptake from existing vegetation and vegetative regeneration (Griffin et al. 2011).

The above and below ground biomass in forested ecosystems represents a large and long-term source of total nitrogen, comprised of organic nitrogen (Kjeldahl) and inorganic nitrate+nitrite to the soil (Knoepp and Swank 1997). While timber harvesting removes vegetation and therefore a large source of nitrogen, large scale tree mortality associated with the MPB epidemic adds a large pool of biomass to the soil surface (and subsurface via root residual) in a relatively short time period. Further, increased soil decomposition rates associated with an open canopy, increased solar radiation, and thereby increased soil temperatures, coupled with increased soil moisture allows a large influx of total nitrogen to the soil (Prescott 2002; Griffin et al. 2011).

Reduced vegetation uptake of nitrate that follows removal of or damage to trees can cause nitrate release into streams. Further, decreased uptake of ammonium caused by vegetative loss may increase the production of nitrate by soil microbes potentially leading to increased nitrate levels in streams (Likens et al. 1970; Vitousek and Melillo 1979; Gundersen et al. 2006). Nitrate export can be suppressed however if enhanced denitrification, caused by decreased evapotranspiration and thereby increased soil moisture, increased soil nitrate content and increased dissolved organic carbon due to enhanced decomposition of organic matter, offsets nitrification (Bremner and Shaw 1958).

Increases in total organic carbon (TOC, comprised of particulate and dissolved carbon) are a concern for drinking water managers because natural organic matter (NOM), a significant portion of TOC, reacts with chlorine during water treatment potentially forming disinfection byproducts (DBP's), such as trihalomenthanes, a human health concern (Nikolaou et al. 2001). TOC can be leached from vegetation via throughfall and stemflow. It can also be pooled in the litter and soil layers by decomposition and leaching of plant and soil organic matter. Whereas TOC concentrations under forest stands reflect litter availability and decomposition rates (Brown and Schreier 2009), residence times and flow paths are among the primary mechanisms affecting TOC concentrations within streams (Boyer et al. 1997). Spring runoff in mountainous watersheds typically flushes TOC into surface waters (Boyer et al. 1997; Mikkelson 2013a).

The amount of nitrogen and carbon released from decomposing matter was observed to be correlated to the carbon:nitrogen ratio (C:N) (Vitousek 1982; Gundersen et al. 1998). When the C:N is low, there is a net release of inorganic nitrogen from decomposing matter leading to excess pools of nitrogen in the soil which could increase stream water nitrogen. When the C:N is high, decomposers are nitrogen limited and therefore microbes consume most available nitrogen and retain it in the biomass (de la Crétaz and Barten 2007; Vitousek 1982), potentially leading to a high C:N in streams. Large nitrogen inputs via needles and large carbon inputs via branches and boles could lead to varied and offsetting responses to overall decomposition rates as the effect of nitrogen on organic matter decomposition depends on the stage of decomposition (Berg and Matzer 1997). Nitrate and ammonium additions stimulate initial decomposition but retard humus decomposition. Significant negative correlations have been shown between nitrogen concentrations and humus respiration rates, prompting lower C:N levels to cause more carbon rich humus to be left within forest soils (Berg and Matzer 1997).

Timber Harvest

Nitrate is generally the only response nutrient in relation to timber harvesting in the Rocky Mountains (MacDonald et al. 1991; NCASI 1994).

Nitrate

Although the removal of forest vegetation disrupts nutrient cycles, at the watershed level water quality changes following timber harvest are minimal (MacDonald and Stednick 2003; Stednick and Troendle 2004).

Timber harvesting may temporarily increase nitrate levels in soils and stream water, however these increases are relatively short lived as vegetation recovers. At Fraser Experimental Forest, a 33% clearcut increased the amount of ammonium and nitrate in the snow pack and nitrate concentrations in subsurface flows. The increases to stream water nitrate were only from 0.006 to 0.06 mg/L N (Reuss et al. 1997; Troendle and Reuss 1997), well below the EPA drinking water standard of 10 mg/L.

A clearcut in central Idaho produced observed nitrate concentrations of 0.06 mg/L, 10 times higher than average, with nitrate levels returning to control levels within 5 years (Clayton and Kennedy 1985). Again, this value is very low compared to state or national water quality standards.

In west central Alberta, Canada, undisturbed and harvested forests were monitored. All concentrations of nitrate remained low, between 0.005 – 0.05 mg/L, with no significant difference between control and harvested watersheds (Singh and Kalra 1975).

Mountain Pine Beetle

The large scale tree mortality associated with the MPB epidemic has altered biogeochemical and physical process, such as organic material decay and hydrological flow paths, potentially enhancing leaching of nitrogen, carbon, and organic material into the soil and subsurface waters (Edburg et al. 2012; Mikkelson et al. 2013b).

Total Nitrogen

Only one study within the Rocky Mountain region has examined the effects of MPB die-back on stream water total nitrogen. Examination of stream water chemistry for three major inlets to the Three Lakes system in Grand County, CO from 2001-2009 revealed an increasing trend over time in total nitrogen concentrations (Clow et al. 2011). This trend was in contrast to dissolved nitrate concentrations, which showed a downward trend over time. The increase in total nitrogen was attributed to a greater flux of particulate organic matter to surface waters caused by litter decomposition under MPB-killed trees (Clow et al. 2011).

Nitrate

The effects of MPB die-back on nitrogen pools, fluxes, and leaching is contingent upon the processes that limit or enhance nitrogen supply (Griffin et al. 2013). Nitrogen delivered by needle additions to the soil litter layer following beetle attack may increase soil nitrate (Cullins et al. 2003; Morehouse et al. 2008; Clow et al. 2011; Griffin et al. 2011), however, increases in microbial immobilization and nitrate uptake by unaffected vegetation could decrease soil nitrate (Fahey et al. 1985; Edburg et al. 2012). Nitrate was found to be significantly higher in the soils under red and gray trees in Grand County, CO, (Clow et al. 2011), and in the litter layer below red and gray stands in Yellowstone National Park (Griffin et al. 2011). However, within Yellowstone National Park, no significant difference in nitrate concentrations were found in the soils under undisturbed, red (2 years post attack), gray (4 years post attack) and 30 year post MPB beetle attack stands despite observed increases in both net nitrogen mineralization and net nitrification. These results were attributed to significantly greater total percent cover of understory vegetation in the red and gray stands, signaling nitrogen retention in the beetle-affected lodgepole pine forests (Griffin et al. 2011).

Studies of insect induced defoliation in N-saturated environments where atmospheric inputs exceed forest growth requirements and microbial demand, have shown an increase in the leaching of nitrate pools into streams for both short term, 1-2 year, defoliations (Swank et al. 1981; Eshleman et al. 1998; Lewis and Liken 2007) and long term, multi-year defoliation by bark beetles (*Ips typographus*) (Huber 2005). In the conifer forests of western North America however, N-inputs via precipitation and fixation are small compared to forest growth requirements (Griffin et al. 2011). Additionally, in contrast to single season defoliation, the pulse of additional litter due to MPB die back occurs over a multiple year time scale (Huber 2005).

There have been no Colorado studies to date that have shown a significant increase in stream nitrate due to MPB in the western Rocky Mountains. All previous studies of the region have focused on a short term response, i.e. within 1-3 years of outbreak onset (e.g. Stednick et al. 2010; Clow et al. 2011; Rhoades et al. 2013). Multiple-regression analysis of recent streamflow nutrient levels in Grand County, CO indicated that the percent of the basin killed by the MPB no significant influence on stream nitrate concentrations, rather basin characteristics such as percent classified as forest and basin relief explained the spatial variation (Clow et al. 2011). It has been hypothesized that in nitrogen-deficient environments, such as the Central Colorado Rocky Mountains, the excess nitrogen observed at the tree scale is being used by surviving vegetation (Griffin et al. 2011; Griffin et al. 2013; Hubbard et al. 2013).

Studies in N-saturated forests (inputs = outputs) have shown a lag time between the defoliation and peak nitrate stream response of several months to a year in the case of short term insect induced defoliation (Eshelman et al. 1998; Lewis and Likens 2007), to five years in the case of the bark beetle with elevated levels 17 years post-infestation (Huber 2005). This provides incentive to evaluate a stream’s nitrate response in an N-poor environment at a longer time step after initial MPB outbreak.

Carbon

One major difference between timber harvest and MPB induced tree mortality is the effect of tree removal versus tree retention on nutrient cycles. Both needle and bole decomposition creates additional TOC that could be transported to streams via overland, subsurface and groundwater flow paths**.** The increase in litter availability that occurs 3-4 years after initial infestation as dead trees drop their needles leads to greater decomposition rates (Edburg et al. 2011), in addition to increasing the litter supply. Pine litter leachate becomes more aromatic and hydrophobic two months after deterioration (Beggs and Summers 2011) allowing for a temporal and prolonged release of organic carbon since not all needle-fall from a dead tree or an infected stand will occur simultaneously. Additionally, the increase of carbon into the soil may be delayed due to the cessation of the chemical cycling associated with the roots and fungi of dead trees (Godbold et al. 2006).

Additions of carbon from boles and other coarse woody debris will stimulate decomposition, though the rate may potentially be smaller than needle drop (Harmon et al. 1986; Edburg et al. 2011). However the rate could last longer because tree fall will occur at varying times over a longer time period allowing carbon to be leached from boles and snags into the soil at a slower rate (Edburg et al. 2012). As boles have abundant amounts of carbon compared to needles (Pearson et al. 1987), TOC could remain elevated for many years before returning to pre-outbreak levels. The timing of tree fall is dependent upon soil type, temperature, and moisture (Lewis and Hartley 2005), and could range between 5 years and 14 years (Schmid et al. 1985; Mitchell and Preisler 1998) after needles have dropped, delaying the potential response of TOC observed in streams. The coupling of decomposing needles and boles could lead to large soil stores of carbon being leached into streams over an extended period of time with a delayed onset.

Few studies have been done related to the change in TOC levels in streams due to bark beetle infestations in the Rocky Mountain region. Water quality data from 2004 to 2011 for water entering treatment facilities in Colorado within watersheds affected and unaffected (control) by the MPB epidemic showed that the TOC levels entering treatment facilities from the affected watersheds were 4 times greater than levels in the control watersheds (Mikkelson et al. 2013a). Additionally, the study found TOC levels peaked 4 years after initial watershed infestation, however, a statistically significant correlation with percent watershed die-back was not found. Similarly, stream TOC levels in the Willow Creek Watershed of Grand County, Colorado were also found to not be related to the percent of the watershed that was affected by the MPB (Stednick et al. 2010).

Stream water dissolved organic carbon (DOC) concentrations for one watershed in Grand County did not increase from 2001-2009 (Clow et al. 2011). While expected seasonal fluctuations were observed, percent of forest killed by the MPB was less correlated to DOC concentrations than typical watershed characteristics such as basin area, precipitation, and percent forest cover.

Carbon:Nitrogen

Lodgepole pine trees reabsorb a portion of nitrogen from needles into their roots before natural needle fall. Therefore premature needlefall from MPB induced tree mortality causes a pulse of low C:N litter to the soil litter layer (Morehouse, Griffin et al. 2011; Keville et al. 2013). When C:N is low there is a net release of inorganic N from decomposing matter (de la Crétaz and Barten 2007; Vitousek 1982). Accumulation of ammonium has been observed in the organic soil horizon beneath red and gray MPB infested trees versus live trees (Clow et al. 2011; Griffin et al. 2011; Xiong et al. 2011; Keville et al. 2013) and nitrate has been observed to increase (Clow et al. 2013) or remain the same (Griffin et al. 2011; Xiong et al. 2011; Keville et al. 2013). Despite the increase in ammonium, evidence suggests soil total nitrogen slightly increases (Clow et al. 2011) or remains the same (Griffin et al. 2011; Xiong et al 2011; Keville et al. 2013). This has led to observed C:N in organic horizon soils beneath red and gray trees to be similar to the ratio beneath living trees (Griffin et al. 2011; Keville et al. 2013). There have been no regional studies to date examining changes in stream water C:N due to the MPB.

HYPOTHESIS AND STUDY OBJECTIVES

The purpose of this study is to provide insight into changes in annual water yield, streamflow generation mechanisms and stream water nitrate concentrations due to the MPB epidemic. To evaluate the possible effect, 18 watersheds with differing amounts of MPB caused tree mortality, ranging from 6 to 78%, in the north-central Rocky Mountains were studied. It is hypothesized that the canopy loss associated with the MPB epidemic in north central Colorado has led to significant changes in annual water yield, streamflow generation mechanisms and stream total nitrogen, nitrate, and TOC concentrations. The objectives of this study were:

1) To determine if annual water yield in MPB affected watersheds is increasing or is stationary via using of historical streamflow records.
2) Using isotopic signatures, ^{18}O and ^{2}H, to determine if the mechanisms for streamflow generation is changing due of MPB.
3) To determine if changes in total nitrogen, nitrate, and TOC concentrations in stream waters are affected by MPB.

METHODS

Site Description

The study area is located in north-central Colorado, including Grand, Eagle and Summit Counties. To compliment previous research in the area and the availability of long term streamflow records, 18 watersheds were selected for study (Figure 1, Table 1). The watersheds of interest are mostly federal lands consisting of mountainous subalpine mixed conifer forests. Annual hydrographs are snowmelt dominated.

A Geographical Information System (GIS) was used to calculate the area killed by the MPB between 1997 and 2013. The MPB kill layer was obtained from the United States Forest Service Aerial Detection Surveys *(http://www.fs.usda.gov).* The forests layer for each watershed was obtained from 2013 National Land Cover Data *(http://landcover.usgs.gov).*

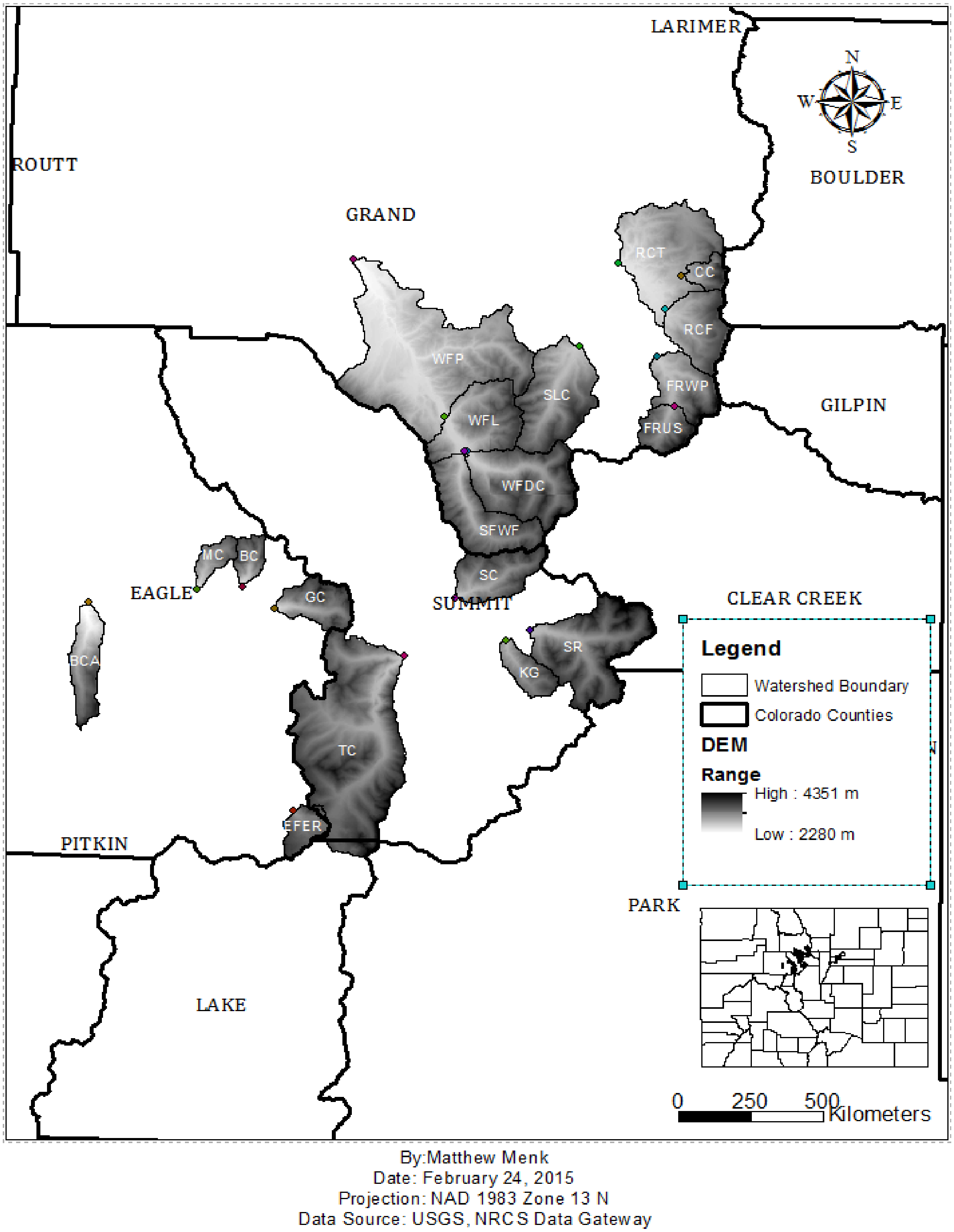

Figure 1: Study watersheds selected across north-central Colorado.

Table 1: Watershed characteristics for the 18 study areas including watershed description, code, gauging station number, area and mean elevation. The study period of record is 1991 to 2013.[1][2]

Watershed Name	Watershed Code	USGS Gauging Station No.	Watershed Area (km^2)	Mean Elevation (m)
Booth Crk nr Minturn, CO	BC	09066200	16	3264
Beaver Crk at Avon, CO	BCA	09067000	38	3157
East Fork Eagle River nr Climax, CO[1]	EFER	09061600	21	3470
Fraser River at Upper Station nr Winter Park, CO	FRUS	09022000	26	3498
Fraser River at Winter Park, CO	FRWP	09024000	72	3273
Gore Crk at Upper Station nr Minturn, CO	GC	09065500	37	3379
Keystone Gulch nr Dillon, CO	KG	09047700	23	3341
Middle Crk nr Minturn, CO	MC	09066300	16	3148
Ranch Crk nr Fraser, CO	RC	09032000	51	3166
Ranch Crk bl Meadow Crk nr Tabernash, CO[2]	RCT		170	2961
Straight Crk bl Laskey Gulch nr Dillon, CO	SC	09051050	48	3419
South Fork of Williams Fork nr Leal, CO	SFWF	09035900	72	3351
St. Louis Creek nr Fraser, CO	SLC	09026500	85	3309
Snake River nr Montezuma, CO[2]	SR		149	3517
Tenmile Crk bl N. Tenmile Crk at Frisco, CO	TMC	09050100	237	3343
Williams Fork ab Darling Crk nr Leal, CO	WFDC	09035700	91	3386
Williams Fork nr Leal, CO	WFL	09036000	232	3160
Williams Fork nr Parshall, CO	WFP	09037500	476	2891

[1] EFER streamflow was analyzed from 2003 to 2013 due historical data availability
[2] RCT and SR streamflow was not analyzed due to incomplete streamflow records

Annual Water Yield

Annual water yield records for gauges were accessed through the USGS National Water Information System (NWIS) for water years 1991 to 2013 (Table 1). This time period allowed for 11 years prior to and post regional beetle-killed area to be analyzed as 2002 is considered the regional break point (Maggart 2014).

Data Stationarity

The nonparametric Mann-Kendall test was used to test for trends in annual water yield because the data do not need to conform to a particular distribution (Gilbert 1987). The null hypothesis, H_o, of no trend was tested against the alternative hypothesis, H_a of a trend. A positive or negative Z-statistic indicates that annual water yield in the preceding year was less or more than the current year, i.e. annual water yield was increasing or decreasing over time. The significance of the trend was assessed by comparing the absolute value of the Z-statistic against the corresponding probability value for the cumulative normal distribution at the alpha, α, significance level (Gilbert 1987). If a linear trend is present, the slope of the trend can be estimated using Sen's nonparametric method. A $100(1- \alpha)\%$ two-sided confidence interval around the true slope is calculated and the estimated slope is statistically different from zero if zero is not within the confidence limits (Gilbert 1987).

The Mann-Kendall test for trend was tested at the α = 0.001, 0.01, 0.05, 0.1 confidence levels while the Sen's slope estimate was tested at the α = 0.01, 0.05 confidence levels using the MAKESENS Excel template (Salmi et al. 2002).

Stable Isotope Analysis to Characterize Streamflow Generation Mechanisms

Sample Collection

Sampling dates were chosen at different times over the annual hydrograph, i.e. the beginning of low flow, low flow and peak runoff, to account for seasonal (temperature) changes in isotopic signatures and to characterize streamflow generation when different source water would be relatively larger contributors (Table 2).

Streamflow samples were collected using 20 mL polypropylene scintillation bottles. Phase change within the sample bottles was prevented by capping the fully submerged bottle to eliminate head space.

Soil water extracted from near surface soil samples (20-50cm depth) from each site were used as a surrogate for groundwater (Table 2). Soil water was extracted from soil samples via the water extraction line at the University of Wyoming Stable Isotope Facility. Groundwater samples were collected when accessible by using a 20mL polypropylene scintillation bottle as above (Table 2). Isotopic signatures of paired soil and groundwater were compared to ensure soil water could be used as a surrogate for groundwater. Regionally averaged isotopic signatures of rain and snow collected in the study area was previously determined as the isotopic signature of rain and snow was determined to not be spatially variable (Maggart 2014). Only isotopic precipitation data collected in the current study watersheds were used. In temperate climates, annual average isotopic values generally do not vary by more than 1‰ (Gat et al. 2001). The University of Wyoming Stable Isotope Facility provided isotopic analysis of steam, soil water, and groundwater samples for $\delta^{18}O$ and $\delta^{2}H$.

Table 2: Sampling dates and type of isotope sample collected for the study watersheds. Symbols correspond to sample type(s) collected: ◊ stream water, ● soil water, + groundwater.

Watershed	**22-Sep-13**	**20-Oct-13**	**14-Jan-14**	**19-May-14**
BC	◊ ●	◊ ●	◊	◊ ● +
BCA	◊ ●	◊	◊	◊
EFER	◊	◊ ●		◊ ● +
FRUS		◊ ●		◊ ●
FRWP		◊	◊	◊ ● +
GC	◊ ●	◊	◊	◊ ● +
KG	◊	◊ ●	◊	◊ ●
MC	◊	◊	◊	◊
RC	◊	◊	◊	◊ ●
RCT		◊		◊ ● +
SC	◊ ●	◊	◊	◊ ●
SFWF	◊ ●	◊	◊	◊ ●
SLC	◊	◊	◊	◊ ● +
SR	◊ ●	◊ ●	◊	◊ ●
TMC	◊ ●	◊ ●	◊	◊ ● +
WFDC	◊ ●	◊		◊ ● +
WFL	◊ ●	◊		◊ ● +
WFP	◊ ●	◊ ●	◊	◊ ● +

Data Analysis

Through the use of isotopic signatures, streamflow can be separated into its source waters (McGuire and McDonnell 2008). Multi-component mixing models can determine bounds on the proportional contributions of differing sources using balance equations when the number of sources (snow, rain, and groundwater) is greater than 1 + number of systems (streamflow). Stable Isotope Analysis in R (SIAR) (Parnell and Jackson 2013) was used to determine the proportional contributions to streamflow of snow, rain, and groundwater via ^{18}O and ^{2}H isotopic signatures. SIAR is design to solve mixing models for stable isotopic data within a Bayesian framework with uncertainty and is especially useful in finding feasible solutions to an undefined system equations (Inger et al. nd.), i.e., having one isotope system (streamflow) and three unknowns (contributions of snow, rain, and groundwater). Originally designed to determine the dietary intake of organisms, input files for SIAR can be used to work for most stable isotopic systems (D. Williams, personal communication 2014). The output of the model is a range of feasible solutions for each source water based upon 95%, 75% and 50% Bayesian credibility intervals.

The model was run independently for each watershed. Inputs required for streamflow source water analysis in R are two separate files: one containing the isotopic composition of stream water, and the other containing the mean and standard error for the signature of the source waters. Stream water isotopic signatures for each individual watershed were used in the first input file (Table 3, 4). In the second input file, regional averages of rain and snow from (Maggart 2014) were used for all watersheds as well as the respective standard errors. Standard error for rain and snow was determined using all available data from Oct 2011 to Nov 2012 for all study watersheds collectively (Table 3, 4). The second input file contained site specific soil water signatures in lieu of groundwater and with the associated standard error (Table 3, 4). Lastly, program default settings with respect to the number of iterations (500,000) and number of the initial iterations to discard (50,000) were used (Inger et al. nd). For this study, SIAR analysis of isotopic signatures was done twice, once using annual averages of all data (Table 3) and once using annual averages for rain and snow and only May data for soil water and stream water to better characterize peak streamflow (Table 4).

Spearman’s rank-order correlation was used to assess the significance of the correlation between the mean annual and peak source water contribution to streamflow and percentage beetle-killed area. The correlation coefficient (ρ) is calculated by first ranking the variables (X_i,Y_i) in descending order then giving a rank (x_i,y_i) to each variable according to the ordinal position. The ranks are then used to calculate ρ (Eqn 3).

$$\rho = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \qquad \text{(Eqn 3)}$$

Significance of ρ is determined using Student's t-distribution. Spearman's correlation coefficient and the resulting *p*-value was determined using statistical software calculator (Wessa 2012).

Table 3: Mean annual isotopic signatures (δ^2H and $\delta^{18}O$) and associated standard errors (SE) based on (n) data available for rain, snow, soil water and stream water. Rain and snow signatures are based on data collected from Oct 2011 to Nov 2012 in the study watersheds from Maggart (2014).

	Rain			**Snow**			**Soil Water**					**Stream Water**		
Watershed	**n**	**δ^2H**	**$\delta^{18}O$**	**n**	**δ^2H**	**$\delta^{18}O$**	**n**	**δ^2H**	**SE(δ^2H)**	**$\delta^{18}O$**	**SE($\delta^{18}O$)**	**n**	**δ^2H**	**$\delta^{18}O$**
BC	33	-58.3	-8.6	28	-147	-19.8	3	-123	3.73	-16.7	0.68	3	-120	-16.1
BCA	33	-58.3	-8.6	28	-147	-19.8	1	-101	5.50	-13.4	0.96	4	-121	-15.9
EFER	33	-58.3	-8.6	28	-147	-19.8	2	-135	8.40	-17.9	1.3	3	-131	-17.4
FRUS	33	-58.3	-8.6	28	-147	-19.8	2	-128	4.65	-17.4	0.71	2	-127	-17.0
FRWP	33	-58.3	-8.6	28	-147	-19.8	1	-140	4.65	-18.4	0.71	3	-126	-16.9
GC	33	-58.3	-8.6	28	-147	-19.8	2	-116	7.19	-15.4	0.94	4	-120	-16.1
KG	33	-58.3	-8.6	28	-147	-19.8	2	-124	6.23	-16.4	1.1	4	-130	-17.1
RC	33	-58.3	-8.6	28	-147	-19.8	1	-105	6.93	-15.1	0.79	3	-118	-15.6
RCT	33	-58.3	-8.6	28	-147	-19.8	1	-135	2.11	-17.6	0.41	3	-118	-15.3
SC	33	-58.3	-8.6	28	-147	-19.8	2	-152	9.17	-20.2	0.51	4	-131	-16.9
SFWF	33	-58.3	-8.6	28	-147	-19.8	1	-127	5.22	-16.0	1.1	4	-128	-16.7
SLC	33	-58.3	-8.6	28	-147	-19.8	1	-131	0.850	-17.4	0.28	5	-127	-16.8
SR	33	-58.3	-8.6	28	-147	-19.8	3	-111	7.53	-14.7	1.1	5	-123	-16.5
TMC	33	-58.3	-8.6	28	-147	-19.8	3	-130	3.06	-17.5	0.51	4	-125	-16.4
WFDC	33	-58.3	-8.6	28	-147	-19.8	1	-140	3.02	-18.6	0.68	4	-126	-16.7
WFL	33	-58.3	-8.6	28	-147	-19.8	2	-123	3.31	-16.8	0.11	3	-127	-16.9
WFP	33	-58.3	-8.6	28	-147	-19.8	3	-120	6.93	-15.8	0.88	5	-127	-16.5
Standard Error		4.63	0.62		2.87	0.35								

Table 4: Isotopic signatures (^{2}H and ^{18}O) of rain, snow, soil water and stream water. Rain and snow signatures are based on (n) data collected from Oct 2011 to Nov 2012 in the study watersheds from Maggart (2014). Soil water and stream water data is from one sample date in May 2014.

	Rain			Snow			Soil Water		Stream Water	
Watershed	**n**	**$\delta^{2}H$**	**$\delta^{18}O$**	**n**	**$\delta^{2}H$**	**$\delta^{18}O$**	**$\delta^{2}H$**	**$\delta^{18}O$**	**$\delta^{2}H$**	**$\delta^{18}O$**
BC	33	-58.3	-8.6	28	-147	-19.8	-131	-18.0	-126	-16.9
EFER	33	-58.3	-8.6	28	-147	-19.8	-130	-17.7	-132	-17.4
FRUS	33	-58.3	-8.6	28	-147	-19.8	-123	-16.9	-131	-17.4
FRWP	33	-58.3	-8.6	28	-147	-19.8	-140	-18.4	-126	-17.7
GC	33	-58.3	-8.6	28	-147	-19.8	-133	-18.0	-128	-16.9
KG	33	-58.3	-8.6	28	-147	-19.8	-134	-17.4	-130	-17.1
RC	33	-58.3	-8.6	28	-147	-19.8	-105	-15.1	-124	-16.1
RCT	33	-58.3	-8.6	28	-147	-19.8	-135	-17.6	-127	-16.8
SC	33	-58.3	-8.6	28	-147	-19.8	-169	-22.4	-134	-17.3
SFWF	33	-58.3	-8.6	28	-147	-19.8	-127	-16.0	-131	-16.9
SLC	33	-58.3	-8.6	28	-147	-19.8	-131	-17.4	-129	-17.2
SR	33	-58.3	-8.6	28	-147	-19.8	-117	-14.7	-129	-17.2
TMC	33	-58.3	-8.6	28	-147	-19.8	-135	-18.1	-130	-17.2
WFDC	33	-58.3	-8.6	28	-147	-19.8	-140	-18.6	-131	-17.5
WFL	33	-58.3	-8.6	28	-147	-19.8	-131	-16.8	-131	-17.3
WFP	33	-58.3	-8.6	28	-147	-19.8	-140	-18.5	-128	-16.9
Standard Error		4.63	0.62		2.87	0.35				

Nutrient Export

Sample Collection and Analysis

Streamflow samples from each watershed were collected from the gauging stations on multiple days and analyzed for nitrate, total nitrogen, and TOC (Table 5). Nitrate samples were collected in 500 mL polyethylene bottles and analyzed at Colorado State University using the a Hach Spectrophotometer with results reported as mg/L NO_3-N. Total nitrogen and TOC samples were collected in 40 mL borosilicate simulation vials and analyzed at the EcoCore Analytical Facility at Colorado State University. Total nitrogen is reported as mg/L N and TOC as mg/L of non-purgeable organic carbon (NPOC).

Data Analysis

Total nitrogen, nitrate, and TOC concentrations were plotted against cumulative beetle-kill percentage. Additionally, nitrate flux out of the watershed (kg/ha/mo), defined as the product of nutrient concentration and streamflow, was plotted against cumulative beetle-kill percentage. Lastly, the concentration and flux C:N, expressed as both TOC/total nitrogen (TOC/TN) and TOC/total Kjeldahl nitrogen (TOC/TKN) for each sampling date were compared to cumulative beetle-killed percentage.

Spearman's rank-order correlation (Eqn 3) was used to assess the significance of the correlation between total nitrogen, nitrate and TOC concentration and flux and TOC/TN and TOC/TKN concentration and flux to the percentage of beetle-killed area.

Long term nitrate balance defined as atmospherically deposited nitrate minus streamflow weighted nitrate exiting the watershed was also compared to cumulative beetle kill percentage. The closest atmospheric deposition site to the watersheds of interest is NTN CO93 Buffalo Pass-Dry Lake located at 40°32′5″, -106°46′52″. Atmospheric deposition data were accessed from the National Atmospheric Deposition Program (NADP) at (*nadp.sws.uiuc.edu/NTN/ntndata.aspx)*. Only the RC watershed was analyzed because that is the only study watershed with long term stream nitrate data available. Stream nitrate concentration for RC was measured bi-monthly by the USGS (NWIS) therefore annual totals were calculated to be twice the cumulative bi-monthly measured totals.

Table 5: Sampling dates and type of nutrient sample collected in stream water for the study watersheds. Symbols correspond to sample type(s) collected: ● nitrate, + total nitrogen, Δ TOC.

Watershed	**22-Sep-13**	**20-Oct-13**	**14-Jan-14**	**19-May-14**
BC	●	● + Δ	● + Δ	●
BCA	●	● + Δ	● + Δ	● + Δ
EFER	●	●		●
FRUS		● + Δ		● + Δ
FRWP		● + Δ	● + Δ	● + Δ
GC	●	● + Δ	● + Δ	●
KG	●	● + Δ	● + Δ	●
MC	● + Δ	●	● + Δ	● + Δ
RC	● + Δ	●	● + Δ	● + Δ
RCT		●		● + Δ
SC	●	●	● + Δ	● + Δ
SFWF	● + Δ	●	● + Δ	● + Δ
SLC	● + Δ	● + Δ	● + Δ	● + Δ
SR	● + Δ	● + Δ	● + Δ	●
TMC	●	● + Δ	● + Δ	● + Δ
WFDC	●	● + Δ		● + Δ
WFL	● + Δ	●		● + Δ
WFP	● + Δ	● + Δ	● + Δ	● + Δ

RESULTS

Cumulative annual MPB killed area as a percent of watershed area from 1997 to 2013 was determined for the study watersheds (Table 6). Cumulative beetle-killed area for the 18 watersheds analyzed from 1997 to 2013 ranged from 6 to 78 percent of watershed area (Table 6)

Annual Water Yield

Data stationarity analysis using the Mann-Kendall test for trend showed no significant trend in annual water yield from 1991-2013 for any of the study watersheds analyzed at the least restrictive α=0.1 confidence level (Table 7). Similarly, Sen's slope estimate was not statistically different from zero for any of the study watersheds at the $\alpha = 0.05$ confidence level (Table 7). By way of illustration, interannual variability of annual water yield is plotted for TMC (29% beetle-killed, 237 km^2 watershed area), RC (58%, 51 km^2), and SLC (72.8%, 85km^2) in order to represent the broad range of beetle-killed areas and watershed areas analyzed (Table 6, Figure 2). Interannual variability of annual water yield shows a variance about the mean pre-beetle outbreak and when compared to cumulative beetle kill percentage indicating minimal impact of MPB induced tree mortality on annual water yield (Figure 2).

Table 6: Cumulative beetle-killed area as a percent of total watershed area from 1997 to 2013. Watersheds are listed in ascending order of the percentage of beetle-killed area in 2013.

		Cumulative Beetle-Killed Area (%)																
Watershed	**Watershed Area km^2**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
EFER	21	0.0	0.1	0.1	2.7	3.1	3.3	3.6	3.8	4.0	4.1	4.5	4.5	4.5	5.0	5.2	5.5	**6.4**
SR	149	0.7	2.3	2.5	3.1	3.7	3.9	4.6	7.2	8.0	8.7	10.1	10.9	13.0	13.8	13.8	14.5	**14.9**
TMC	237	0.2	2.9	3.5	4.8	4.9	6.0	6.5	8.7	9.9	11.0	13.0	18.0	20.0	22.0	23.0	26.8	**29.3**
GC	37	2.9	7.4	9.0	10.1	13.1	14.1	18.1	23.1	25.1	26.1	27.1	30.2	32.2	32.2	33.2	33.9	**36.9**
FRUS	26	1.2	1.3	1.8	4.9	7.3	9.4	10.1	16.2	19.2	23.3	25.3	28.3	30.3	32.4	32.4	36.2	**41.7**
BC	16	7.1	8.9	10.5	15.1	18.4	24.9	27.0	32.4	33.5	35.7	36.8	38.9	40.0	41.1	41.1	41.3	**41.8**
FRWP	72	0.6	3.0	3.5	4.9	6.4	7.6	8.2	11.9	14.9	19.9	29.8	33.8	37.8	38.8	39.8	41.9	**45.3**
KG	23	0.0	0.4	1.1	1.8	1.8	1.9	2.1	5.8	12.1	14.1	18.1	35.2	46.2	48.2	48.2	49.5	**49.5**
SFWF	72	0.9	2.9	5.1	6.3	7.3	9.9	16.9	27.8	32.8	35.8	38.7	39.7	42.7	43.7	43.7	46.4	**50.9**
WFDC	91	1.0	1.6	2.2	2.8	2.9	4.2	10.6	27.5	31.2	33.0	34.9	36.7	36.7	42.2	42.2	46.9	**51.1**
BCA	38	1.3	4.7	5.8	8.1	10.4	11.8	11.8	16.3	17.7	28.1	28.8	30.3	31.8	35.5	36.2	44.1	**51.5**
WFP	476	0.8	4.2	6.6	8.5	11.1	21.5	29.4	40.8	44.2	46.4	46.4	47.6	47.6	48.7	48.7	50.8	**53.1**
WFL	232	1.3	3.3	4.6	5.6	6.2	11.1	19.7	36.7	40.6	43.2	44.6	47.2	48.5	51.1	51.1	54.0	**57.4**
RC	51	0.2	3.2	3.3	3.7	4.4	5.4	8.0	13.1	19.1	29.2	46.3	50.3	53.3	55.3	55.3	55.7	**58.0**
SC	48	4.4	4.6	4.6	4.8	4.9	6.3	9.1	27.9	37.8	41.8	49.8	51.8	53.8	53.8	53.8	56.5	**58.5**
RCT	170	0.2	2.5	2.7	2.8	3.6	4.8	7.3	12.8	26.6	40.4	56.9	58.7	62.4	63.3	63.3	64.1	**66.5**
SLC	85	4.4	10.0	11.0	14.0	15.0	15.0	23.0	30.0	40.0	47.1	52.1	55.1	56.1	59.1	59.1	64.6	**72.8**
MC	16	17.7	23.6	24.5	25.5	27.5	30.4	40.2	51.0	58.9	61.8	63.8	67.7	71.6	72.6	72.6	73.1	**77.5**

Table 7: Results of the Mann-Kendall test for trend and Sen's slope estimate.[3] No watershed exhibited a significant trend in annual water yield using Mann-Kendall or Sen's Slope.

Watershed	Water Years Analyzed	n	Mann-Kendall Z-score	Sen's Slope Estimate
BC	1991-2013	23	-0.58	-0.32
BCA	1991-2013	23	-0.61	-0.20
EFER	2003-2013	11	0.47	1.0
FRUS	1991-2013	23	-0.48	-0.14
FRWP	1991-2013	23	-1.1	-0.25
GC	1991-2013	23	-0.48	-0.41
KG	1991-2013	23	-0.63	-0.24
MC	1991-2013	23	-0.16	-0.072
RC	1991-2013	23	-0.11	-0.12
SC	1991-2013	23	0.45	0.094
SFWF	1991-2013	23	0.21	0.18
SLC	1991-2013	23	-0.37	-0.14
TMC	1991-2013	23	-0.79	-0.24
WFDC	1991-2013	23	-0.53	-0.33
WFL	1991-2013	23	-0.11	-0.040
WFP	1991-2013	23	0.48	0.24

[3] EFER streamflow was analyzed from 2003 to 2013 due historical data availability

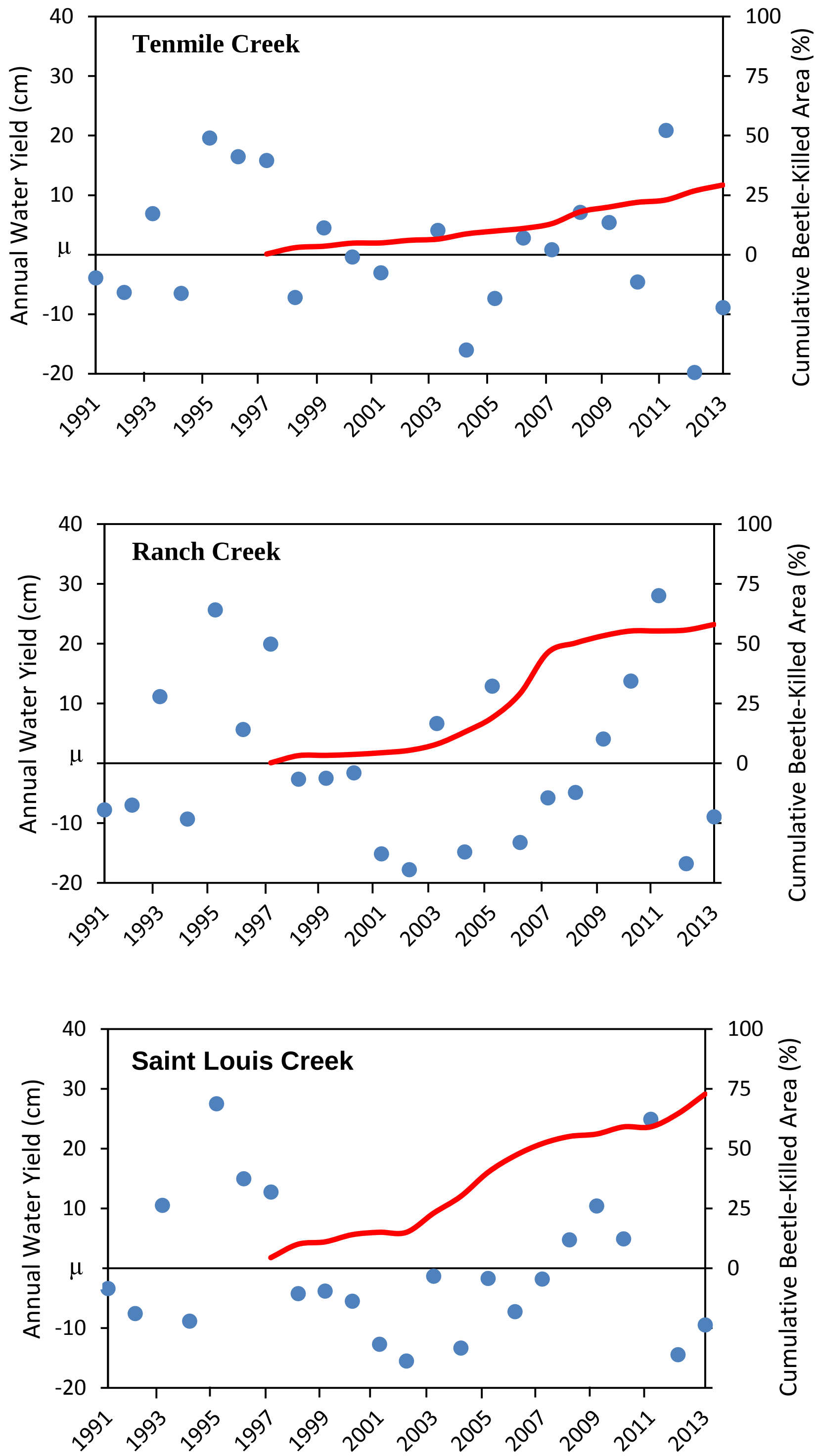

Figure 2: Annual water yield (cm) departure from the mean and cumulative beetle-killed area (%, solid red line) for Tenmile Creek (TMC), Ranch Creek (RC) and Saint Louis Creek (SLC). Mean annual water yield was calculated from 1991 to 2013. The MPB had already impacted SLC before the beetle kill census began in 1997 therefore cumulative beetle-killed area is greater than zero in 1997.

Streamflow Generation

A LMWL was established from rain and snow data collected from Oct 2011 to Nov 2012 in the study watersheds (Maggart 2014) (Figure 3). The distribution of data in $\delta^{18}O$-$\delta^{2}H$ space indicates little spatial variability throughout the study area though differences in the signature of snow versus rain are evident. The relative location of rain and snow data on the LMWL are indicative of the condensation temperature. Vapor that condenses at cooler temperatures (snow) is composed of relatively more light isotopes than rain, and appear on the left-hand side of the LMWL (Figure 3)

Stream water isotopic data generally lies along the LMWL (Figure 4). The line of best fit from soil water data plotted from all study watersheds, known as the evaporation line, demonstrates a slight departure (less slope) from the LMWL (Figure 4). The lower slope of the evaporation line than the LMWL is the result of water becoming increasingly enriched in both ^{18}O and ^{2}H during evaporation as the transformation from liquid to vapor in the soil fractionates the relative components of ^{18}O and ^{2}H (Gonfiantini 1986; Singh and Kumar 2005). Using available groundwater data in place of soil water did not significantly change the slope of the evaporation line, indicating that soil water was a reasonable proxy for groundwater (Figure 5).

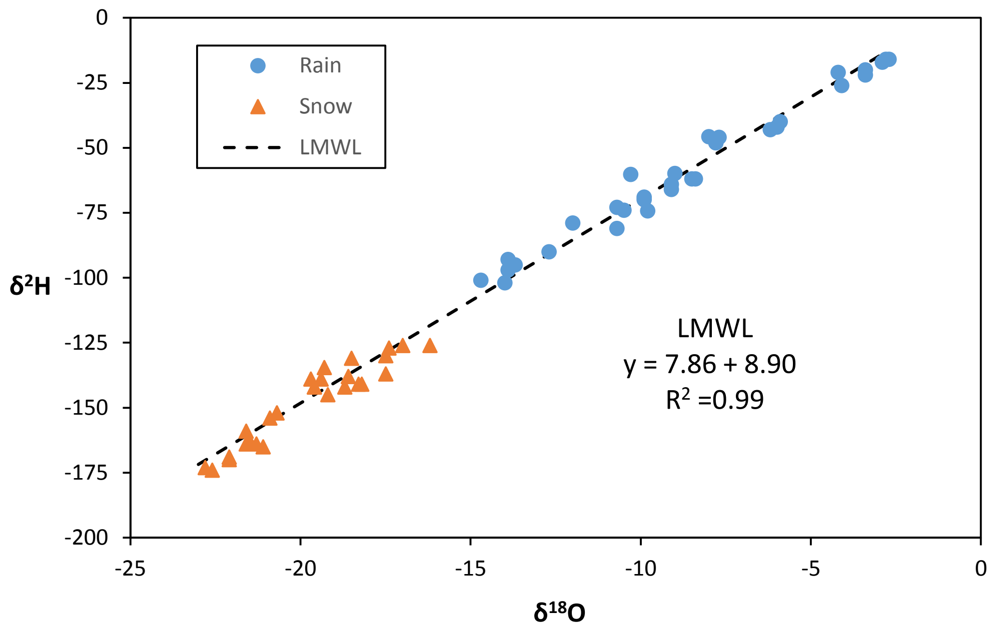

Figure 3: Rain and snow isotopic signatures (δ^2H and δ^{18}O) for precipitation collected within the study watersheds from Oct 2011 to Nov 2012 (Maggart 2014).

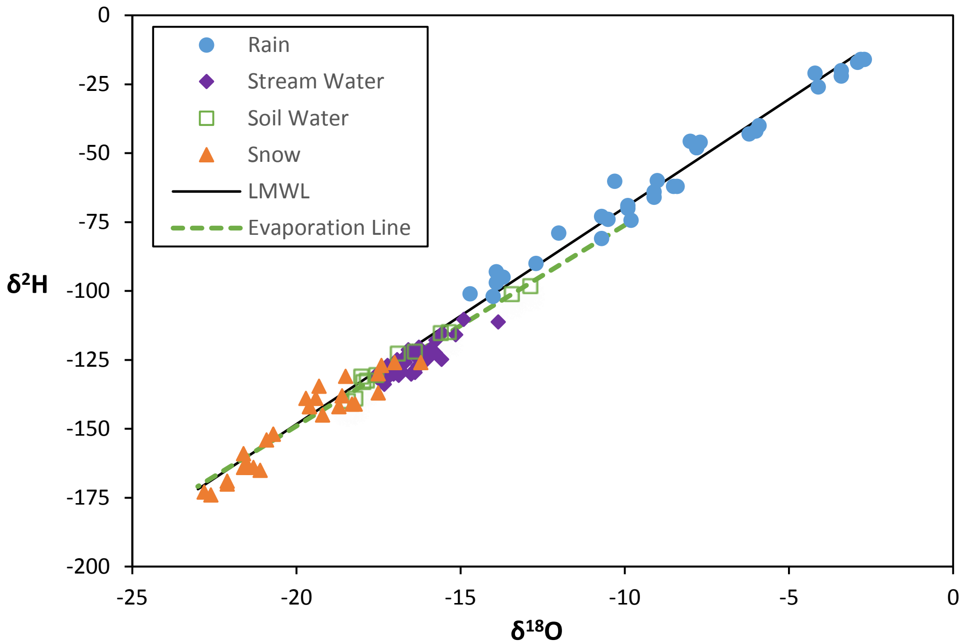

Figure 4: Isotopic signature (δ^2H and δ^{18}O) of rain, stream water, soil water and snow. The LMWL and evaporation line are also shown.

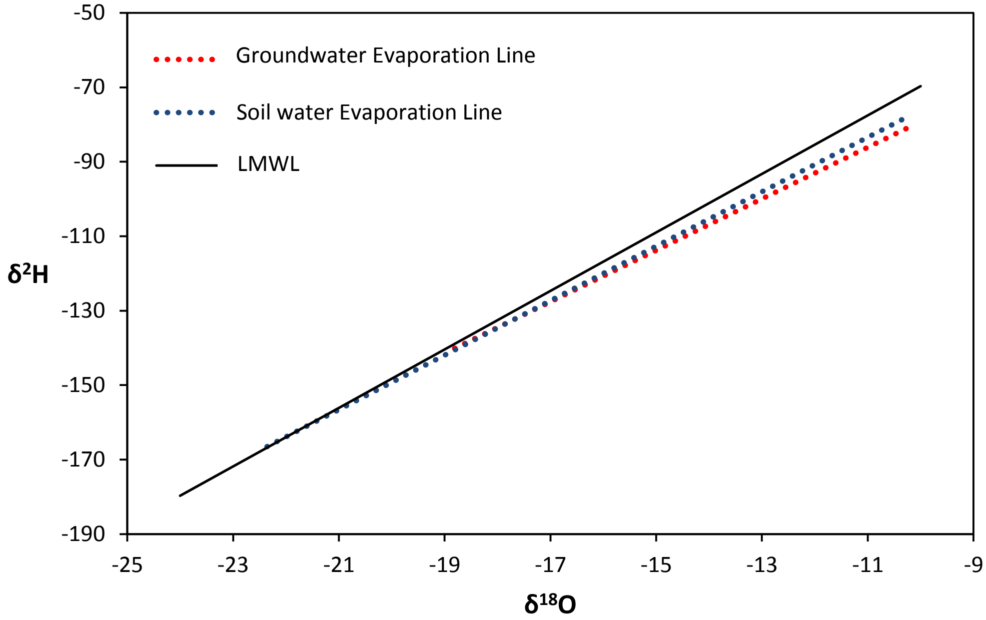

Figure 5: The evaporation line formed by available groundwater data versus the evaporation line from paired soil water data. The LMWL is also shown for reference.

Source Water Separation

Mean Annual Contribution

SIAR was used to find a range of feasible solutions for the mean annual relative contribution to streamflow of rain, snow and ground (soil) waters (Table 8). The mean annual source water contribution (%) was determined by averaging the range of feasible solutions determined by SIAR (Table 8). Averaging across all watersheds, the mean annual source water contribution to streamflow of rain, snow and soil water for the 4 sampling dates was 18%, 44% and 38%, respectively (Table 8). Rain proved to be the smallest contributor to streamflow in all study watersheds ranging from 10% (BCA, 38 km2, 51% beetle-killed and KG, 23 km^2, 49.5%) to 30% (RCT, 23km^2, 49.5%). Snow contributed the highest proportion to streamflow in most watersheds (14 out of 17) with snow and soil water being within 2% in 4 out of 17 watersheds (Table 8). MC was not analyzed due to insufficient soil water data.

When mean source water contribution was plotted against percent beetle-killed area, snow showed a decreasing slope (-0.15) while rain and soil water exhibited slightly increasing slopes (0.06 and 0.05, respectively) (Figure 6). Regression analysis using Spearman’s rank-order correlation (Eqn 3) of the source waters indicate that none of the mean annual source waters are significantly correlated to percent beetle-killed (Table 9).

Table 8: Range of feasible solutions for the mean annual contributions of snow, rain and soil waters to the mean isotopic signature of streamflow. Values may not add to 100% due to rounding. MC was not analyzed due to insufficient soil water data.

		Mean Annual Source Water Contribution (%)								
		Rain			Snow			Soil Water		
Watershed	**Kill%**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
BC	42	18	26	22	33	51	42	24	49	37
BCA	52	3.9	16	10	45	55	50	30	51	41
EFER	6.4	13	19	16	37	59	48	24	50	37
FRUS	42	13	19	16	36	55	46	26	51	39
FRWP	45	20	24	22	32	53	43	25	47	36
GC	37	15	25	20	39	54	47	23	48	36
KG	50	7.0	15	11	41	59	50	28	52	40
RC	58	14	26	20	40	55	48	20	46	33
RCT	67	27	32	30	23	42	33	26	50	38
SC	59	20	25	23	30	49	40	28	47	38
SFWF	51	10	17	14	39	55	47	29	51	40
SLC	73	13	19	16	30	52	41	29	56	43
SR	15	8.6	20	14	45	59	52	23	49	36
TMC	29	16	23	20	30	51	41	28	54	41
WFDC	51	20	24	22	30	49	40	27	49	38
WFL	57	10	17	14	36	56	46	27	53	40
WFP	53	8.5	17	13	38	54	46	31	53	42
Average				**18**			**44**			**38**

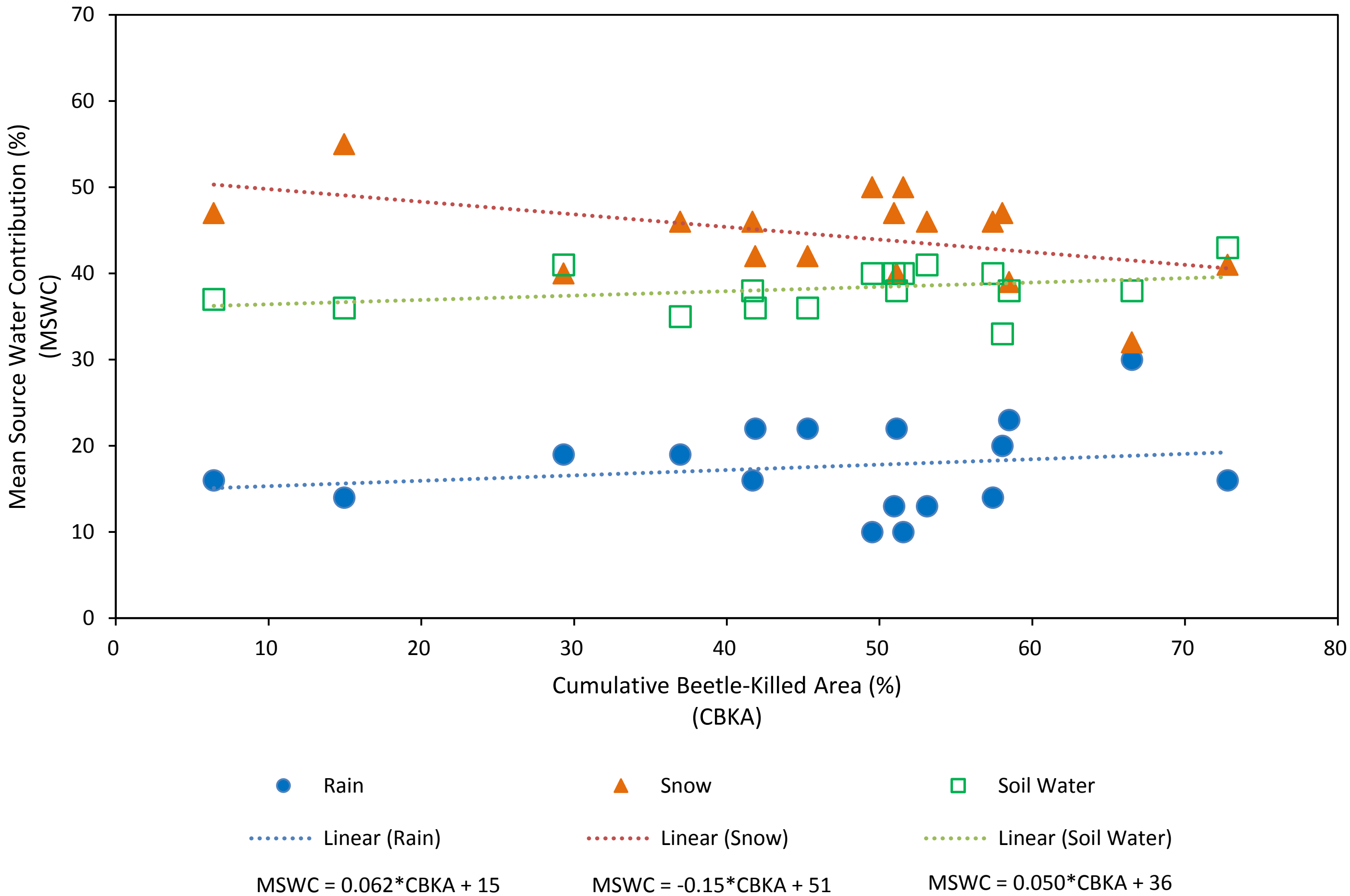

Figure 6: Mean annual source water contribution (%) versus beetle-killed area (%) for rain, snow and soil water. None of the source waters are significantly correlated to percent beetle-killed area.

Table 9: Results of Spearman's rank-order correlation assessing the strength between cumulative beetle-killed percent and mean annual source water contirbution to streamflow, where n is the sample number and ρ is the correlation coefficient. MC was not analyzed due to insufficient soil water data.

	Cumulative Beetle-Killed (%)		
	n	***ρ***	***p*-value**
Rain	17	0.16	0.52
Snow	17	-0.38	0.13
Soil Water	17	0.34	0.18

Peak Flow Contribution

The mean source water contribution (%) by watershed for peak flow was determined by using soil water and streamflow data from May 2014 and calculated by averaging the range of feasible solutions determined by SIAR (Table 10). Averaging across all watersheds, the mean source water contribution to May streamflow of rain, snow and soil water was 14%, 42% and 43%, respectively (Table 10). Rain proved to be the smallest contributor to streamflow in all study watersheds ranging from 4% (SR, 149 km^2, 14.9% beetle-killed) to 27% (RC, 51 km^2, 58.0% and SC 48 km^2, 58.5%). Snow and soil water contribution to streamflow was roughly equal with snow being the largest contributor to streamflow in 7 out of 16 watersheds (Table 10).

When mean source water contribution is plotted against percent beetle-killed area, snow showed a decreasing slope (-0.21) while rain and soil water exhibited slightly increasing slopes (0.11 and 0.09, respectively) (Figure 7). Spearman's correlation coefficient (Eqn 3) was used to determine the significance of source water contribution as it relates to cumulative beetle-killed percentage. Results of this test indicate that snow's decreasing contribution to streamflow is significantly correlated to increasing beetle-killed percentage ($p = 0.02$) (Table 11). Neither rain nor soil water is significantly correlated to percentage beetle-killed area (Table 11).

Table 10: Range of feasible solutions and the average of all watersheds for the contributions of rain, snow and soil water to peak (May) streamflow. Values may not add to 100% due to rounding. BCA and MC were not analyzed due to insufficient soil water data.

		Peak Flow Source Water Contribution (%)								
		Rain			Snow			Soil Water		
Watershed	**Kill%**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
BC	42	12	16	14	36	53	45	31	52	42
EFER	6.4	10	15	13	42	60	51	26	48	37
FRUS	42	2.2	8.2	5.2	37	53	45	36	61	49
FRWP	45	17	19	18	27	48	38	33	56	45
GC	37	17	20	19	38	55	47	25	45	35
KG	50	9.1	14	12	22	47	35	38	68	53
RC	58	24	29	27	63	69	66	0.63	10	5
RCT	67	14	19	17	25	47	36	34	61	48
SC	59	24	29	27	29	57	43	19	42	31
SFWF	51	2.0	7.9	5.0	27	42	35	50	71	61
SLC	73	4.0	15	9.5	6.3	49	28	36	90	63
SR	15	0.89	7.5	4.0	43	54	49	38	57	48
TMC	29	14	17	16	36	54	45	29	50	40
WFDC	51	13	16	15	25	51	38	33	61	47
WFL	57	7.4	12	9.7	36	51	44	37	56	47
WFP	53	18	21	20	28	49	39	30	53	42
Average				**14**			**42**			**43**

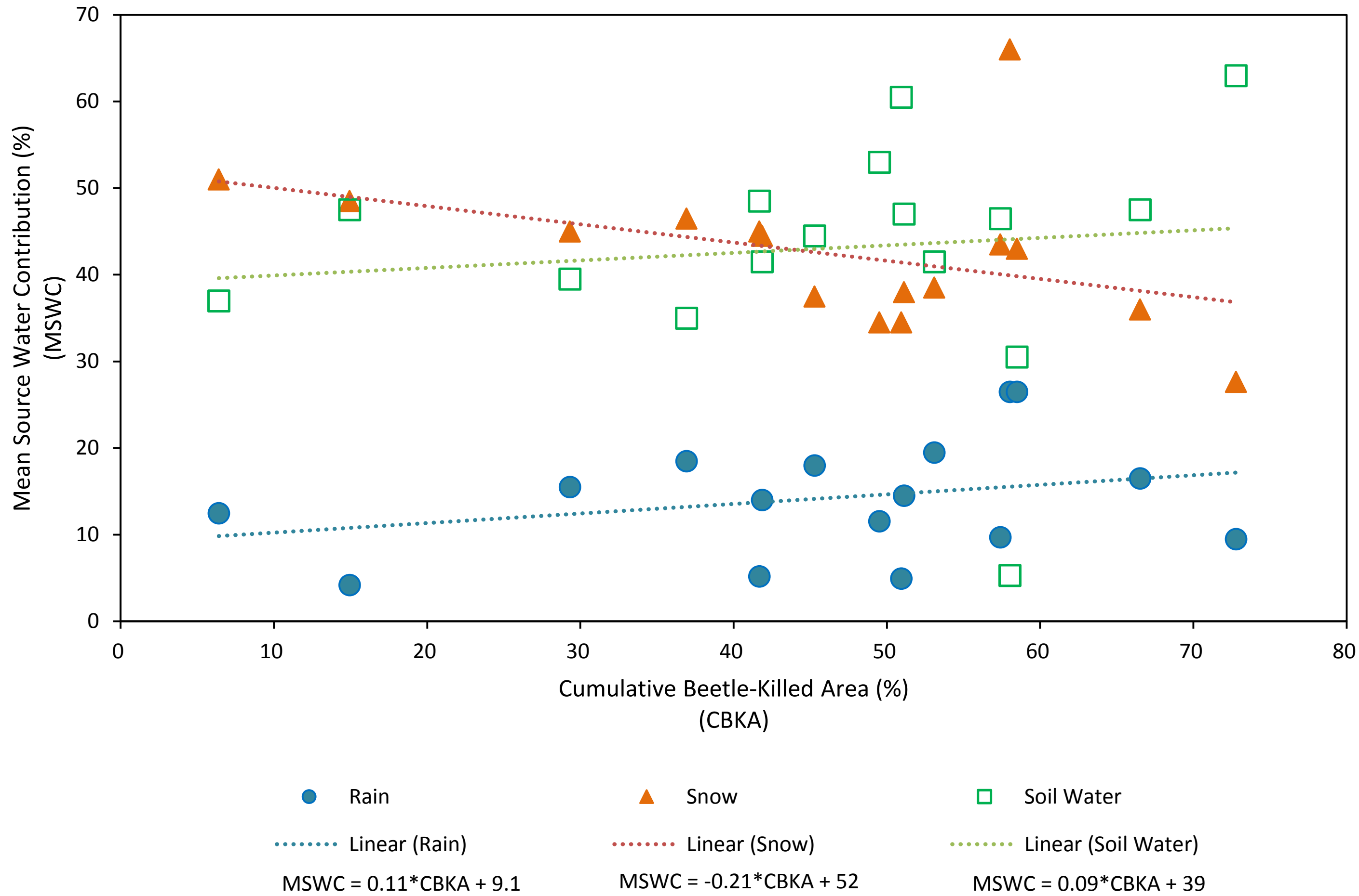

Figure 7: Cumulative beetle-killed area (%) versus mean source water contribution to peak (May) streamflow (%) for rain, snow and soil water. Snow as a source water significantly decreased with respect to percent beetle-killed area ($p = 0.02$).

Table 11: Results of Spearman's rank-order correlation assessing the strength between cumulative beetle-killed percent and source water contirbution to peak (May) streamflow, where n is the sample number and ρ is the correlation coefficient. BCA and MC were not analyzed due to insufficient soil water data.

	Cumulative Beetle-Killed (%)		
	n	**ρ**	***p*-value**
Rain	16	0.29	0.21
Snow	16	-0.56	0.02
Soil Water	16	0.16	0.55

Nutrient Export

Nutrient Concentration

No temporal trend was seen for stream total nitrogen over the sampling dates (Table 12). Stream nitrate concentrations were generally highest in Jan with Sep, Oct and May concentrations being approximately equal (Table 12). TOC concentrations were generally highest during the higher flow months of Sep and May, Oct and Jan TOC concentrations were relatively equal or lower in lower flow months (Table 12).

When total nitrogen, nitrate and TOC concentrations were plotted against cumulative beetle-killed area, Spearman's correlation coefficient (Eqn 3) (Wessa 2012) found significant trends in Jan for total nitrogen ($p = 0.03$) and nitrate ($p = 0.03$), and Oct for TOC ($p = 0.04$) (Table 13, Figure 8).

Using Spearman's correlation coefficient, a significant upward trend was determined for Jan ($p = 0.02$) and May ($p = 0.02$) sampling dates when TOC/TN concentration was plotted against cumulative beetle kill percent (Table 13) (Eqn 3) (Wessa 2012). An upward trend was also seen in May ($p = 0.04$) for the concentration of TOC/TKN, calculated as total nitrogen minus nitrate, when plotted against beetle kill percent. No trend in TOC/TKN versus beetle-killed percentage was determined for the other three sampling months (Table 13).

Table 12: Measured total nitrogen, nitrate, and TOC concentrations from streamflow samples for each sampling date.

	Total Nitrogen (mg/L N)				Nitrate (mg/L N)				TOC (mg/L)			
Watershed	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**
BC		0.15	0.35		0.02	0.02	0.08	0.05		1.0	1.0	
BCA		0.15	0.15	0.27	0.02	0.02	0.09	0.04		1.7	1.2	3.0
EFER					0.01	0.02		0.04				
FRUS		0.22		0.22		0.08		0.06		1.2		1.9
FRWP		0.21	2.4	0.32		0.05	2.1	0.12		1.2	1.6	3.0
GC		0.16	0.33	0.23	0.04	0.07	0.16	0.04		1.2	1.2	3.6
KG		0.16	0.09		0.00	0.02	0.06	0.01		1.1	0.86	
MC	0.08				0.01	0.02		0.13	1.7		1.1	
RC	0.19		0.19	0.18	0.03	0.06	0.07	0.01	3.1		1.2	4.6
RCT				0.21	0.01	0.01		0.01				5.7
SC			0.21	0.24	0.05	0.08	0.07	0.06			0.83	2.9
SFWF	0.11		0.17	0.19	0.03	0.05	0.09	0.02	2.1		0.83	3.1
SLC	0.21	0.27	0.21	0.22	0.05	0.08	0.05	0.03	2.9	1.4	1.3	3.5
SR	0.13	0.23	0.28		0.11	0.10	0.17	0.13	0.66	0.49	0.53	
TMC		0.24	0.52	0.35	0.07	0.07	0.22	0.10		1.4	1.1	2.7
WFDC		0.20		0.16	0.02	0.04		0.01		1.7		3.3
WFL	0.12			0.15	0.02	0.04		0.01	2.7			2.9
WFP	0.11	0.15	0.19	0.22	0.01	0.02	0.08	0.0	2.21	1.6	1.2	5.5

Table 13: Results of Spearman's rank-order correlation assessing the strength between cumulative beetle-killed percent and total nitrogen (TN), nitrate (NO3), TOC, TOC/TN and TOC/TKN, where n is the sample number and ρ is the correlation coefficient.

	Cumulative Beetle-Killed (%)		
TN	**n**	ρ	***p*-value**
Sep	7	0.00	1.0
Oct	11	-0.27	0.42
Jan	13	-0.61	0.30
May	13	-0.34	0.25
NO3			
Sep	16	-0.17	0.53
Oct	18	-0.21	0.38
Jan	13	-0.60	0.03
May	18	-0.34	0.17
TOC			
Sep	7	0.43	0.35
Oct	11	0.64	0.04
Jan	12	0.27	0.39
May	13	0.44	0.14
TOC/TN			
Sep	7	0.32	0.50
Oct	11	0.40	0.21
Jan	12	0.66	0.02
May	13	0.62	0.02
TOC/TKN			
Sep	7	-0.64	0.14
Oct	11	0.21	0.53
Jan	12	0.53	0.08
May	13	0.57	0.04

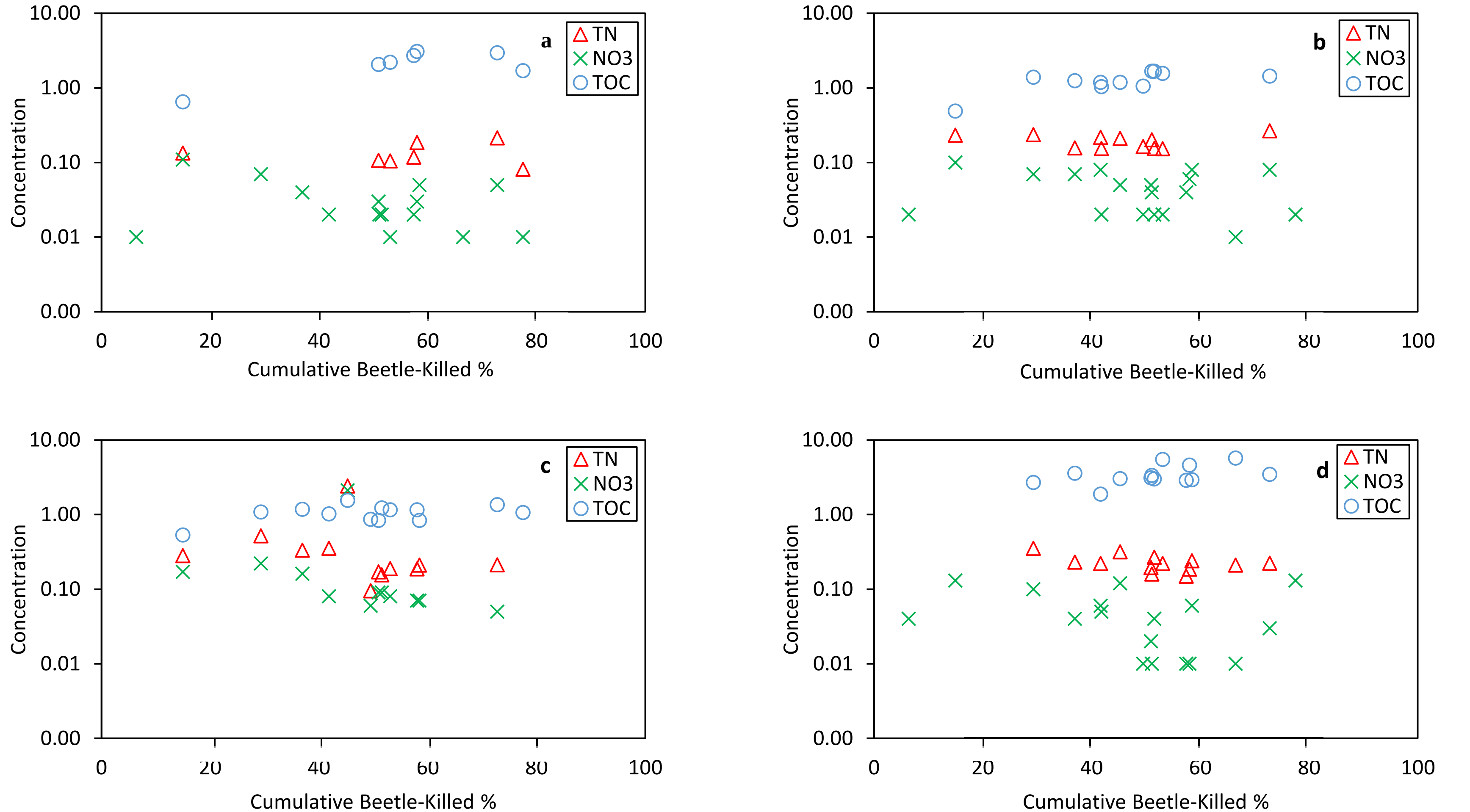

Figure 8: Total nitrogen (mg/L N), nitrate (mg/L N), and TOC (mg/L) concentrations versus cumulative beetle-killed percent for (**a**) Sep-13, (**b**) Oct-13, (**c**) Jan-14, and (**d**) May-14.

Nutrient Flux

Nutrient flux was calculated when both streamflow and nutrient concentration data were available, which were surprisingly few. During the sampling period, nutrient flux for the three nutrients studied was highest during May and lower and relatively constant during the lower flow sampling months of Sep, Oct, and Jan (Table 14). Similar to nutrient concentrations, nutrient flux for total nitrogen, nitrate and TOC showed few significant correlations to cumulative beetle-killed area (Table 15, Figure 9).

No significant correlation was seen for any sampling date for TOC/TN flux with cumulative beetle-killed percent (Table 15). Similarly, no significant correlation was found when TOC/TKN (calculated as total nitrogen minus nitrate) flux was correlated to cumulative beetle-killed percentage (Table 15).

Table 14: Total nitrogen, nitrate, and TOC flux (kg/ha/mo) for each sampling date. Flux data is only available when both nutrient concentration and streamflow data are available.

	Total Nitrogen (kg/ha/mo)				Nitrate (kg/ha/mo)				TOC (kg/ha/mo)			
Watershed	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**	**Sep-13**	**Oct-13**	**Jan-14**	**May-14**
BC		0.04	0.02		0.00	0.00	0.00	0.08		0.26	0.06	
BCA		0.02		0.18	0.00	0.00		0.03		0.18		2.0
EFER					0.00	0.00	0.00	0.03				
FRUS		0.06		0.21		0.02		0.06		0.34		1.8
FRWP		0.06	0.16	0.28		0.01	0.14	0.11		0.31	0.10	2.7
GC		0.04		0.33	0.01	0.02		0.06		0.35		5.1
KG		0.02			0.00	0.00		0.01		0.14		
MC	0.00				0.00	0.00		0.08	0.09		0.01	
RC	0.03		0.01	0.24	0.01	0.01	0.00	0.01	0.56		0.05	5.9
RCT												
SC			0.01	0.13	0.01	0.01	0.00	0.03			0.06	1.6
SFWF	0.02			0.18	0.01	0.01		0.02	0.43			2.9
SLC	0.03	0.05	0.01	0.18	0.01	0.01	0.00	0.02	0.48	0.26	0.09	2.8
SR	0.06	0.06			0.05	0.02		0.09	0.28	0.12		
TMC		0.03		0.38	0.02	0.01		0.11		0.20		2.9
WFDC		0.04		0.14	0.00	0.01		0.01		0.35		2.9
WFL	0.02			0.13	0.00	0.01		0.01	0.51			2.5
WFP	0.01	0.02		0.20	0.00	0.00		0.00	0.21	0.22		5.0

Table 15: Results of Spearman's rank-order correlation assessing the strength between cumulative beetle-killed percent and the flux of total nitrogen (TN), nitrate (NO3), TOC, TOC/TN and TOC/TKN, where n is the sample number and ρ is the correlation coefficient.

	Cumulative Beetle-Killed (%)		
TN Flux	**n**	ρ	***p*-value**
Sep	7	-0.32	0.50
Oct	11	-0.41	0.21
Jan	5	-0.50	0.45
May	12	-0.67	0.02
NO3 Flux			
Sep	15	-0.18	0.51
Oct	17	-0.20	0.43
Jan	5	-0.30	0.68
May	17	-0.45	0.07
TOC Flux			
Sep	7	0.04	0.96
Oct	11	0.07	0.84
Jan	6	-0.49	0.35
May	12	-0.15	0.63
TOC/TN Flux			
Sep	7	0.32	0.50
Oct	11	0.41	0.21
Jan	5	0.80	0.13
May	12	0.56	0.63
TOC/TKN Flux			
Sep	7	-0.53	0.23
Oct	11	0.21	0.54
Jan	5	0.70	0.23
May	12	0.50	0.10

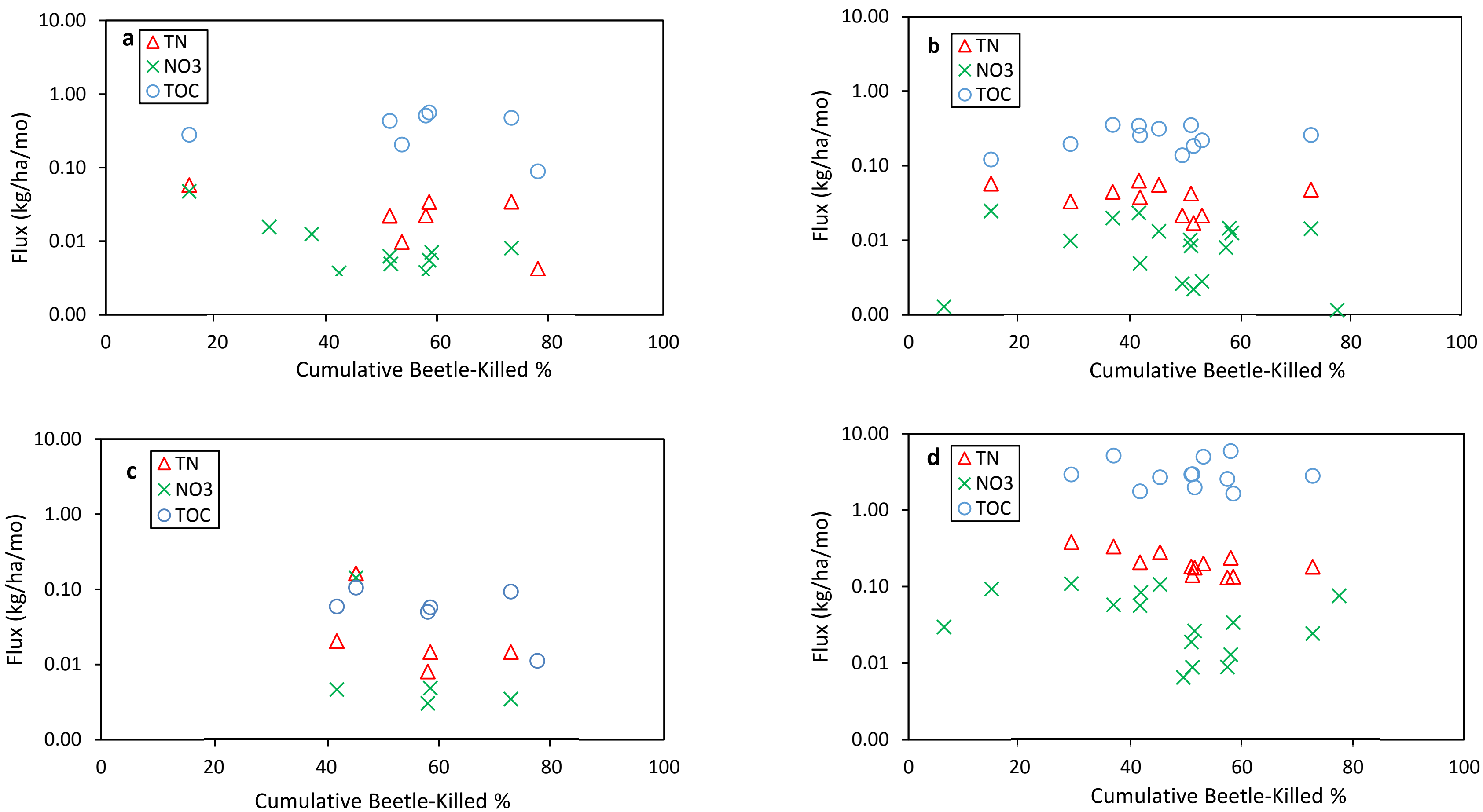

Figure 9: Total nitrogen, nitrate, and TOC flux (kg/ha/mo) versus cumulative beetle-killed percent for (**a**) Sep-13, (**b**) Oct-13, (**c**) Jan-14, and (**d**) May-14. Nutrient flux could only be calculated when both nutrient concentration and streamflow data were available.

Long-term nitrate

The temporal trends in nitrate from four sampling dates are investigated by plotting nitrate concentrations and fluxes in the RC watershed from 1997 to 2013 (Figure 11). The long-term nitrate balance in the RC watershed, calculated as atmospheric deposition minus streamflow weighted flux, did not exhibit any apparent change or trend since the local onset of the beetle kill infestation in 1997 (Figure 12). Further, atmospheric deposition in the area was greater than stream transport of nitrate out of RC with roughly 99.5% of atmospherically deposited nitrate being retained.

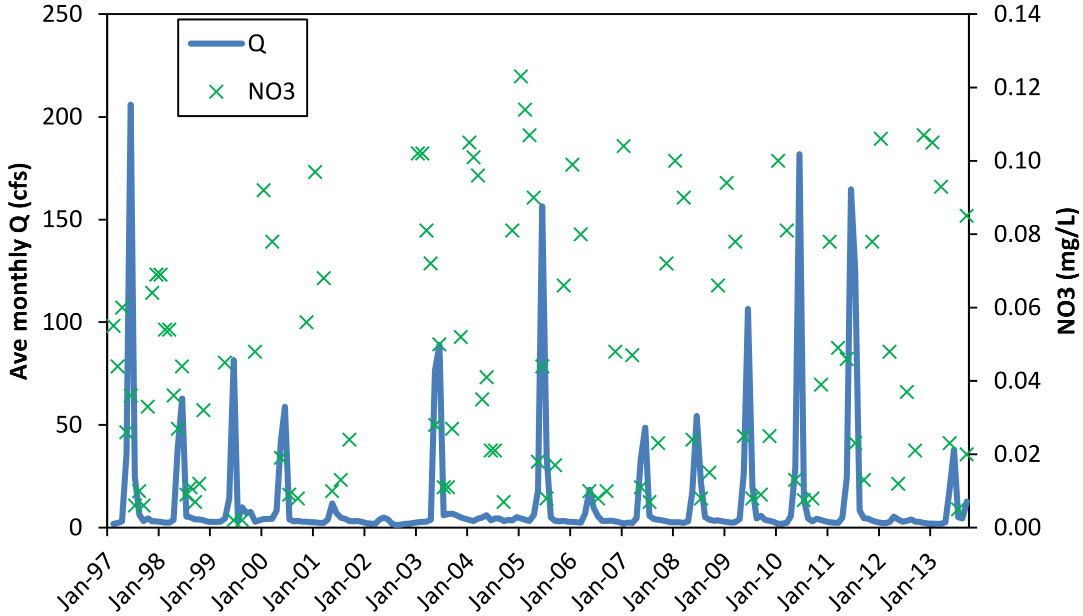

Figure 10: Average monthly streamflow (cfs) and nitrate concentration (mg/L) for Ranch Creek from Feb 1997 to Sep 2013.

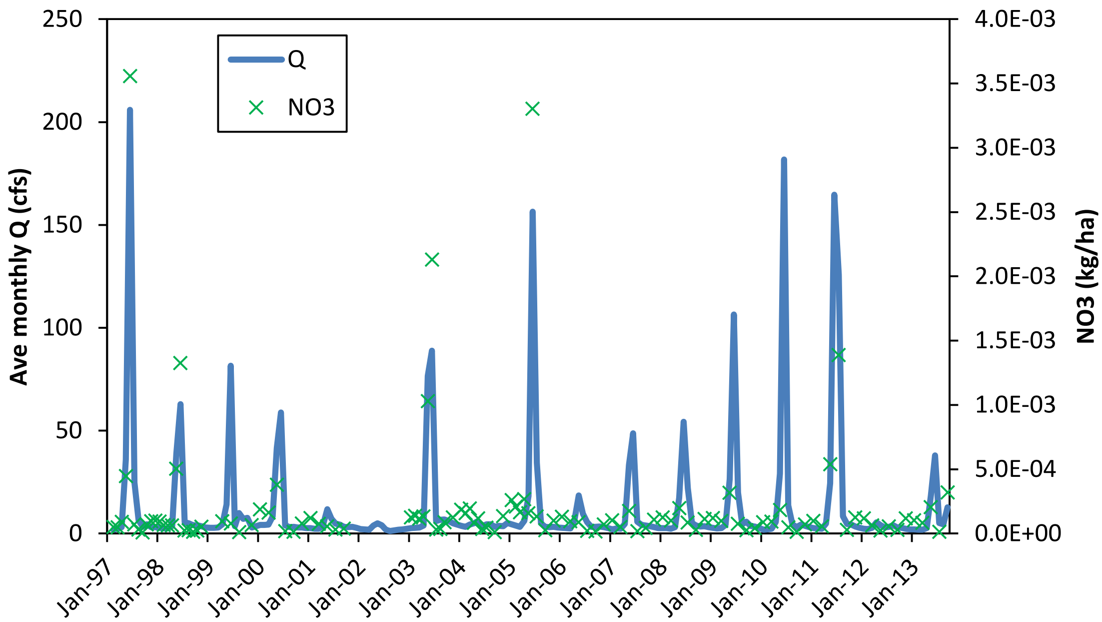

Figure 11: Average monthly streamflow (cfs) and nitrate flux (kg/ha/mo) for Ranch Creek from Feb 1997 to Sep 2013.

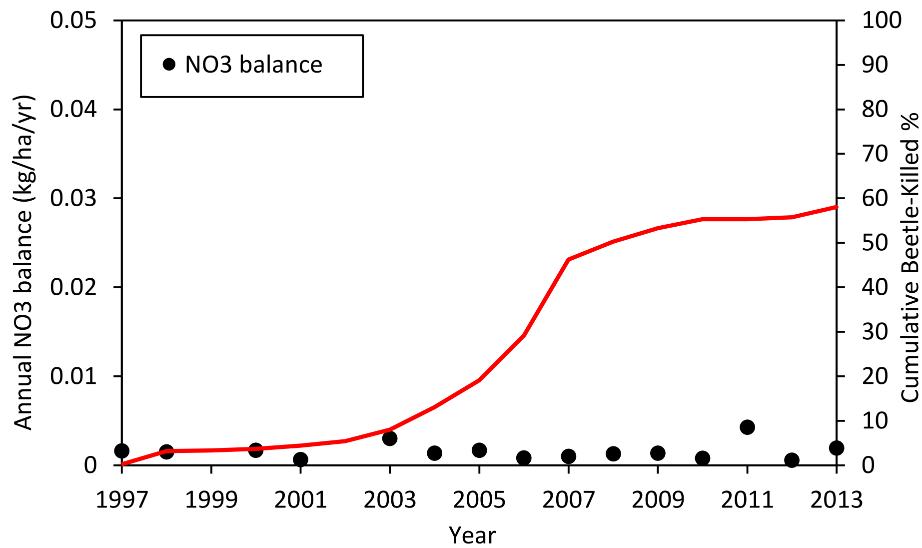

Figure 12: Annual nitrate balance (kg/ha/yr) out of the Ranch Creek watershed, calculated as atmospheric deposition minus streamflow weighted flux, and cumulative beetle-killed percent from 1997 to 2013.

DISCUSSION

A large number of watersheds with varying areas and percent beetle killed area were used to determine the effect of the MPB in the northern Colorado Rockies. The US Forest Service conducts annual aerial detection surveys to determine the extent of tree mortality caused by the MPB (Johnson nd). It is important to note that insect caused tree mortality is highly variable and dynamic. As a result, aerial detection survey data is better used for trend analysis, not as precise measurements (Johnson nd).

Annual Water Yield

The Mann-Kendall test for trend and Sen's slope estimate were used to identify trends in annual water yield from 1991 to 2013. None of the study watersheds exhibited a significant trend (α=0.05) in annual water yield (Table 7).

These results are contradictory to annual water yield increases seen after timber harvesting even though the minimum canopy removal via timber harvest of 20% for the Rocky Mountain region before annual water yield increase is detectable (Stednick 1996). An increase in water yield after timber harvesting is usually seen within the first year and decreases as vegetation recovers (Troendle and King 1985, 1987; Troendle and Kaufmann 1987). The seemingly contradictory hydrologic response between timber harvest and the current MPB epidemic is likely attributed to the differing canopy structure changes. Timber harvesting results in the total removal of the canopy and the selected trees with accompanying damage to the understory. MPB inducted tree mortality leaves the entire tree on site and does not impact the understory. While snow interception decreases after harvest (Troendle and Kaufmann 1985; Troendle 1987; Troendle and Ruess 1997), the needles, branches and boles that remain after a MPB infestation can still intercept precipitation, leading to higher sublimation rates relative to timber harvest (Edburg et al. 2012; Mikkleson at al. 2013b). Additionally, the heterogeneous, selective advancement of MPB tree mortality allows unsuitable host trees and understory vegetation to utilize transpirational savings from dead trees.

The annual water yield results from the current MPB epidemic differ from those found in the White River and Yampa River watershed after a spruce beetle outbreak in the early 1940's (Bethlahmy 1974, 1975). Bethlahmy reported increases in these watersheds to be the smallest within the first five years with the largest increase 15 years after the peak infestation. It is possible that not enough time has lapsed since the current MPB epidemic water yield to trigger a water yield response. More likely the differing results are caused by the differing amounts of reported regeneration between the two studies. The increase in water yield in the White River and Yampa River watersheds was partially attributed to slow regeneration after the peak of the spruce beetle outbreak. Significant tree mortality coupled with slow vegetative regrowth provided additional water via transpirational savings to enter streams. Conversely, in the current study watersheds, the reported abundant vegetative regeneration of saplings and the understory (Diskin et al. 2011) are utilizing the transpirational savings from the dead trees.

Streamflow Generation

A LMWL was developed from the isotopic signature of precipitation data (Figure 3). Snow appears on the left side of the LMWL as snow condenses at cooler temperatures than rain and is therefore composed of relatively lighter isotopes (Phillips and Gregg 2003). As expected, stream water samples lie on the LMWL between rain and snow and around the intersection with the evaporation line since stream water is a volume weighted result of the three source waters (Figure 4). The evaporation line developed via the line of best fit through soil water data, exhibited a slight, downward departure from the LMWL (Figure 4, 5). The smaller slope of the evaporation line is a result of the enrichment of both ^{18}O and ^{2}H as soil water evaporation fractionates the relative composition of heavy and light isotopes (Gonfiantini 1986; Singh and Kumar 2005). The magnitude of departure of the evaporation line from the LMWL differs from the large departure found from data collected in 2011-2012 (Maggart 2014). This difference is expected however as Colorado endured a significant drought year in 2012 with most headwater watersheds receiving less than 50-70% of average precipitation (Lukas et al. 2012), leading to high rates of soil evaporation (Vegetation Drought Response Index 2012) and the large departure from the evaporation line.

Source Water Separation

SIAR is a multi-component mixing model that can be used to find a range of feasible solutions to undefined systems. Average annual isotopic signatures of rain and snow in addition to the signatures of soil water and stream water were used to determine mean annual source water contribution to streamflow and mean source water contribution to peak (May) streamflow (Table 3, 4). The resulting outputs from SIAR was a range of feasible solutions (Table 8, 10).

Annual Contribution

Mean annual isotopic signatures of rain, snow, soil water and stream water were used in the model. Annual averaging of data from different temperature conditions, i.e., different fractionation rates of heavy and light isotopes led to fairly large range of feasible solutions some watersheds (Table 8).

Mean annual contribution to streamflow is a result of 18% rain, 44% snow and 38% soil water (Table 8, Figure 6). These results differ slightly from the previous study in area which found that stream water resulted from 20% rain, 60% snow and 20% soil water (Maggart 2014). The difference in results could be indicative of comparing an average water year to a drought year. During a drought year, soil water stores are overly depleted, compared to a normal water year, as the rate of evapotranspiration greatly exceeds that of precipitation (Lukas et al. 2012). Larger amounts of snowmelt are needed to recharge depleted soil moisture and therefore relatively less snowmelt is available for streamflow generation. In addition, as seen by the similar slope of the evaporation line to the LMWL (Figure 4, 5), the isotopic signatures of soil water samples were not greatly different that the other source waters, leading to tighter results from the two major contributors.

With respect to percent beetle-killed area, snow as a source water decreased with increasing beetle killed area while rain and soil water's contribution increased with increasing beetle-killed area (Figure 6). None of the mean annual contributions to streamflow were significantly correlated to percent beetle killed area (Table 9).

Peak Flow Contribution

Mean source water contribution to peak (May) streamflow was determined by using annual average rain and snow isotopic signatures as well as signatures from soil water and stream water collect in May 2014 (Table 4). On average, May streamflow was a result of 14% rain, 42% snow and 43% soil water (Table 10, Figure 7). These results differ from the previous study in area (Maggart 2014) and from the mean annual source water contribution. Using mean annual isotopic signatures of soil water and stream water can result in large standard errors due to annual temperature fluctuations and the resulting different fractionation rates of heavy and light isotopes. Using only soil water and stream water data from May allows for a better representation relative source water contribution to peak streamflow.

Snow's contribution to peak streamflow was negatively correlated ($p = 0.02$) to percent beetle-killed (slope = -0.21) (Table 11, Figure 7). Rain and soil water as source waters were not significantly correlated to percent beetle-killed (Table 11). The negative correlation of snow as a source water and percent beetle-killed suggests that as the amount of beetle kill increases and more of the forest canopy is removed, increases in snowpack sublimation more than offset interception sublimation savings (Biederman et al. 2012) and accumulation increases (Pugh and Small 2011), resulting in a slightly lower amount of available snow water (Biederman et al. 2012; Mikkelson et al. 2013a; Mikkelson et al. 2013b).

Nutrients

Canopy structure changes dues to the MPB epidemic in northern Colorado has the potential to alter nutrient cycling within beetle-affected watersheds. Canopy characteristics in forested watersheds greatly influence the amount of nutrient availability by dictating the type and amount of forest litter and decomposition and nutrient mineralization rates via temperature and moisture regulation (Prescott 2002). Not only does the canopy structure regulate hydrological conditions by redirecting precipitation throughfall and thereby impacting nutrient redistribution via leaching and overland flow, but boles, branches and foliage act as a major nutrient source and sink (Vitousek and Reiners 1975; Laino and Prescott 1999; Prescott 2002).

Nitrogen

High tree mortality due to forest disturbance such as timber harvest and the current MPB epidemic has the potential to increase nitrogen levels in streams due to decreases in vegetative uptake (Likens et al. 1970; Vitousek and Melillo 1979; Gundersen et al. 2006; Griffin et al. 2011). Further, increases to stream nitrogen concentrations after forest disturbance may be followed by a period of decreased levels as the forest regrows and nitrogen is retained in the ecosystem (Vitousek and Reiners 1975; Binkley 2001; Binkley et al. 2004). The increase signal could be muted however in the nitrogen-deficient northern Colorado Rocky Mountains as the increase in nitrogen availability is utilized by residual vegetation (Griffin et al. 2011; Griffin et al. 2013; Hubbard et al 2013).

After a 33% clearcut at Fraser Experimental Forest, stream water nitrate increased from 0.006 to 0.06 mg/L N (Reuss et al. 1997; Troendle and Reuss 1997). Similarly, a clearcut in central Idaho lead to nitrate concentrations that were 10 times higher than average, though concentrations remained well below the EPA standard of 10 mg/L and returned to control levels within 5 years (Clayton and Kennedy 1985).

Similar to the findings of this study (Figure 10, 12), little evidence exists for long term increasing stream water nitrogen concentrations as a result of the current MPB epidemic (Mikkelson et al. 2013b; Stednick et al. 2010; Clow et al. 2011). An increasing trend in stream water total nitrogen was found in Grand County, Colorado however the magnitude and the confidence level was not reported. Additionally, this same system showed a decreasing trend in nitrate over the same time period (Clow et al. 2011). Similarly, the current MPB epidemic was also shown to not significantly increase stream water nitrate concentrations at Fraser Experimental Forest (Rhoades et al. 2013).

In addition to no long term trend in nitrate concentrations in the current study (Figure 10, 12), concentrations were not shown to be related to the percentage of MBP die-back within a watershed (Table 13,

Figure 8). These findings are similar to those found in Grand County (Clow et al. 2011) and in the Willow Creek watershed (Stednick et al. 2010) and further the idea that additional available nitrogen due to large scale tree death is being used by new and residual vegetation to meet forest growth requirements.

Although stream water nitrogen concentrations were not shown to be influenced by the MPB epidemic (Table 13, Figure 8), concentrations were shown to generally follow vegetative growing cycles and flushing caused by spring snowmelt. Stream nitrate concentrations are typically lowest during the growing season as available stores are used to meet vegetative demand (de la Crétaz and Barten 2007). This was seen in the current study as stream water nitrate concentration was highest in Jan and lowest in May, Sep and Oct. This seasonal variation was also seen in Grand County (Clow et al. 2011). Additionally, nitrate flux generally increases during spring snowmelt as soil nitrate pools accumulated during winter dormancy are flushed (de la Crétaz and Barten 2007). This phenomenon were seen in this study and stream water nitrate flux highest in May and lower but relatively constant during the lower flow sampling months of Sep, Oct and Jan (Table 14).

Carbon

The large influx of forest litter caused by the MPB epidemic has the potential to cause a prolonged increase to stream water carbon concentrations due to increased litter availability and the potential for increased decomposition rates (Edburg et al. 2011). Since boles have abundant amounts of stored carbon relative to needles and decay more slowly (Pearson et al. 1987), stream water carbon levels could remain elevated for an extended amount of time before returning to pre-outbreak levels.

Water entering Colorado treatment facilities originating in MPB infested watersheds have been shown to have 4 times the concentration of TOC than waters not originating from infested watersheds (Mikkelson et al. 2013a). Conversely, DOC concentrations have not shown to be elevated in MPB infested watersheds in Grand County (Clow et al. 2011). Similar to this current study, neither of the aforementioned studies found a relation between the percentage of MPB-killed and stream water carbon concentrations (Table 13, Figure 8). One explanation for conflicting results could be the major hydrological flow paths sampled. Waters sampled for this study and the one in Grand County consisted of surface waters where as the water entering the treatment facilities was a combination of surface and groundwaters. Thus, groundwater flow could be an important factor in carbon transport (Mikkelson et al. 2013b).

Carbon:Nitrogen

The amount and rate of N and C released from decomposing matter had been observed to be highly correlated to the C:N ratio (Vitousek 1982; Gundersen et al. 1998). Premature needlefall from MPB induced tree mortality caused a pulse of low C:N litter to the forest floor (Morehouse et al. 2008; Griffin et al. 2011; Keville et al. 2013). The large input of N coupled with decreased N uptake due to the dead trees leads to an initial increase in mineralization which has been associated with higher ammonium concentrations under beetle killed trees (Clow et al. 2011; Griffin et al. 2011; Xiong et al. 2011; Keville et al. 2013).

Limited N and C uptake due to tree death, coupled with increased substrate from needles and roots and increased soil temperature and moisture, causes decomposition rates to increase (Edburg et al 2012, Keville et al. 2013). Increased decomposition leads to higher levels of N immobilized in microbial biomass (Brooks et al. 1998; Edburg et al. 2012) and higher rates of N to be absorbed by the understory (Griffin et al. 2013). Additions of C from snags, boles and roots will further increase decomposition and thus the immobilization of N (Edburg et al. 2012; Mikkelson et al 2013b). Overall, this may lead to the flushing of available C stores while N transport could be limited, especially during spring snowmelt when stores of C are typically flushed from catchment soils and organic matter (Boyer et al. 1997).

Some evidence of this was seen in the stream water TOC/TN and TOC/TKN as the ratio during spring runoff (May) increased as beetle-killed percentage increased (Table 13, 15). Carbon available for leaching increased as more organic matter was available for decomposition due to higher rates of beetle kill. Leachable TKN decreased with increasing beetle-killed percent as organic N was retained in the biomass due to high rates of N immobilization. Immobilization of TKN coupled with inorganic N (ammonium and nitrate) being used by residual vegetation caused available total nitrogen leaching to also decrease with increasing beetle-killed area. Overall, increasing stores of available mobile C combined with smaller stores of mobile TKN plus inorganic N being retained by residual vegetation, resulted in TOC:TN and TOC:TKN to increase with increasing beetle-killed area, though the correlation was not significant in all months (Table 13, 15).

CONCLUSIONS

Since 1996, the MPB epidemic in the northern Colorado and southern Wyoming Rocky Mountains has killed 1.6 million hectares of pine forest, altering not only the canopy structure, but also hydrological processes and nutrient cycling. Eighteen watersheds in the northern Colorado Rockies were analyzed for changes due to the MPB epidemic in the areas of annual water yield, source water streamflow generation and nutrient movement. Study watersheds ranged in size from 16 to 476 km^2 with cumulative beetle-killed area ranging from 6 to 78% of the watershed area.

After applying the Mann-Kendall test for trend from 1991 to 2013, none of the study watersheds exhibited a significant trend in annual water yield. Sen's slope estimate showed no trend in annual water yield. Thus, the current MPB epidemic has had no apparent effect on annual water yield.

Isotopic signature (^{18}O and ^{2}H) analysis of rain, snow, soil water and stream water from the study watersheds showed that little soil evaporation occurred as the evaporation line formed from soil water data only slightly departed from the LMWL. Mean annual streamflow contribution resulted from 14% rain, 44% snow and 38% soil water. None of the source waters were significantly correlation to percent beetle-killed area. Peak streamflow (May) was a result of 18% rain, 43% snow and 44% soil water. Snow's peak streamflow contribution was significantly correlated ($p = 0.02$) to percent beetle-killed ($slope = -0.21$) indicating that snow as a source water decreased as a watershed had a higher fraction of MPB impacted trees. No significant correlation was found between rain or soil water's contribution to peak streamflow and percent beetle-killed area.

Stream water total nitrogen, nitrate and TOC concentrations were not significantly impacted by the current MPB epidemic. Except for certain months, there was no apparent change in total nitrogen, nitrate or TOC concentrations or flux when plotted against percentage of beetle killed area. Nutrient concentrations followed growing season cycles and nutrient flux mirrored the snowmelt hydrograph. Larger C stores may be available with increasing beetle-killed percent as both TOC/TN and TO/TKN increased with increasing beetle-killed percent during snowmelt induced C flushing. Long term nitrate balance (atmospheric deposition minus stream water flux) in the RC watershed did not exhibit any change since the MPB onset within the watershed with 99% of atmospheric deposition being retained.

RECOMMENDATIONS

For future studies, it is recommended that annual water yield data be examined at a longer (forward) time step. A previous study of a spruce beetle outbreak in the White River and Yampa River watersheds reported the largest water yield increases to be 15 years after the initial infestation (Bethlahmy 1974, 1975). Additional studies could also calculate annual water yield with the inclusion of upstream diversions. It is possible that water entering the stream was not accounted for properly at the gauging station if water rights users diverted significantly different amounts of water year to year.

Future studies on source water contribution to streamflow should include more frequent sampling dates throughout the year. Loss of certainty in this study was partially due to a lack of available data. Future researchers should also look to take multiple samples on each sample day. These two sources of additional data would allow for source water contributions to be looked at seasonally instead of on an annual basis.

Due to the fact that the north-central Colorado Rocky Mountain forests are nitrogen deficient and the general accepted theme of excess nitrogen stores being used by regeneration, future studies should look to identify the amount of regeneration within a watershed to determine if any correlations exist between percent beetle-killed, amount of regeneration and stream water nutrient concentrations. Additionally, soil nutrient concentrations should be examined to determine if any anomalies exist in nutrient pools and fluxes. Finally, data sets of long term stream water nutrient concentrations with restricted access should be examined for further insight into nutrient balances among a variety of beetle-killed percentages.

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APPENDIX

Appendix 1: Isotope data by location, sample type and sample month

USGS Station	Location	Sample Type	Sample Month	δ2H	$\delta^{18}O$
9066200	BC	stream	Sep-13	-115	-15.7
9066200	BC	stream	Oct-13	-116	-15.3
9066200	BC	stream	May-14	-129	-17.4
9066200	BC	soil water	Sep-13	-115	-15.6
9066200	BC	soil water	Oct-13	-122	-16.4
9066200	BC	soil water	May-14	-131	-18.0
9067000	BCA	stream	Sep-13	-118	-15.8
9067000	BCA	stream	Oct-13	-118	-14.6
9067000	BCA	stream	Jan-14	-124	-16.4
9067000	BCA	stream	May-14	-126	-16.9
9067000	BCA	soil water	Sep-13	-101	-13.4
9061600	EFER	stream	Sep-13	-133	-17.8
9061600	EFER	stream	Oct-13	-128	-17.1
9061600	EFER	stream	May-14	-132	-17.4
9061600	EFER	soil water	Oct-13	-139	-18.2
9061600	EFER	soil water	May-14	-130	-17.6
9061600	EFER	rain	Jun-12	-90	-12.7
9061600	EFER	rain	Aug-12	-26	-4.1
9061600	EFER	rain	Nov-12	-79	-12
9061600	EFER	snowpit	Mar-12	-159	-21.6
9022000	FRUS	stream	Oct-13	-123	-16.3
9022000	FRUS	stream	May-14	-131	-17.7
9022000	FRUS	soil water	Oct-13	-132	-17.9
9022000	FRUS	soil water	May-14	-123	-16.9
9022000	FRUS	rain	May-12	-60.2	-10.3
9022000	FRUS	rain	Jun-12	-101	-14.7
9022000	FRUS	rain	Jul-12	-46	-7.7
9022000	FRUS	rain	Aug-12	-21	-4.2
9022000	FRUS	rain	Nov-12	-70	-9.9
9022000	FRUS	snowpit	Apr-12	-138	-18.6
9024000	FRWP	stream	Oct-13	-121	-16.5
9024000	FRWP	stream	Jan-14	-127	-17.0
9024000	FRWP	stream	May-14	-128	-17.1
9024000	FRWP	soil water	May-14	-140	-18.4
9024000	FRWP	snowpit	Apr-12	-139	-19.7
9065500	GC	stream	Sep-13	-116	-15.8
9065500	GC	stream	Oct-13	-116	-15.5
9065500	GC	stream	Jan-14	-120	-16.1
9065500	GC	stream	May-14	-128	-16.9
9065500	GC	soil water	Sep-13	-98	-12.9

USGS Station	Location	Sample Type	Sample Date	δ2H	$\delta^{18}O$
9065500	GC	soil water	May-14	-133	-18.0
9065500	GC	rain	May-12	-62	-8.5
9065500	GC	rain	Jun-12	-93	-13.9
9065500	GC	rain	Jul-12	-48	-7.8
9065500	GC	rain	Aug-12	-22	-3.4
9065500	GC	rain	Nov-12	-66	-9.1
9065500	GC	snowpit	Apr-12	-131	-18.5
9047700	KG	stream	Sep-13	-128	-17.1
9047700	KG	stream	Oct-13	-130	-17.0
9047700	KG	stream	Jan-14	-131	-17.2
9047700	KG	stream	May-14	-130	-17.1
9047700	KG	soil water	Oct-13	-115	-15.4
9047700	KG	soil water	May-14	-134	-17.4
9047700	KG	rain	May-12	-72.9	-10.7
9047700	KG	rain	Jun-12	-102	-14
9047700	KG	rain	Jul-12	-48	-7.8
9047700	KG	rain	Aug-12	-17	-2.9
9047700	KG	rain	Nov-12	-62	-8.4
9047700	KG	snowpit	Jan-12	-174	-22.6
9047700	KG	snowpit	Jan-12	-173	-22.8
9047700	KG	snowpit	Mar-12	-141	-18.2
9066300	MC	stream	Sep-13	-122	-16.5
9066300	MC	stream	Oct-13	-125	-16.9
9066300	MC	stream	Jan-14	-127	-16.9
9066300	MC	stream	May-14	-128	-17.0
9032000	RC	stream	Sep-13	-110	-14.9
9032000	RC	stream	Jan-14	-121	-15.9
9032000	RC	stream	May-14	-124	-16.1
9032000	RC	soil water	May-14	-105	-15.1
9032000	RC	snowpit	Apr-12	-139	-19.4
9033100	RCT	stream	Sep-13	-111	-13.9
9033100	RCT	stream	Oct-13	-116	-15.1
9033100	RCT	stream	May-14	-127	-16.8
9033100	RCT	soil water	May-14	-135	-17.6
9033100	RCT	snowpit	Apr-12	-127	-17.4
9051050	SC	stream	Sep-13	-131	-17.4
9051050	SC	stream	Oct-13	-130	-16.4
9051050	SC	stream	Jan-14	-130	-16.5
9051050	SC	stream	May-14	-134	-17.3
9051050	SC	soil water	Sep-13	-134	-18.1
9051050	SC	soil water	May-14	-169	-22.4
9051050	SC	rain	May-12	-74.3	-9.8

USGS Station	Location	Sample Type	Sample Date	δ2H	$\delta^{18}O$
9051050	SC	rain	Jun-12	-97	-13.9
9051050	SC	rain	Jul-12	-43	-6.2
9051050	SC	rain	Aug-12	-20	-3.4
9051050	SC	rain	Nov-12	-64	-9.1
9051050	SC	snowpit	Jan-12	-164	-21.3
9051050	SC	snowpit	Jan-12	-165	-21.1
9035900	SFWF	stream	Sep-13	-123	-16.3
9035900	SFWF	stream	Oct-13	-128	-16.4
9035900	SFWF	stream	Jan-14	-131	-17.3
9035900	SFWF	stream	May-14	-131	-16.9
9035900	SFWF	soil water	May-14	-127	-16.0
9035900	SFWF	rain	May-12	-59.9	-9
9035900	SFWF	rain	Jun-12	-81	-10.7
9035900	SFWF	rain	Jul-12	-40	-5.9
9035900	SFWF	rain	Aug-12	-16	-2.8
9035900	SFWF	rain	Nov-12	-69	-9.9
9035900	SFWF	snowpit	Jan-12	-142	-19.6
9035900	SFWF	snowpit	Jan-12	-142	-19.6
9035900	SFWF	snowpit	Mar-12	-142	-18.7
9026500	SLC	stream	Sep-13	-121	-16.6
9026500	SLC	stream	Oct-13	-127	-17.2
9026500	SLC	stream	Jan-14	-130	-17.5
9026500	SLC	stream	Jan-14	-125	-15.6
9026500	SLC	stream	May-14	-129	-17.2
9026500	SLC	soil water	May-14	-131	-17.4
9026500	SLC	rain	May-12	-45.7	-8
9026500	SLC	rain	Jun-12	-95	-13.7
9026500	SLC	rain	Jul-12	-42	-6
9026500	SLC	rain	Aug-12	-16	-2.7
9026500	SLC	rain	Nov-12	-74	-10.5
9026500	SLC	snowpit	Jan-12	-154	-20.9
9026500	SLC	snowpit	Jan-12	-152	-20.7
9026500	SLC	snowpit	Jan-12	-152	-20.7
9026500	SLC	snowpit	Apr-12	-126	-17
9026500	SLC	snowpit	Apr-12	-134.6	-19.3
9047500	SR	stream	Sep-13	-115	-15.6
9047500	SR	stream	Oct-13	-120	-16.3
9047500	SR	stream	Jan-14	-126	-16.7
9047500	SR	stream	Jan-14	-127	-16.9
9047500	SR	stream	May-14	-129	-17.2
9047500	SR	soil water	Sep-13	-81	-10.2
9047500	SR	soil water	Oct-13	-137	-18.6

USGS Station	Location	Sample Type	Sample Date	δ2H	$\delta^{18}O$
9047500	SR	soil water	May-14	-117	-15.4
9047500	SR	snowpit	Jan-12	-170	-22.1
9047500	SR	snowpit	Jan-12	-169	-22.1
9047500	SR	snowpit	Mar-12	-145	-19.2
9050100	TMC	stream	Sep-13	-125	-16.7
9050100	TMC	stream	Oct-13	-125	-16.0
9050100	TMC	stream	Jan-14	-122	-15.8
9050100	TMC	stream	May-14	-130	-17.2
9050100	TMC	soil water	Sep-13	-124	-16.9
9050100	TMC	soil water	Oct-13	-130	-17.5
9050100	TMC	soil water	May-14	-135	-18.1
9050100	TMC	snowpit	Jan-12	-164	-21.6
9050100	TMC	snowpit	Jan-12	-163	-21.5
9050100	TMC	snowpit	Mar-12	-137	-17.5
9050100	TMC	snowpit	Mar-12	-141	-18.3
9035700	WFDC	stream	Sep-13	-118	-15.7
9035700	WFDC	stream	Oct-13	-123	-16.0
9035700	WFDC	stream	May-14	-131	-17.5
9035700	WFDC	stream	May-14	-131	-17.4
9035700	WFDC	soil water	May-14	-140	-18.6
9036000	WFL	stream	Sep-13	-124	-16.7
9036000	WFL	stream	Oct-13	-127	-16.9
9036000	WFL	stream	May-14	-131	-17.3
9036000	WFL	soil water	Sep-13	-115	-16.8
9036000	WFL	soil water	May-14	-131	-16.8
9035700	WFL	snowpit	Mar-12	-130	-17.5
9036000	WFL	snowpit	Mar-12	-126	-16.2
9037500	WFP	stream	Sep-13	-123	-16.4
9037500	WFP	stream	Oct-13	-126	-16.2
9037500	WFP	stream	Jan-14	-129	-16.8
9037500	WFP	stream	Jan-14	-127	-16.3
9037500	WFP	stream	May-14	-128	-16.9
9037500	WFP	soil water	Sep-13	-92	-12.0
9037500	WFP	soil water	Oct-13	-130	-17.0
9037500	WFP	soil water	May-14	-140	-18.5