University of Nevada, Reno

Seasonal responses of carbon, nitrogen, and phosphorus cycling to woodland reduction and different organic matter additions in the semiarid Great Basin

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Abstract

Disturbances that impact microbially-mediated processes of decomposition and mineralization of organic matter carry ecosystem-level consequences because soil microorganisms can immobilize nutrients or render them available for plant uptake. The stochastic nature of resource availability in drylands may cause these ecosystems to be particularly susceptible to disturbance. We used two common fuel reduction treatments—mastication and cut-and-lop—in piñon-juniper encroached sagebrush ecosystems in the semiarid Great Basin to quantify how organic matter additions affect pools and fluxes of C, N, and P. We found that organic matter additions from woodland reduction altered microbial substrates and C dynamics but that microbial functions that regulate cycling of N and P were resilient to disturbance two years following implementation of the treatments. Our results shed light on how fuels treatments that alter organic matter inputs can in turn influence the ratios of C to nutrients in soil. These findings improve our understanding of how environmental perturbations affect nutrient dynamics in semiarid ecosystems and provide insight on the degree to which intra-annual seasonal variation regulates biogeochemical dynamics. Our results can help land managers predict how piñon-juniper encroached sagebrush ecosystems and other arid or semiarid ecosystems might respond to fuels treatments or other disturbances that alter organic matter additions to the soil.

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Introduction

Microbially-driven processes of decomposition are governed by a suite of biotic and abiotic factors. Microbes are constrained by rigid stoichiometric requirements (Cleveland and Liptzin 2007) necessary to maintain homeostasis. As microbes decompose organic matter (OM), they process nutrients together through integration of respiration, immobilization of organic carbon (C), nitrogen (N), and phosphorus (P), mineralization of inorganic N, and release of P from ester bonds (Parton et al. 2007; Manzoni et al. 2010; Falkowski et al. 2008; Schlesinger et al. 2011). Cycles of C and N are coupled because C and N are directly bonded and when microbes decompose OM to obtain C for energy or biomass, N is either immobilized into microbial biomass or mineralized if the element is not needed (McGill and Cole 1981). Cycles of C and P on the other hand, are thought to be less coupled during decomposition because C and P form ester bonds that microbes can cleave by producing extracellular phosphatase enzymes (McGill and Cole 1981).

Beyond the chemical composition of OM, microbial access to resource substrates is driven by physical characteristics of the soil microenvironment, such as soil moisture, temperature, pH, and texture. Organic matter decomposes faster when soil conditions are warm and wet because hydrologic connectivity enhances substrate diffusion (Stark and Firestone 1995). The effects of temperature on microbial activity can vary seasonally in response to precipitation, where warmer temperatures increase decomposition when soil moisture is also suitable for microbial growth (Chapin 2011). Soil pH can limit microbial access to P because most P is in occluded forms at acidic and alkaline pH values (Brady and Weil 2001). Additionally, soils with higher percentages of clay minerals have higher cation exchange capacity and by attracting cations in OM to negatively charged sites, they reduce the ability of enzymes to attach to substrates (Chapin 2011). When soil conditions are suitable, and microbes can access resource substrates, they have a higher affinity for decomposing litter with a lower C:N ratio and a higher affinity for decomposing litter with a higher concentration of labile versus recalcitrant C (Chapin 2011). Taken together, interactions between chemical and physical characteristics of the soil environment regulate terrestrial nutrient dynamics and the C balance.

Disturbances and environmental changes that alter plant-derived OM inputs to the soil and/or physical characteristics of the soil can disrupt nutrient dynamics (Wieder et al. 2015). The ratio of C relative to N and P in OM exceeds what is required for microbes to maintain a stoichiometric balance of C, N, and P (McGroddy et al. 2004). As a result of this imbalance, the availability of N and P can impact the feedbacks between aboveground litter inputs and belowground soil processes that regulate ecosystem functions. Research has suggested that increased aridity decouples cycles of C, N, and P (Delgado-Baquerizo et al. 2013; Delgado-Baquerizo et al. 2018). This is because moisture limits net primary productivity (NPP) thus cycles of C and N (de Graaff et al. 2014), and P availability may increase due to reduced plant uptake of P and greater physical weathering from abiotic controls exerted on P minerals in more arid ecosystems. These shifts in biotic and abiotic controls result in narrowing ratios of C:P and N:P (Delgado-Baquerizo et al. 2013; Delgado-Baquerizo et al. 2018). Limitation of just one of these elements can slow the cycling of the others, potentially disrupting ecosystem services such as nutrient cycling and stabilization of soil C.

Despite drylands comprising nearly 41% of the global terrestrial surface, (Pointing & Belnap 2012; Sinsabaugh et al. 2015) and 35% of the land area in the western United States (Pointing and Belnap 2012; Sinsabaugh et al. 2015), studies that explore C, N, and P cycles in drylands are limited relative to studies in wetter ecosystems (Schimel 2010). In part, this is due to a longstanding paradigm in ecology that suggests biogeochemical cycles in drylands are inconsequential because water availability constrains these processes and the magnitude of fluxes in these ecosystems (Sala et al. 1988; Comstock and Ehleringer 1992; Schlesinger 1996; Robertson and Groffman 2007; Austin 2011); whereas nutrient availability limits these processes in wetter ecosystems. (Sala et al. 1988; Comstock and Ehleringer 1992; Schlesinger 1996; Robertson and Groffman 2007). Yet, recently, studies have shown that drylands may have unique microbial communities adapted to these ecosystems and that the environmental conditions that limit microbial activity in more humid ecosystems may not always apply in drylands (Fierer et al. 2012; Sullivan et al. 2012).

Drylands may be particularly susceptible to disturbance because the conditions required for recruitment of plant species vary temporally and spatially due to the stochastic nature of resource availability these ecosystems (Svejcar and Kildisheva 2017), and microbes are highly sensitive to episodic precipitation, responding to ephemeral increases in soil moisture by rapidly mineralizing C and immobilizing N (Schimel et al. 2007). A disturbance that disrupts microbial stoichiometry could exacerbate temporal asynchrony between nutrient availability and plant phenology, potentially decoupling biogeochemical cycles of C, N, and P (Augustine and McNaughton 2004). That being so, dryland ecosystems may be more sensitive to environmental perturbations or even minor disturbances (Svejcar and Kildisheva 2017).

Due to the discontinuous nature of resource availability in dryland ecosystems, vegetation cover tends to be patchy (Austin et al. 2004; Austin et al. 2011). Microbial activity tends to be higher in vegetated patches than in unvegetated inter-canopy zones (Gonzales-Polo and Austin 2009; Cable et al. 2009; Maestre et al. 2009, Austin 2011) because microbes degrade and incorporate accumulating senescent plant material into OM (Charley and West 1975; Klopatek 1987; Burke et al. 1989; Schlesinger et al. 1990; Schlesinger and Pilmanis 1998; Austin et al. 2004; Cregger et al. 2014), resulting in the hotspots of resource availability, or "islands of fertility," that are characteristic of dryland ecosystems (Schlesinger et al. 1996; Schlesinger & Pilmanis 1998; Austin *et al*. 2004; Cregger *et al.* 2014).

Piñon-Juniper (*Pinus monophylla-Juniperus osteosperma; PJ*) woodlands are a semiarid ecosystem that encompasses approximately 40 million hectares within the western US (Romme et al. 2009). Over the past 200 years, the range of PJ woodlands has expanded into sagebrush shrublands, and PJ woodland density has increased. As a result, managers have sought to reduce their extent and density to enhance forage for livestock grazing, improve wildlife habitat for sagebrush obligate species, and mitigate the risk of wildfire (Chambers and Wisdom 2009 Miller et al, 2014; Filippelli et al. 2020). Woodland reduction can change soil chemical and physical characteristics by altering the quality, quantity, and distribution of plant-derived OM inputs to the soil, potentially impacting microbial functions, and thus nutrient availability (Overby and Gottfried 2017).

Disturbance from woodland reduction could come with unintended consequences that may have implications for emergent plant communities. For example, competition is less important in recently disturbed ecosystems and establishment of non-native species is common due to increases in the availability of nutrient resources (Grime 1979). Therefore, woodland reduction treatments may facilitate invasion of exotic annual grasses (Davis et al. 2000) that can alter the structure and function of ecosystems (D'Antonio and Vitousek 1992). In part, the theory of fluctuating resource availability posits that ecosystems subjected to stochastic resource supply from external sources or by release from intact species are more susceptible to invasion than ecosystems with a consistent supply of resources (Rejmanek et al. 1989; Davis et al. 2000). Changes in nutrient dynamics following disturbance, especially increases in soil N, the most limiting nutrient in semiarid ecosystems (Germino et al. 2016), could potentially increase the susceptibility of these treated sites to cheatgrass (*Bromus tectorum)* invasion, the most widespread invasive annual grass in the western US (Germino et al. 2016).

In drylands, the temporal coupling of C, N, and P cycles may become interrupted by the role of seasonal water scarcity (Delgado-Baquerizo 2013). As a result, nutrient dynamics and plant production may become temporally asynchronous (Augustine and McNaughton 2004). Given the extent to which drylands encompass the terrestrial surface and the uncertainty surrounding C, N, and P cycling in these ecosystems, it is pertinent to understand how disturbance affects nutrient dynamics and the extent to which climatic variation regulates these dynamics in these ecosystems. Disturbance from woodland reduction may increase asynchrony among C, N, and P cycles by prolonging or intensifying decoupling. Soils are the foundation of terrestrial ecosystems; therefore, it is pertinent to consider how alterations to the soil environment might influence management outcomes. For this project, we used two common woodland reduction treatments—mastication and cut-and-lop—in piñon-juniper ecosystems in the semiarid Great Basin to quantify how plant-derived OM additions to the soil environment affect microbial physiology and ultimately, pools and fluxes of C, N, and P and microbial biomass. Here we asked: 1) what are the seasonal responses of microbial biomass, C, N, and P pools and fluxes to reduction treatments (i.e., cut-and-lop and mastication) 2) do coupled cycles of C, N, and P change in response to season or to treatment? These insights may help land managers predict how piñon-juniper encroached sagebrush ecosystems and other dryland ecosystems might respond to treatments or other disturbances that alter OM additions to the soil.

Methods

Study Site

Our study sites were located in two watersheds, Porter Canyon (N39°27’47.0016” W117°37’18.7”) and Dalton Canyon (N39°27’36.2” W117°32’9”), in the Desatoya mountain range of the central Great Basin, NV, USA (Figure 1). The region is characterized by a semiarid climate and most precipitation falls during winter months. Both study sites were characterized by two unique ecological sites – a mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*), Idaho fescue (*Festuca idahoensis*), and bluebunch wheatgrass (*Pseudoroegneria spicata* ssp. *spicata*) dominated site and a low sagebrush (*Artemisia arbuscula* ssp. *arbuscula*), Idaho fescue, and bluebunch wheatgrass dominated site. The potential vegetation composition of the mountain big sagebrush ecological site is 65% grasses, 10% forbs, and 25% shrubs and the potential vegetation composition of the low sagebrush ecological site is 60% grasses, 10% forbs, and 30% shrubs (Soil Survey Staff, 2021). Both study sites have been encroached by piñon pine and Utah juniper trees, which are now the dominant overstory.

Description of research units

The following information is to clarify terms used for levels of research units in this field study. A *site* refers to either Porter Canyon or Dalton Canyon. A *treatment* refers to either the cut-and-lop woodland reduction technique or the mastication woodland reduction technique. A *plot* refers to one of 8 randomly placed, replicated sampling locations within cut-and-lop and mastication treatments. Plots were randomly selected prior to treatment using elevation, dominant tree species, dominant understory species, and tree density as criteria. A *subplot* refers to sampling units within each plot. In Porter Canyon, each plot contained three treated subplots and one control subplot. Each plot in Dalton Canyon contained four treated subplots. In Porter Canyon, plot sizes are not uniform, and three subplots were established where trees fell in the plot area and one control subplot was established at an intact tree. In Dalton Canyon, plots were 20 x 20 m and subplots were position in one of four cardinal directions extending from plot center. A *sample* is a unit of soil collected from an individual subplot at a given season, totaling three lop samples per plot and one control sample per plot in Porter Canyon and four mastication samples per plot in Dalton Canyon. In 2016, a cut-and-lop treatment was implemented at Porter Canyon and a mastication treatment was implemented at Dalton Canyon.

Field sampling

We estimated mean annual precipitation and mean annual temperature using PRISM 30-y normals (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 5 April 2021), soil descriptions using the Web Soil Survey (Soil Survey Staff, 2021), and soil texture using hydrometer textural analysis (Gee and Bauder 1979). Plant density data were collected before treatment in October 2015 and after treatment in August 2019 (T. Stringham and D. Snyder, unpublished data). In each plot, trees were censused, shrub density was calculated within six 10 m x 2 m belt transects, and herbaceous species were measured within 18 50 cm^2 quadrats.

We installed soil temperature and volumetric water content (VWC) probes (5TM, METER, Pullman, WA) at each subplot attached to EM50 dataloggers (METER) at each plot that were set to record soil temperature and VWC every two hours. Using specifications from METER, we buried the 5TM probes vertically such that the deepest part of the sensor was 15 cm below the mineral soil, which allowed the sensor to measure a soil volume of approximately the top 20 cm. To characterize seasonal differences in soil characteristics, we collected soil samples in August 2018 (dry and warm soil conditions), November 2018 (dry and cold soil conditions), and June 2019 (cool and wet soil conditions). At all subplots, we collected one soil sample to a 10 cm mineral soil depth with a 1.8 cm diameter Oakfield sampler (Oakfield Apparatus, Fond du Lac, Wisconsin, USA), excluding the O-horizon. We stored samples in plastic bags and transported them to the laboratory at ambient temperatures. We processed soil samples within 12 hours of field collection.

Laboratory analyses

We measured pools of soil dissolved organic C (DOC), microbial biomass carbon (C_{mic}), inorganic N, and phosphate (PO_4^3) within 12 hours of field collection and again after a seven-day incubation in jars under field moist conditions at 20°C. We measured net fluxes of DOC, ammonification, nitrification, N mineralization, PO_4^3- mineralization, and C_{mic} as the difference between pools on day seven and day one. The following equation was used to determine, estimated of soil organic C (SOC) flux:

(1) $\Delta DOC + \Delta C_{mic}$ + Net C mineralization = Estimated organic C flux

We used chloroform fumigation extraction (Vance et al. 1987) to measure DOC and C_{mic}. Briefly, we fumigated approximately 8 g of each soil sample with chloroform for five days and then used 40 mL 0.5 M potassium sulfate (K_2SO_4) to extract chloroform labile DOC. Extractions were shaken for one hour then the suspensions were refrigerated for approximately 12 hours, after which, we filtered them through Whatman No. 2 filter paper. We also repeated the extraction process for a corresponding unfumigated subsample. We determined total organic carbon (TOC) for all samples using a TOC analyzer (TOC-V-CSN Shimadzu Scientific Instruments, Kyoto, J). We calibrated this instrument using standards that ranged from 0 to 50 ppm potassium hydrogen phthalate and 0 to 15 ppm potassium nitrate. The difference between fumigated and non-fumigated values represents the chloroform-extractable pool of DOC and is proportional to microbial biomass C (C_{mic}). Our C_{mic} values are raw as we did not use correction factors to convert chloroform-extractable DOC to C_{mic}.

We measured soil inorganic N (NH_4^+ and NO_3^-) by extracting 8 g soil with 50 mL of 1 M potassium chloride (KCl; Maynard *et al.,*1993). Samples were shaken for one hour, suspensions were refrigerated for approximately 12 hours, then samples were filtered with Whatman No. 2 filter paper. We analyzed NH_4^+ and NO_3^- by colorimetric analysis using a QuickChem 8500 Series 2 Flow Injection Analyzer (FIA) System (Lachat Instruments, Loveland, CO, USA). The instrument was calibrated using a combined NH_4^+ and NO_3^- standard ranging from 0 ppm – 2 ppm and a nitrite (NO_{2-}) standard that ranged from 0 ppm to 2ppm. We used resin to measure PO_4^{3-} based on the method of Tiessen and Moir (2008) and described by Sullivan and Hart (2013). We determined PO_4^{3-} colorimetrically with the QuickChem 8500 FIA using an anhydrous potassium phosphate monobasic standard curve ranging from 0 – 2000 parts per billion (ppb).

To estimate C mineralization and nitrous oxide (N_2O) production, we incubated 25 g of soil at field moist conditions in a jar equipped with a septum. We collected 17 mL of headspace gas five times over the course of the incubation. Gas samples were stored in over-pressurized 12 mL exetainers (Labco Limited, Lampeter, Wales, UK). We analyzed gas samples for carbon dioxide (CO_2) and N_2O concentrations using a gas chromatograph (GC-2014, Shimadzu Scientific Instruments, Columbia, MD, USA) with a flame ionization detector (FID), equipped with a methanizer, and an electron capture detector (ECD) for CO_2 and N_2O, respectively. CO_2 results were calibrated with 300 ppm – 10000 ppm CO_2 standards and N_2O results were calibrated with 0 – 2 ppm N_2O standards.

We used blank correction, check standards, and replicates to ensure accuracy and precision in our data. For gas chromatography and FIA, we included results in our analyses if check standards were within 10% of the standard curve value and if the residual standard error of replicates was 5% or less. Due to issues with the instrument, we adjusted our quality assurance and quality control (QA/QC) standards for TOC analyses. We used results for statistical analyses if check standards were within 15% of the standard curve and if the residual standard error of replicates was 7% or less.

Statistical analyses

We used R version 1.2.2019 (R Core Team 2020) with an *a priori* $\alpha = 0.05$ for all statistical analyses. We first evaluated if volumetric water content (VWC), soil temperature, and concentrations of available N in Porter Canyon and Dalton Canyon were similar prior to treatment using two-dimensional non-metric multidimensional scaling (NMDS) ordination analyses with a Bray-Curtis distance measure followed by permutational analysis of variance (PERMANOVA) test using the “vegan” package (Oksanen *et al.* 2020). To address our first question (i.e., what were the impacts of treatment and season on pools and fluxes of C, N and P availability), we used an NMDS ordination analysis with a Bray-Curtis distance measure to visualize and explore overall treatment and seasonal differences in pools and fluxes of DOC, NH_4^+, NO_3^-, $PO4_3^-$, and C_{mic} after treatments were implemented. We computed dissimilarity indices using the Bray dissimilarity index and then used a PERMANOVA test to determine if the centroids and dispersion of data points differed among seasons, between treatment groups, and between treatment groups as a function of season. To assist with visualizing our data, we fit vector arrows that represent significant correlations between predictor variables and NMDS scores. Prior to analyses, we removed outliers beyond 1.5 times the interquartile range to reduce noise in our data. We tested for violations of sphericity, homogeneity of variance, and normality using Mauchly’s tests, Fligner-Killeen tests, and Shapiro tests, respectively. Data that did not meet assumptions were log transformed (Net C mineralization, Net N mineralization, DOC:inorganic N, DOC:PO_4^{3-}, inorganic N:PO_4^{3-}, ΔDOC:inorganic N, DOC, NO_3^-, NH_4^+, PO_4^{3}). For ease of interpretation, means were back transformed to their original units in figures. We used two-way repeated measures analysis of variance (RMANOVA) followed by a Tukey's HSD post-hoc test to determine if season, treatment, or a season by treatment interactions affected pools and fluxes of C, N, and P. To answer our second question (i.e., do coupled cycles of C, N, and P change in response to season or to treatment?), we explored relationships between response variables using Spearman's rho rank correlation coefficients.

Results

Pretreatment results

Prior to fuels treatments, Porter canyon and Dalton canyon had similar vegetative species composition, but Porter Canyon had higher densities of trees, shrubs, and herbaceous vegetation than Dalton Canyon. Soil physical characteristics and soil available N concentrations were similar among sites (Table 1; Figure 2).

Post-treatment results

To determine the seasonal responses of C, N, and P pools and fluxes to OM addition from woodland reduction we compared pools and fluxes of C, N, and P between soils sampled from plots within the zone of influence of an intact piñon pine (control plots), a cut-and-lopped tree (lop plots), and masticated debris (mastication plots) in June, August, and November. Seasons were represented by the month when we sampled soil. Soil conditions were warm and wet in June, warm and dry in August, and cool and dry in November (Tables 2 and 3). Pools and fluxes of C, N, and P, soil VWC, and soil temperature were significantly different among seasons and treatments, but the effect of treatment did not vary by season. All pools and fluxes were significantly associated with seasons and treatments with the exception of net ammonification (Figure 3A and 3B).

Seasonal changes in C, N and P pools and fluxes in control plots

In control plots under intact trees, all C, N, and P pools and fluxes varied seasonally (Tables 5, 7), but not always in consistent ways (Tables 2-3). For example, in control plots, DOC concentrations were 1.6 times higher in August than in June and 1.9 times higher in August than in November (Table 2, Figure 4; August = 91.1 mg kg^{-1}; June = 58.3 mg kg^{-1}; November = 47.3 mg kg^{-1}), mean soil NH_4^+, NO_3^-, and inorganic N, and C_{mic} concentrations were higher in June than in August and November. Ammonium levels in June exceeded levels in August and November by a factor of 4.9 and 2.4, respectively (Table 2, Figure 4; June = mg kg^{-1} 3.6; August = 0.74 mg kg^{-1}; November = 1.5 mg kg^{-1}). Nitrate concentrations were 3.6 and 1.7 times higher in June than in August and November (Table 2, Figure 4; June = 0.5 mg kg^{-1}; August = 0.14 mg kg^{-1}; November = 0.3 mg kg^{-1}). Taken together, inorganic N concentrations in June exceeded concentrations in August by a factor of 4.7 and exceeded concentrations in November by a factor of 2.3 (Table 2; June = 4.1 mg kg^{-1}; August = 0.88 mg kg^{-1}, November = 1.8 mg kg^{-1}). Soil phosphate concentrations were similar in all seasons (Table 2, Figure 4; June = 23.5 mg kg^{-1}; August = 24.5 mg kg^{-1}; November = 20.5 mg kg^{-1}). Microbial biomass C pools were 1.2 to 1.7 times greater in June than in August and November (Table 2; June = 178.6 mg kg^{-1}; August = 149.8 mg kg^{-1}; November = 102.7 mg kg^{-1}).

Control soil DOC declined more during the seven-day incubations in August and June than in November (Table 3; August ΔDOC = -18.4 mg kg^{-1}, June ΔDOC = -18.0 mg kg^{-1}) and ΔC_{mic} increased most in November, decreased most in August, and was intermediate in June (November ΔC_{mic} = 50.7 mg kg^{-1}; August ΔC_{mic} = -134.6 mg kg^{-1}; June ΔC_{mic} = -26.6 mg kg^{-1}). Net C mineralization, net ammonification, net nitrification, and net N mineralization were highest in June when soil conditions were warm and wet. Microbes respired 11.3 times more CO_2 in June than in August and 3.7 times more CO_2 in June than in November (Table 3; June = 102.0 mg kg^{-1}; August = 9.0 mg kg^{-1}; November = 27.6 mg kg^{-1}). Net ammonification was positive in June, but negative in August and November (Table 3; June = 0.7 mg kg^{-1}; August = -0.002 mg kg^{-1}; November = -0.24 mg kg^{-1}). Net nitrification was 4.6 times higher in June than in November and nitrate was immobilized in August (Table 3; June = 5.5 mg kg^{-1}; November = 1.2 mg kg^{-1}; August = -0.03 mg kg^{-1}). Net N mineralization was 6.9 times higher in June than in November and on average, inorganic N was immobilized in August (Table 3; June = 6.2 mg kg^{-1}; November = 0.9 mg kg^{-1}; August = -0.028 mg kg^{-1}). Net N_2O flux was 57 times and 5.1 times greater in June than in August and November, respectively (June = 5.1 μ kg^{-1}; August = 0.09 μ kg^{-1}; November = 1.0 μ kg^{-1}). Net P mineralization was 34 times greater in June than in August and on average, was negative in November (June = 3.4 mg kg^{-1}, August = 0.1 mg kg^{-1} ; November = -0.1 mg kg^{-1}). At the end of the August incubation, estimated soil organic C flux was negative but was positive in June and

November, respectively (Table 3; August = -144.0 mg kg^{-1}; June = 57.4 mg kg^{-1} ; November = 75.7 mg kg^{-1}).

Coupled pools of soil DOC:available N, and DOC:PO_4^{3-} varied seasonally, but soil available N:PO_4^{3-} was unaffected (Table 8). The ratio of soil DOC:available N was highest in August intermediate in November and lowest in June (Table 4; August = 425.6 mg kg^{-1}; November = 112.8 mg kg^{-1}; June = 14.9 mg kg^{-1}). Season did not change coupled fluxes (Table 8).

Effects of OM addition from woodland reduction on C, N and P pools and fluxes

Disturbance from OM inputs impacted all pools of C, N, and P (Table 5). The most notable changes included lower soil NH_4^+ concentrations in mastication plots (mean = 0.86 mg kg^{-1}) compared to control plots (mean = 1.95 mg kg^{-1}), with lop plots being intermediate (mean = 1.33 mg kg^{-1}), and concentrations of soil NO_3^- that were approximately 9.5-10.9-fold higher in mastication plots (mean = 2.93 mg kg^{-1}) than in control (mean = 0.31 mg kg^{-1}) and lop plots (mean = 0.27 mg kg^{-1}; Table 2). Lop plots had lower concentrations of PO_4^{3-} (mean = 15.9 mg kg^{-1}) than control (mean = 22.8 mg kg^{-1}) or mastication plots (mean = 27.9 mg kg^{-1}), which had similar PO_4^{3-} concentrations (Table 2). Microbial biomass C pools were consistently lower in mastication plots (mean C_{mic}: 96.0 mg kg^{-1}) compared to control (mean C_{mic}: 143.7 mg kg^{-1}) and lop plots (mean C_{mic}: 100.3 mg kg^{-1}; Table 2). Only ΔC_{mic} and estimated SOC flux changed due to OM addition (Table 6) but consistent differences independent of season are not apparent (Table 3).

The only coupled pool that was altered by OM addition from woodland reduction was soil DOC:PO_4^{3-} (Table 8), which was lowest in mastication plots (mean = 2.3 mg kg^{-1}), highest in lop plots (mean = 5.0 mg kg^{-1}), and intermediate in control plots (mean = 3.6 mg kg^{-1}; Table 4). Disturbance from woodland reduction did not change coupled fluxes of C, N, and P (Table 8).

Effect of season by treatment interaction on pools and fluxes of C, N, and P

Of the pools we measured, only soil NH_4^+ and C_{mic} did not have a significant season by treatment interaction (Table 5). Otherwise, notable interactions included the difference in soil NO_3^- which increased from June through November in soils in mastication plots (June = 1.0 mg kg^{-1}; August = 3.1 mg kg^{-1}; November = 4.7 mg kg^{-1}), but was unaltered in control plots (June = 0.5 mg kg^{-1}; August = 0.14 mg kg^{-1}; November = 0.3 mg kg^{-1}) and lop plots (June = 0.4 mg kg^{-1}; August = 0.2 mg kg^{-1}; November = 0.4 mg kg^{-1}), and soil PO_4^{3-} that was higher in mastication plots than in control or lop plots in June (June: mastication = 35.3 mg kg^{-1}; control = 23.5 mg kg^{-1}; lop = 24.5 mg kg^{-1}) and November (November: mastication = 31.5; control = 20.5 mg kg^{-1}; lop = 15.9 mg kg^{-1}), and highest in control plots, intermediate in mastication plots, and lowest in lop plots in August (August: control = 24.5 mg kg^{-1}; mastication = 17.0 mg kg^{-1}; lop = 7.4 mg kg^{-1}; Table 2).

Of the fluxes we measured during the incubations, ΔC_{mic}, net C mineralization, estimated SOC flux, and net N_2O flux had significant season by treatment interactions (Table 6-7). Over the course of the June incubation, C_{mic} increased substantially in lop plots, but decreased in control and mastication plots (lop = 19.5 mg kg^{-1} ; control = -26.6; mg kg^{-1} mastication = -11.6 mg kg^{-1} ;), whereas C_{mic} decreased in all treatments in

August incubations (control = -134.6 mg kg^{-1} ; lop = -32.0 mg kg^{-1} ; mastication = -21.6 mg kg^{-1}), and in November incubations, C_{mic} increased in all treatments (control = 50.7 mg kg^{-1} ; mastication = 43.0 mg kg^{-1} ; lop = 49.6 mg kg^{-1} ; Table 3). Net C mineralization during June was roughly twice as high in control plots as mastication plots, with lop plots intermediate (control = 102.0 mg kg^{-1} ; mastication = 52.0 mg kg^{-1} ; lop = 62.3 mg kg^{-1}), whereas mastication plots had higher mean net C mineralization than control or lop plots in August (mastication = 13.6 mg kg^{-1} ; control = 9.0 mg kg^{-1} ; lop = 8.9 mg kg^{-1}), and lop plots had higher mean net C mineralization than control or mastication plots in November (lop = 43.4 mg kg^{-1} ; control = 27.6 mg kg^{-1} ; mastication = 28.5 mg kg^{-1} ; Table 3). In August, estimated soil organic C was more negative in control plots than lop or mastication plots (control = -144.0 mg kg^{-1} ; lop = -32.2 mg kg^{-1}; mastication = -16.9 mg kg^{-1}). The ratio of DOC:inorganic N in August was 6.3 and 20 times higher in control plots compared to lop and mastication plots, respectively (control = 425.6 mg kg^{-1}; lop = 67.2 mg kg^{-1}; mastication = 21.2 mg kg^{-}). The ratio of inorganic N:PO_4^{3-} was 1.7 times lower in control plots than lop plots and 3.3 times lower than mastication plots in August (control = 0.06 mg kg^{-1}; lop = 0.1 mg kg^{-1}; mastication = 0.2 mg kg^{-1}). The interaction of season and treatment did not impact coupled fluxes of C, N, and P.

Seasonal correlations among soil characteristics under different OM inputs

In June, the warm and wet season, pools of net C mineralization were positively correlated with pools of NH_4^+, inorganic N, and PO_4^{3-}. Microbial biomass C was negatively correlated with net ammonification. Soil DOC: inorganic N was positively correlated with ΔC_{mic} and ΔDOC was negatively correlated with net P mineralization (Supplementary Figure 1). In lop plots in June, DOC and PO_4^{3-} were positively correlated with net C mineralization which was positively correlated with net nitrification and NH_4^+, inorganic N, and PO_4^{3-} were negatively correlated with ΔDOC (Supplementary Figure 2). In mastication plots in June, inorganic N was negatively correlated with ΔDOC, positively correlated with net P mineralization, and negatively correlated with ΔDOC:PO_4^{3-}, which was positively correlated with net ammonification, and estimated SOC flux was positively correlated with net N_2O flux, (Supplementary Figure 3).

In control plots in August, estimated SOC flux and ΔC_{mic} were negatively correlated with net N mineralization, soil VWC was positively correlated with estimated SOC flux and net N_2O flux and soil temperature was positively correlated with ΔC_{mic} and negatively correlated with net N_2O flux (Supplementary Figure 4). In lop plots in August, ΔDOC was positively correlated with inorganic N, net C mineralization was negatively correlated with soil PO_4^{3-} and net ammonification, net nitrification was positively correlated with soil PO_4^{3-}, net N mineralization was negatively correlated with soil PO_4^{3-} and net C mineralization, and inorganic N:PO_4^{3-} was negatively correlated with ΔDOC, ΔC_{mic}, and estimated SOC flux. The change in inorganic N:PO_4^{3-} was positively correlated with ΔDOC and soil temperature was positively correlated with net N_2O flux in lop plots in August (Supplementary Figure 5). In mastication plots in August, PO_4^{3-} and ΔDOC were negatively correlated with ΔC_{mic}, net nitrification and net N mineralization were positively correlated with soil PO_4^{3-}, and C_{mic} pools were positively correlated with net P mineralization (Supplementary Figure 6).

In control plots in November, soil PO_4^{3-} was positively correlated with ΔDOC, ΔC_{mic}, and estimated SOC flux, soil inorganic N was positively correlated with net C mineralization, and soil VWC was positively correlated with net ammonification and net N_2O flux (Supplementary Figure 7). In lop plots in November, inorganic N was positively correlated with estimated SOC flux, and $\Delta DOC{:}PO_4^{3-}$, estimated SOC flux was positively correlated with net ammonification, and soil temperature was positively correlated with net P mineralization (Supplementary Figure 8). In masticated plots in November, soil NO_3^- was positively correlated with ΔC_{mic}. soil DOC was positively correlated with net N mineralization, soil PO_4^{3-} was positively correlated with ΔC_{mic}, estimated SOC flux, net N_2O and Net C mineralization, and soil moisture was negatively correlated with net P mineralization (Supplementary Figure 9).

Discussion

Our results demonstrate that OM addition from woodland reduction impacts microbial substrates and C dynamics. Microbial functions that regulate nutrient dynamics were responsive to intra-annual seasonal variation but were resilient to disturbance from woodland reduction. These findings suggest that water availability and temperature may constrain microbial functions to a greater extent than disturbances that alter their resource substrates. Our findings contribute to our understanding of biogeochemical cycling in drylands, provide insight into the response of these ecosystems to disturbance and intra-annual seasonal variability, and have implications for management of piñon and juniper encroached sagebrush ecosystems in the Great Basin.

C, N, and P in untreated controls under warm and wet soil conditions in June

Higher concentrations of NH_4^+, NO_3^-, and greater net nutrient fluxes, and net C mineralization were observed in June (near the peak of the growing season) compared to August or November. This is consistent with well-established theories in soil biogeochemistry that suggest that soil moisture regulates microbial functions and promotes resource availability for plants and microbes (Stark and Firestone 1995; Schimel et al. 2007; Bailey et al. 2017; Tecon and Or 2017; Schimel 2018). Further, several aspects of our data point to evidence of C-limitation of microbial activity during the peak growing season when soil conditions were moist (June). First, rates of net N mineralization, net N_2O efflux, and P mineralization that were higher in June than in other seasons suggest that inorganic N and PO_4^{3-} exceeded microbial stochiometric requirements. Second, the soil DOC:available N ratio that was lowest in June, was positively correlated to net ΔC_{mic} (Supplementary Table 1), but net ΔC_{mic} declined the most in June (Table 3), indicating that microbes may have had more access to inorganic N to maintain their stoichiometry than DOC. In addition, the negative correlation between net PO_4^{3-} mineralization and ΔDOC may further substantiate C-limitation of microbial activity but is particularly interesting because a longstanding paradigm in soil biogeochemistry suggests that P cycles independently of C (McGill and Cole 1981); however, more recent research in humid ecosystems has shown that C-limitation may drive mineralization of P (Spohn and Kuzyakov 2013; Heuck et al. 2015; Spohn et al. 2015; Wang et al. 2016).

In June control plots, we found that, on average, ΔDOC was negative, net P mineralization was positive, and that there was a negative correlation between the two fluxes (Supplementary Table 1; Supplementary Figure 1). The McGill and Cole (1981) model suggested that cycles of C and P are decoupled because microbes can bypass oxidation of C when they need P by producing extracellular phosphatases that cleave P from ester bonds (P driven mineralization). Our results corroborate more recent research in mesic ecosystems that show that mineralization of organic P can be driven by microbial C-limitation (C-driven mineralization), thus coupling cycles of C and P. More recent research suggests that phosphatase is not only key to mineralization of P from ester bonds, but that dephosphorylation mediated by phosphatase may also play a part in C acquisition (Spohn et al. 2015). Our research in a semiarid ecosystem suggests that cycles of C and P are linked when soil conditions are warm and wet, but further investigation is required to determine how P is linked to C. Tracer experiments by Spohn and Kuzyakov (2013) and Heuck et al. (2015) used isotopically labeled glucose-6-phosphate (^{14}C and ^{33}P) to show that P_o mineralization can be driven by microbial need for C in temperate forests. Conducting a similar study in PJ woodlands may be able to confirm our finding that microbial P cycling is limited by C during the warm and wet season. Taken together these results substantiate evidence of C-limitation of microbial activity in June and highlight the need for a closer examination of C and P dynamics in semiarid ecosystems. In addition, our findings may suggest that microbes in this ecosystem may be capable of adjusting their P acquisition strategy in times of C-limitation and that disturbances that impact the quantity or quality of C inputs to soil may have implications for the supply of P available to plants.

C, N, and P in lop plots under warm and wet soil conditions in June

Lop plots were similar to control plots in that warm and wet soil conditions promoted resource availability and microbial activity, but we observed differences in relationships between net C mineralization and nutrients between treatments. Microbial respiration of CO_2 is a byproduct of enzyme production (R_e), maintenance (R_m), or growth (R_g) (Schimel and Weintraub 2003). When microorganisms decompose soil organic C (SOC), nutrient availability regulates whether the resulting DOC is partitioned between biomass or respiration (Poeplau et al. 2016; Soong et al. 2018). In June, lop plots were different than control plots in that, changes in inorganic N availability did not significantly affect net C mineralization, but microbes were more responsive to concentrations of DOC (Supplementary Table 1 and 2). The negative correlation we observed between ΔDOC and NH_4^+ and ΔDOC and available N in June may indicate N-limitation of enzyme production and as a result, N-limitation of SOC decomposition (Supplementary Table 2). When more N was available (NH_4^+ and/or inorganic N), more DOC was available for R_e, R_m, and R_g. We observed a substantial increase in ΔC_{mic} in lop plots relative to control or mastication plots during the warm and wet season (Table 2), and it may be that when available, a considerable amount of DOC was partitioned to ΔC_{mic}.

C, N, and P in masticated plots under warm and wet soil conditions in June

Unlike soils in control and lop plots, there were no relationships between microbial resource substrates and net C mineralization (Supplementary Tables 1, 2, 3), but interesting relationships existed between C and N. The higher net production of nitrous oxide we observed in June (relative to control and lop plots) was positively correlated with estimated SOC flux (Supplementary Table 3). Nitrification and denitrification are indicators of excess N availability, but denitrification requires a source of DOC for energy while nitrification is performed by chemoautotrophs (Sullivan et al. 2012). The relationship between N_2O production and estimated SOC flux may indicate that denitrification was occurring where soil conditions were anaerobic.

C, N, and P in untreated controls under warm and dry soil conditions in August

Lower concentrations of inorganic N in the dry season (August) compared to the wet season (June) contradicts research in other ecosystems. A meta-analysis of drought manipulation studies across biomes demonstrated that inorganic N typically accumulates with decreased precipitation (Homyak et al. 2017). Non-manipulative studies specific to other semiarid ecosystems also show that inorganic N concentrations accumulate in seasons with dry soil conditions. Even when soil conditions are dry in the summer, inorganic N can accumulate because it is mineralized hydrologically disconnected microsites; therefore, diffusion of inorganic N to plant roots or microbes is limited (Parker and Schimel 2011; Homyak et al. 2014; Schaeffer et al. 2017). Discrepancies between our observations and those in other semiarid ecosystems could be attributed to several mechanisms including differences in plant functional group, differences in precipitation regime and growing season, soil taxonomical differences between our study site and the sites of others, or losses of N as N_2O via nitrification or denitrification. For example, Great Basin PJ woodlands have a peak growing season in the spring and are dominated by perennial vegetation (Comstock and Ehleringer 1992) whereas California grasslands favor annual vegetation, the predominant growing season is in winter (Silver et al. 2010; Schaeffer et al. 2017), and above-and belowground OM inputs and nutrient uptake from living vegetation are nearly non-existent during the dry season (Parker and Schimel 2011; Schaeffer et al. 2017). In both ecosystems, diffusion of resources may be low when soil conditions are dry (Stark and Firestone 1995), but competition for inorganic N between soil microorganisms and plants may persist in Great Basin PJ woodlands even in times of low soil moisture if plants and microbes are both still metabolically active. Dormancy of vegetative growth is known to occur in many Great Basin species during the dry season, but plants may still be metabolically active because episodic precipitation allows for the continuation of root growth (Hodgkinson et al. 1978; Comstock and Ehleringer 1993) and increased reproduction (Evans et al. 1991; Comstock and Ehleringer 1993). Specific to *Artemisia* species (an evergreen plant genus of concern in the Great Basin that reproduces midsummer), carbohydrates necessary for filling reproductive structures are derived from inflorescences and photosynthate from vegetation supports root growth during the dry season (Comstock and Ehleringer 1993). In addition, research has shown that microbial growth may persist under conditions of low soil moisture within water films adsorbed to soil particles, albeit slowly (Parker and Schimel 2011; Sullivan et al. 2012; Schaeffer et al. 2017). If perennial plants are metabolically active in the dry season, root exudation of a labile C substrate like DOC rather than aboveground litter inputs of more recalcitrant material may continue to support microbial activity. While these differences between our study sites and the sites of others could potentially contribute to the mechanisms underlying inorganic N availability, it is difficult to suggest a definitive mechanism having inorganic N measurements from one time point.

Dissolved organic C is derived from live plant inputs, degraded plant matter, and microbial processes (Neff and Asner 2001). In August, soil DOC pools were 1.6 times larger than those in June and 1.9 times larger than those in November (Table 2). Our findings are consistent with those of Schaeffer et al. (2017) who showed increased DOC concentrations when soils were dry. Still, the differences between our study site and the grassland site in Schaeffer et al. (2017) necessitate an assessment of the potential mechanisms behind DOC accumulation under dry soil conditions specific to our study site. More than one mechanism may play a role in the change in DOC pool size that we observed. First, soil moisture limits microbial activity before it limits enzyme activity (Schaeffer et al. 2011). As a result, depolymerization of SOM to DOC can persist under conditions of low soil moisture assuming that substrate is available for enzyme activity. Second, limitation of substrate diffusion under dry soil conditions may limit the capacity of microbes to capitalize on accumulated soil DOC (Schaeffer et al. 2017).

Prior to the incubation 84% of the inorganic N pool was comprised of NH_4^+ and at the end of the incubation 87% of the inorganic N pool was comprised of NH_4^+ (Table 2, Table 3). This makes sense because higher concentrations of DOC would favor heterotrophic microorganisms that rely on soluble C sources over ammonia oxidizing chemotrophs (Booth et al. 2005). Thus, it is likely that higher DOC concentrations in August (compared to other seasons) promoted formation of NH_4^+ because DOC was required for the energy and enzyme production necessary to liberate NH_4^+ from organic N (Schimel and Weintraub 2003). Yet, on average soil microorganisms assimilated (negative flux) inorganic N (Table 3), meaning that microbial metabolism likely required inorganic N and that there was enough inorganic N available to promote a positive ΔC_{mic} at the end of the incubation (Table 3).

C, N, and P in mastication plots under warm and dry soil conditions in August

In both August and November, over 90% of the inorganic N pool was NO_3^- (Table 2). Findings from previous studies are not always consistent with ours but site-specific and/or methodological differences complicate comparisons between results. Inconsistent with our findings, research on management of expanding PJ woodlands in southwestern Colorado conducted in August by Owen et al. (2009) showed that mastication treatments increased soil NH_4^+ concentrations and did not change NO_3^- concentrations, and research by Overby and Gottfried (2017) showed no change to inorganic N due to OM input from mastication treatments. Yet, comparisons between our findings and theirs are confounded by differences in precipitation regime because summers in southwestern Colorado are characterized by monsoonal moisture. In addition, Overby and Gottfried (2017) noted that their results may be confounded by a mass tree die off that occurred prior to mastication treatment. Research by Young et al. (2014) in a juniper woodland in the Great Basin showed similar results to ours which they attributed to reduced uptake of inorganic N by trees and increased soil moisture after mastication treatments. However, they did not partition inorganic N species, and differences in mycorrhizal associations among piñon trees (ectomycorrhizal fungi; EMF) and juniper trees (arbuscular mycorrhizal fungi; AMF) may impact nutrient dynamics (Gehring et al. 2017) because

EMF and AMF associations can cause differences in litter quality among hosts (Phillips et al. 2013).

We interpret our results similarly to the interpretation of research by Young et al. (2014) but we extend our interpretation to include mechanisms behind nitrate being the dominant N species. Removal of vegetation may release soil microorganisms from competition with plants for nutrients (Kaye and Hart 1997) and OM on the soil surface can ameliorate soil moisture (Young et al. 2013; Overby and Gottfried 2017) thus promoting microbial activity to some extent (Stark and Firestone 1995; Schimel et al. 2007; Bailey et al. 2017; Tecon and Or 2017; Schimel 2018). Diffusional limitation may have limited access to DOC, but microbes may have been stuck within water films where NH_4^+ was bound to soil particles, allowing nitrification to persist even in times of low soil moisture (Parker and Schimel 2011; Sullivan et al. 2012; Schaeffer et al. 2017). While removing vegetation may have enhanced nutrient availability, inputs of labile C sources from living plant roots would have also been reduced. Research has shown that nitrification increases under microbial C limitation because the coupled cycling of C and N can lead to mineralization of NH_4^+, a substrate for nitrifiers, when microbes need C and N is in excess (Chen and Stark 2000).

C, N, and P in all plots under cool and dry soil conditions in November

A noteworthy difference between our incubations in November and incubations in June and August may have confounded our findings. In November, samples underwent drastic fluctuations in temperature. The mean seven-day antecedent soil temperature was -3.2° C

(26° F), the soil temperature at the time of sampling was 0.65° C (33.2° F), soils were brought to an air temperature of -11.1° C (12° F) for approximately three hours, brought to an air temperature of 20° C (68° F) for approximately 12 hours, then were incubated at 20° C (68° F) for seven days. Q_{10} incubation studies in temperate systems have demonstrated that increases in temperature stimulate microbial activity (Cheng et al. 2015, Zhang et al. 2019), thus our results from our November incubation should be taken with caution.

Implications for management

Soils are the foundation of terrestrial ecosystems. Understanding how management may alter the soil environment is important for making decisions that will meet treatment objectives. A primary ecological concern with woodland reduction is the impact on the response of vegetation after treatment. Organic matter additions from woodland reduction treatments altered soil nutrient availability and C, N, and P stoichiometry. The supply and proportion of these belowground resources constrain microbial processes. The impacts of woodland reduction on soil physical and chemical characteristics could facilitate conditions that favor invasion by non-native species, leading to unintended plant community trajectories, the persistence of soil legacies that promote reestablishment of piñon and juniper trees, overall losses of soil C, N, and P from the system, or impact the resilience of emerging plant species by facilitating changes to C allocation strategies in plants.

Disturbance that reduces intraspecific competition for resources between plants and/or interspecific competition for resources between plants and soil microbes may promote the establishment and dominance of invasive plant species (Grime 1979; Davis et al. 2000). Phenological variation exists between cheatgrass and native perennial vegetation with cheatgrass often getting a head start on establishment by germinating before other vegetation in the fall or early spring (Thill et al. 1979; Aguirre and Johnson 1991) reducing resources for native species. Successful establishment of cheatgrass is responsive to increased soil moisture (Link et al. 1995), and to elevated levels of inorganic N (Lowe et al, 2002; Roundy et al. 2007), particularly NO_3^- (Chambers et al. 2007). Invasion by cheatgrass often occurs in soils subjected to mechanical tree removal (Chambers et al. 2014; Roundy et al. 2014; Flake and Weisberg 2021). Changes in soil characteristics caused by disturbance from mastication in our site may facilitate soil conditions that increase the competitive advantage of cheatgrass by extending the duration of soil moisture, availability of soil NO_3^- in the fall, and soil PO_4^{3-} in the spring and fall.

Soil legacies of dominating plant communities may carry long-term and unforeseen consequences. Two common strategies for nutrient acquisition by vegetation are symbioses with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF). Piñon trees are a host for EMF while juniper trees and understory vegetation, including sagebrush, are hosts for AMF, obligate symbionts (Pepe et al. 2018). Ectomycorrhizal fungi mineralize nutrients from OM, while AMF scavenge for nutrients released by saprotrophic microbes. The potential for recovery of native perennial vegetation on soils previously occupied by piñon trees may be difficult if microbial communities associated with piñon trees persist. Research in PJ woodlands nine years after a large-scale tree mortality showed that sites that were once dominated by piñon left behind a legacy of EMF inoculum in the soil that was comparable to inoculum levels in living woodlands, but that EMF diversity and community composition changed (Mueller et al. 2019). Not all EMF species have long-lasting propagules that can survive in the absence of a host (Bruns et al. 2009), as a result, disturbance could potentially impact the functional diversity of EMF dormant in the soil (Mueller et al. 2019). By contrast, research 2.5 years after removal of piñon and juniper trees using a mastication treatment showed that AMF spore abundance was comparable between untreated and treated sites, but that AMF diversity declined significantly in mastication sites compared to untreated sites (Owen et al. 2009). The potential for persistence of EMF inoculum following disturbance may contribute to the reestablishment and survival of piñon pines (Gehring et al.1998; Mueller et al. 2019), while diversity losses of AM, which are associated with 80% of terrestrial plants (Phillips et al. 2013) may have consequences for the establishment of native perennial vegetation.

Some research in drylands suggests that photodegradation may exert a strong control on decomposition of aboveground litter with C inputs from aboveground primary productivity being lost to the atmosphere as inorganic C before circulating through the SOM pool (Austin and Vivanco 2006). Redistribution of standing live vegetation to masticated OM spread across the soil surface increases the surface area directly exposed to photodegradation. Compared to biotic decomposition, photodegradative decomposition may have a stronger control on decomposition in areas where masticated OM was distributed within former intercanopy spaces where microbial activity is presumably low (Gonzales-Polo and Austin 2009; Austin 2011; Cable et al. 2009; Maestre et al. 2009). Evidence suggests that decomposing soil microorganisms are specialized at degrading litter specific to the area they inhabit (Ayres et al. 2009; Austin et al. 2014). The potential for C loss may be greater within former intercanopy zones if the microbial community is not adapted to decomposing the recalcitrant OM associated with mastication. Further, research in expanding PJ woodlands has shown that half of the existing SOM under intact PJ woodland canopies is in non-mineral associated organic fractions that are prone to destabilization. Thus, disturbance from mastication could also trigger the loss of previously stabilized soil C and N (Neff et al. 2009).

As previously mentioned, soil NO_3^- accumulation can be a sign of microbial C limitation because low demand for N can lead to ammonification of NH_4^+ during decomposition of SOM providing a substrate for nitrification (Chen and Stark 2000). The solubility of NO_3^- can facilitate plant uptake when vegetation is intact, but when vegetation is inactive, damaged, or removed and paired with sufficient soil moisture, leaching can lead to losses of N from the system (Manzoni et al. 2019). This is because pulses of N mineralization associated with episodic precipitation in semiarid ecosystems may occur at times when plants are unable to capitalize on the resource, decoupling N mineralization from plant uptake and possibly increasing abiotic losses of N (Austin et al. 2004; Augustine and McNaughton 2004; Dijkstra et al. 2012).

Following disturbance, plants allocate new growth to the tissues needed to acquire the most limiting nutrients (Chapin 2011). Research has demonstrated that plants may adjust their patterns of aboveground and belowground allocation when soil inorganic N concentrations are high, potentially shifting allocation from root biomass or adjusting the extent of mycorrhizal colonization in favor of shoot growth (Allen et al. 2010; Chapin

2011). Changes to patterns of aboveground and belowground allocation may have implications for the resilience of species to drought conditions (Allen et al. 2010).

Future directions

We acknowledge that incubation studies do not represent field conditions and that measurements of net fluxes do not account for all the turnover that may have occurred over a seven-day incubation. Future research should include use of *in situ* methods to obtain gross fluxes of C, N, and P to determine if our findings hold under field conditions. To better understand the impacts of woodland reduction on soil processes over longer timescales, we recommend that future studies elucidate the relationships between C, N, and P in multiple sites over a range of times since woodland reduction. We suggest that future research examines the microbial communities present in piñon and juniper encroached sagebrush ecosystems. It is possible that encroaching trees (specifically piñon trees that have symbioses with EMF) could change the microbial community to benefit their establishment at the expense of other species. One idea is to evaluate the effect of mycorrhizae on establishment and survival of both EMF associated species such as piñon trees and AMF associated species such as sagebrush using hyphal exclusion experiments (Liang et al. 2020).

Table 1. Site descriptions of Porter Canyon and Dalton Canyon watersheds. Plants are shown as density. Abbreviations: Mean annual precipitation = MAP, mean annual temperature = MAT.

Site	Latitude/ Longitude	Elevation (m)	MAP (mm)	MAT (°C)	Soil classification[2]	Soil texture	% Sand	% Silt	% Clay	Piñon+ juniper (#ha^{-1})	Sagebrush (#m^{2})	Herbaceous Plants (#m^{2})
Porter Canyon	N39°27’47.0016” W-117°37’18.7”	2236	305	8.9	Lithic Argixerolls	Loam	38	48	14	469	0.5	1.2
Dalton Canyon	N39°27’36.2” W-117°32.9”	2243	278	8.1	Lithic Argixerolls	Loam	36	48	16	308	0.04	0.4

[1] 30-y normal from PRISM Climate Group (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 5 April 2021).

[2] From Web Soil Survey (Soil Survey Staff, 2021).

Table 2. Surface (0-10 cm) mineral soil carbon (C), nitrogen (N), and phosphorus (P) pools and seven-day antecedent soil temperatures and volumetric water contents in control, lopped, and masticated treatments in different seasons represented by the month when we sampled soil (June = warm and wet, August = warm and dry, November = cool and dry). Values are means ± standard error. Abbreviations: DOC = dissolved organic C, NH_4^+= ammonium, NO_3^- = nitrate, PO_4^{3-} = phosphate, C_{mic} = microbial biomass C, VWC = volumetric water content. Temperature and VWC values are seven-day antecedent means prior to sampling date.

	June			August			November		
Concentrations (mg kg^{-1})	Control	Lopped	Masticated	Control	Lopped	Masticated	Control	Lopped	Masticated
DOC	58.3 ± 12.2	32.1 ± 1.0	49.1 ± 2.7	91.1 ± 19.7	30.5 ± 3.2	43.4 ± 4.4	47.3 ± 5.5	38.0 ± 2.0	57.2 ± 3.8
NH_4^+	3.6 ± 0.8	2.9 ± 0.4	1.9 ± 0.2	0.74 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	1.5 ± 0.6	0.6 ± 0.1	0.3 ± 0.1
NO_3^-	0.5 ± 0.1	0.4 ± 0.1	1.0 ± 0.2	0.14 ± 0.1	0.2 ± 0.04	3.1 ± 0.5	0.3 ± 0.1	0.4 ± 0.1	4.7 ± 1.1
Inorganic N	4.1 ± 0.9	3.3 ± 0.4	2.9 ± 0.3	0.88 ± 0.3	0.7 ± 0.1	3.4 ± 0.5	1.8 ± 0.6	1.1 ± 0.1	5.0 ± 1.1
PO_4^{3-}	23.5 ± 5.5	24.5 ± 2.2	35.3 ± 2.1	24.5 ± 6.1	7.4 ± 0.6	17.0 ± 1.5	20.5 ± 4.2	15.9 ± 1.7	31.5 ± 2.6
C_{mic}	178.6 ± 110.5	106.7 ± 14.1	98.5 ± 14.2	149.8± 44.0	110.1±18.8	101.3 ± 8.6	102.7 ± 26.7	84.2 ± 10.3	88.3 ± 15.7
Temperature (°C)	12.0 ± 0.9	10.9 ± 0.6	12.7 ± 0.34	20.8 ± 0.57	20.2 ± 0.29	20.7 ± 0.47	-3.2 ± 0.64	-5.5 ± 0.34	2.1 ± 0.4
VWC (%)	16.3 ± 0.02	17.6 ± 0.01	21.3 ± 0.01	5.4 ± 0.01	7.0 ± 0.004	12.1 ± 0.01	4.5 ± 0.01	7.5 ± 0.01	11.1 ± 0.01

Table 3. Surface (0-10 cm) mineral soil carbon (C), nitrogen (N), and phosphorus (P) net fluxes over seven days in control, lopped, and masticated treatments in different seasons represented by the month when we sampled soil (June = warm and wet, August = warm and dry, November = cool and dry). Abbreviations: DOC = dissolved organic C, C_{mic} = microbial biomass C, N_2O = nitrous oxide. Values are means ± standard error.

	June			August			November		
Concentrations (mg kg^{-1})	Control	Lopped	Masticated	Control	Lopped	Masticated	Control	Lopped	Masticated
ΔDOC	-18.0 ± 5.7	-7.1 ± 1.1	-16.4 ± 2.9	-18.4 ± 10.2	-9.1 ± 2.4	-8.9 ± 2.4	-2.6± 2.0	-7.2 ± 1.2	0.59 ± 3.4
Δ C_{mic}	-26.6 ± 80.7	19.5 ± 31.4	-11.6 ± 19.7	-134.6 ± 87.0	-32.0 ± 46.2	-21.6 ± 52.3	50.7± 33.9	49.6 ± 18.5	43.0 ± 16.4
Net C mineralization	102.0 ± 27.6	62.3 ± 5.7	52.0 ± 5.8	9.0 ± 1.5	8.9 ± 1.8	13.6 ± 2.0	27.6± 3.1	43.4 ± 4.2	28.5 ± 3.5
Estimated soil organic C flux	57.4 ± 75.0	74.7 ± 35.1	24.1 ± 20.6	-144.0 ± 81.2	-32.2 ± 47.4	-16.9 ± 52.4	75.7± 36.6	85.8 ± 18.5	72.0 ± 15.6
Net ammonification	0.7 ± 2.8	-0.3 ± 0.6	-0.9 ± 0.3	-0.002 ± 0.3	-0.16 ± 0.1	-0.02 ±0.1	-0.24± 0.7	-0.2 ± 0.1	0.4 ± 0.3
Net nitrification	5.5 ± 1.3	3.1 ± 0.5	3.0 ± 0.6	-0.03 ± 0.04	0.05 ± 0.03	1.1 ± 0.9	1.2± 0.4	0.8 ± 0.2	1.4 ± 0.8
Net N mineralization	6.2 ± 3.7	2.8 ± 0.7	2.1 ± 0.7	-0.028 ± 0.3	-0.1 ± 0.1	1.1 ± 0.9	0.9± 1.0	0.6 ± 0.2	1.8 ± 0.7
*Net N_2O flux	5.1 ± 1.4	6.1 ± 0.9	10.9 ± 1.9	0.09 ± 0.04	0.89 ± 0.6	1.5 ± 0.7	1.0± 0.3	1.6 ± 0.7	0.7 ± 0.3
Net P mineralization	3.4 ± 2.9	4.0 ± 2.6	-0.88 ± 2.1	0.1 ± 1.5	-0.8 ± 0.4	-0.7 ± 0.9	-0.1± 4.3	-2.6 ± 2.6	-6.2 ± 3.1

Table 4. Ratios of surface (0-10 cm) mineral soil carbon (C), nitrogen (N), and phosphorus (P) pools and net fluxes over seven days in control, lopped, and masticated treatments in different seasons represented by the month when we sampled soil (June = warm and wet, August = hot and dry, November = cool and dry). Abbreviations: DOC = dissolved organic C, PO_4^{3-} = phosphate. Values are means ± standard error.

	June			August			November		
Concentrations (mg kg^{-1})	Control	Lopped	Masticated	Control	Lopped	Masticated	Control	Lopped	Masticated
DOC:available N	14.9 ± 2.1	13.5 ±1.6	19.0 ± 2.3	425.6 ± 207.3	67.2 ± 16.8	21.2 ± 4.4	112.8± 68.3	48.5 ± 5.5	102.0 ± 52.2
DOC :PO_4^{3-}	2.6 ± 0.3	1.5 ± 0.1	1.5 ± 0.1	5.0 ± 1.5	5.1 ± 1.1	3.3 ± 0.5	3.2 ± 0.7	8.4 ± 3.5	2.2 ± 0.2
Available N:PO_4^{3-}	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.01	0.06 ± 0.03	0.1 ± 0.01	0.2 ± 0.03	0.1 ± 0.04	0.2 ± 0.1	0.2 ± 0.03
ΔDOC:available N	-8.8 ±12.6	-8.1 ± 1.2	-7.6 ± 3.3	-77.8 ± 171.4	23.1 ± 23.2	4.6 ± 9.9	-78.9 ± 10.6	-10.3 ± 13.7	-30.3 ± 32.2
ΔDOC:PO_4^{3-}	-0.75 ± 0.4	-0.39 ± 0.1	-0.33 ± 0.1	-0.97 ± 1.0	-1.2 ± 0.8	-0.86 ± 0.3	0.58 ± 2.1	-5.5 ± 41.4	0.76 ± 0.4
ΔAvailable N: PO_4^{3-}	0.6 ± 0.5	0.1 ± 0.1	0.1 ± 0.03	-0.01 ± 0.02	0.02 ± 0.03	0.2 ± 0.1	0.1 ± 0.1	2.2 ± 2.2	0.1 ± 0.04

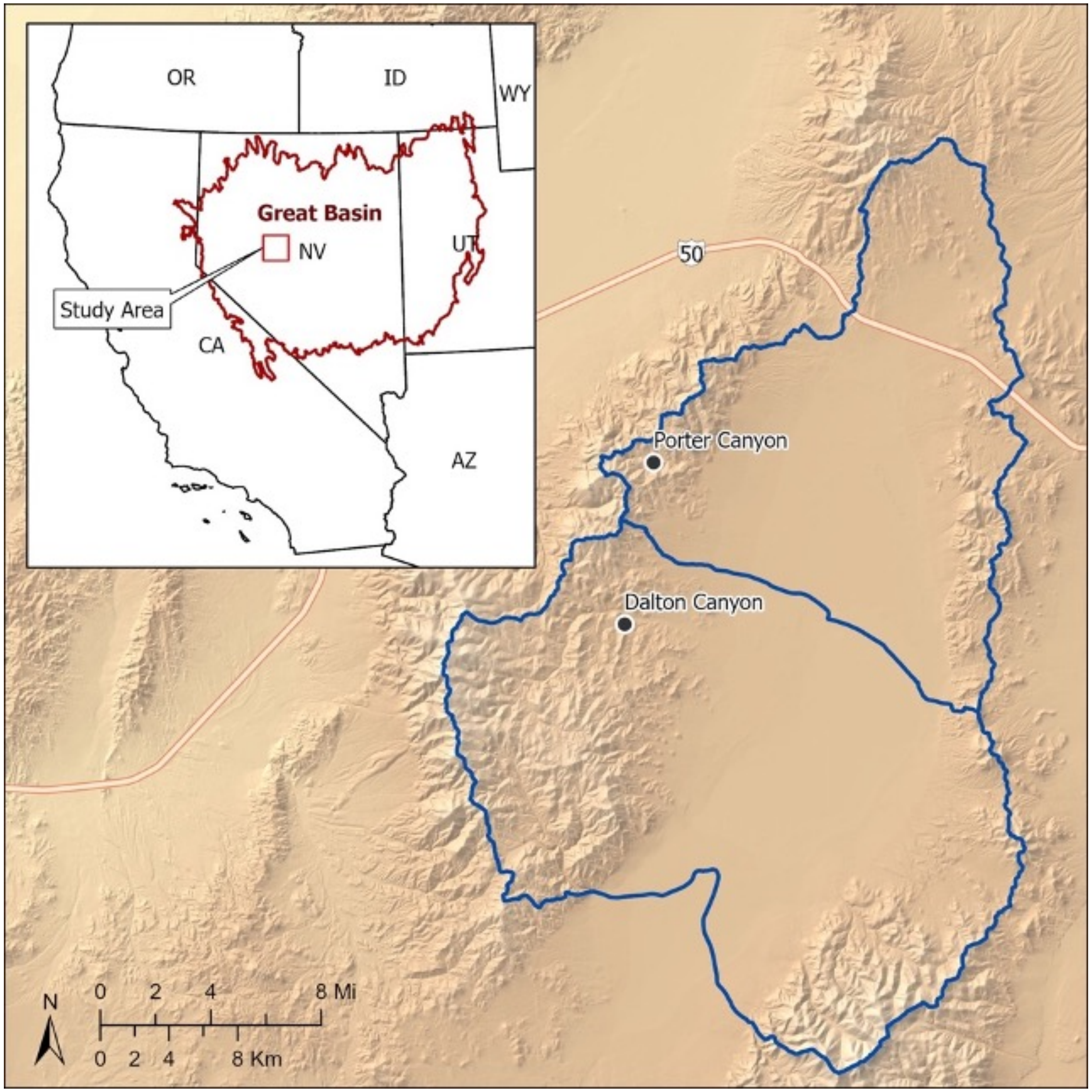
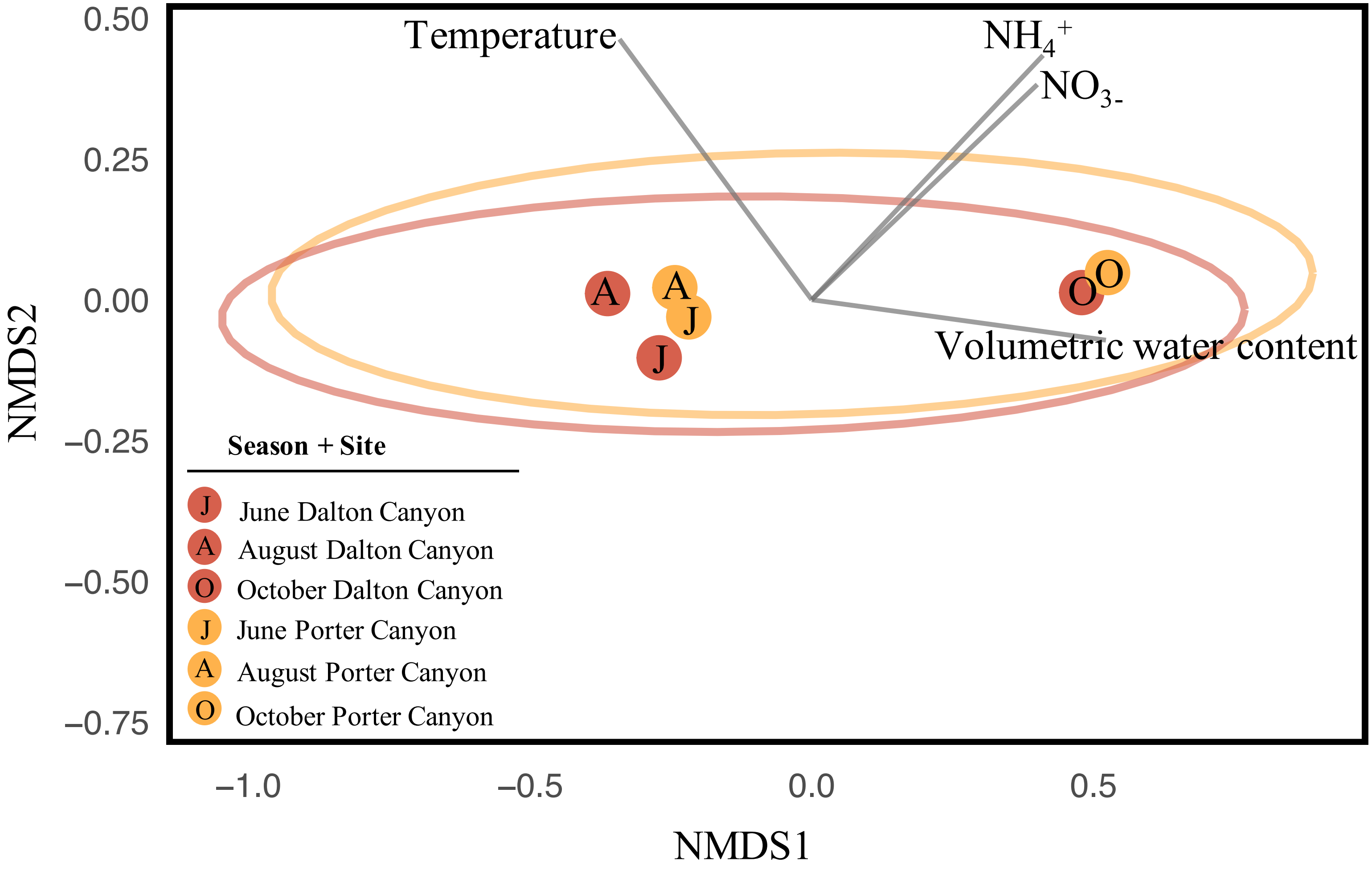


Figure 1. Study sites were in two adjacent watersheds (denoted by blue lines) in the Desatoya Range in the central Great Basin, USA. Porter Canyon had uncut control trees and the lopping treatment; Dalton Canyon had a mastication treatment.

Figure 2. Nonmetric multidimensional scaling plot of pre-treatment soil characteristics (soil temperature, soil volumetric water content, and soil available nitrogen (as ammonium (NH_4^+) plus nitrate (NO_3^-) in Porter and Dalton Canyons. The two sites were not significantly different from each other based on pretreatment soil characteristics in each site were not significantly different based on permutational analysis of variance (F = 0.56, R2 = 0.009, P = 0.53



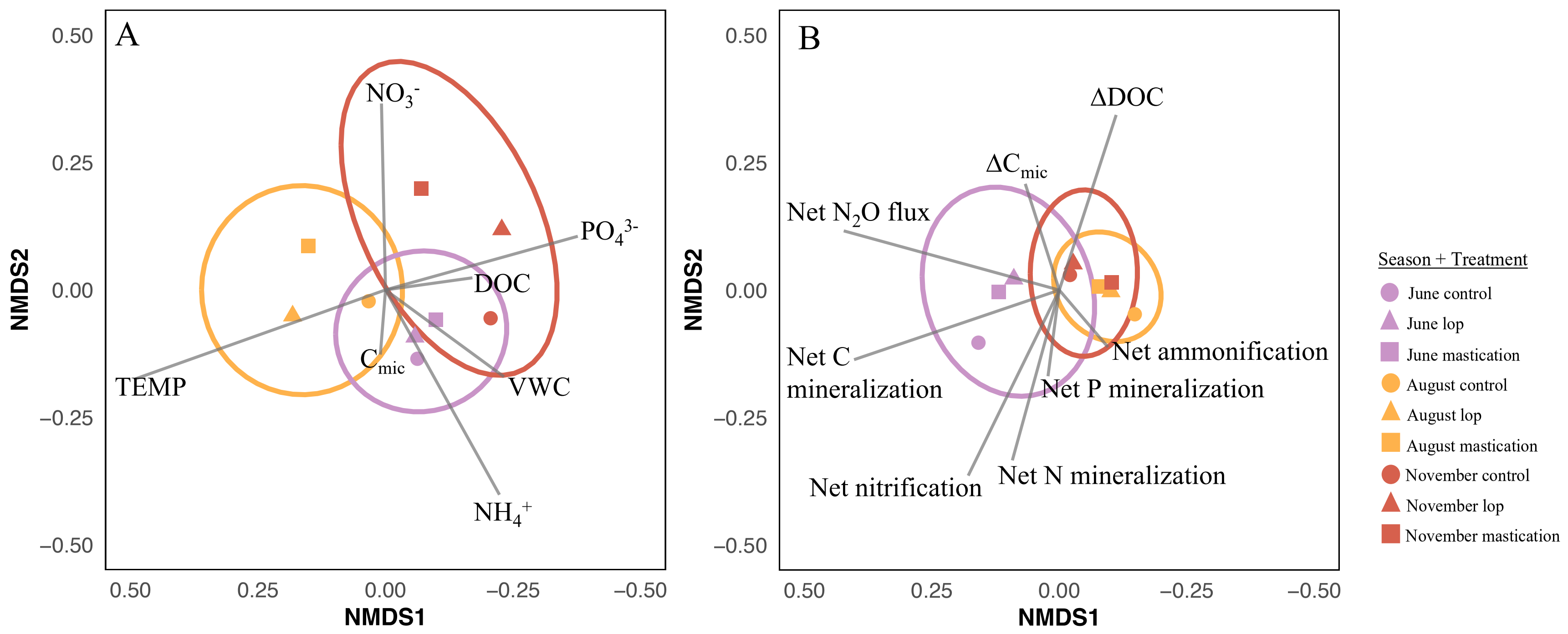


Figure 3. Nonmetric multidimensional scaling (NMDS) ordinations show the variability in soil carbon (C), nitrogen (N), and phosphate (P) pools (panel A; stress = 0.17896) and fluxes (panel B; stress = 0.153156). Permutational analysis of variance (PERMANOVA) tests indicated a significant season effect (F = 20.9, R^2 = 0.25 , P < 0.001), treatment effect (F = 11.1 ; R^2 = 13.3; P < 0.001) , but no significant interaction of season and treatment in pools of C, N, and P (F = 1.1; R^2 = 0.026; P = 0.382). Fluxes of C, N, and P were different among seasons (F = 18.23; R^2 = 0.22; P < 0.001), treatment (F = 5.1; R^2 = 0.06; P < 0.001), and season by treatment interaction (F = 3.1; R^2 = 0.08; P = 0.003). Circles, squares, and triangles represent the centroids of each treatment within a season and ellipses represent the 95% confidence intervals around each centroid. Significant correlations (P < 0.05) between predictor variables and NMDS scores are shown by vector arrows. Asterisks indicate a predictor variable that was not significantly correlated with NMDS scores. Abbreviations: C_{mic} = microbial biomass C, DOC = dissolved organic C, NH_4^+ = ammonium, NO_3^- = nitrate, VWC = volumetric water content, TEMP = temperature.

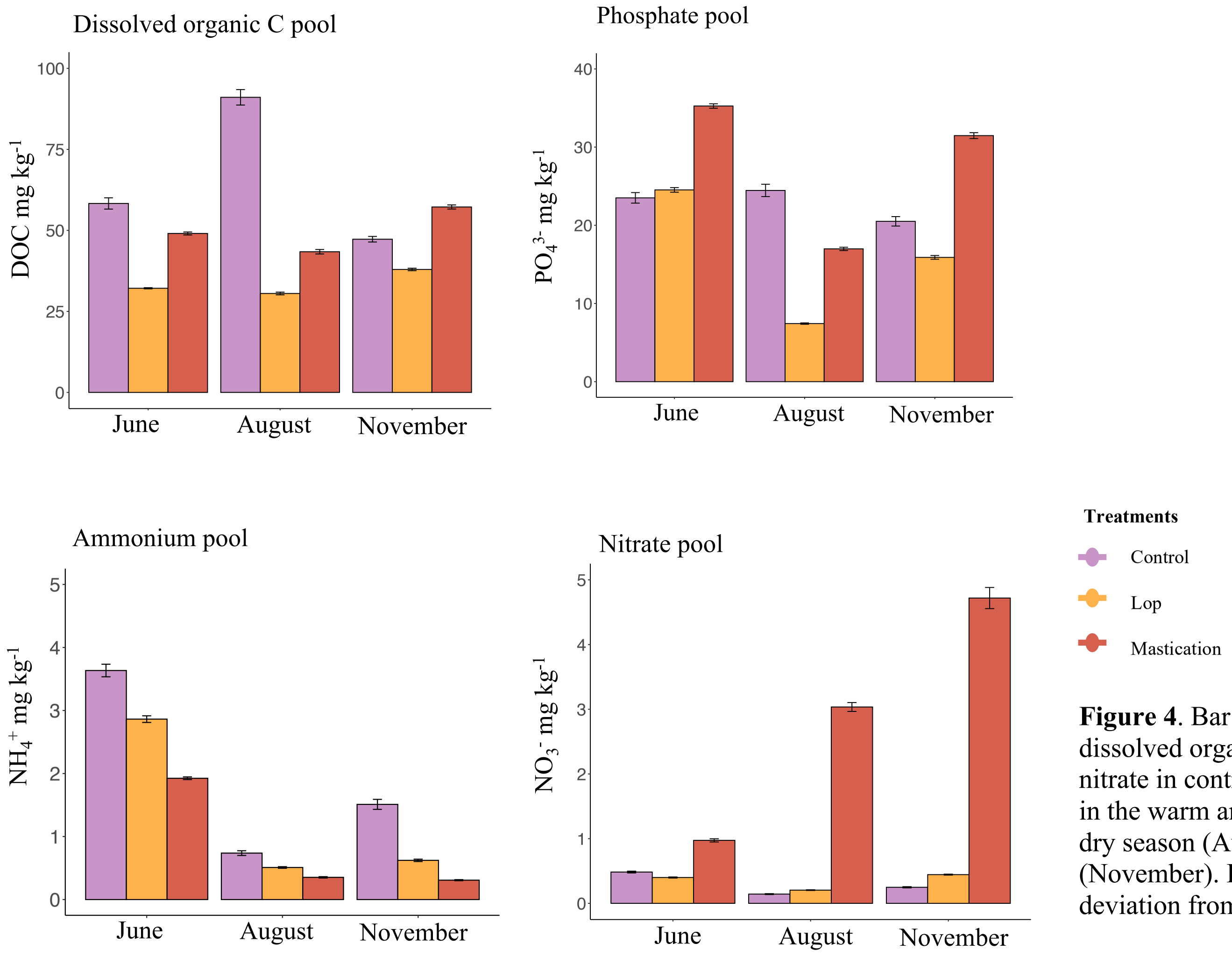


Figure 4. Bar plots show mean pools of dissolved organic C, phosphate, ammonium, and nitrate in control, lop, and mastication treatments in the warm and wet season (June), the warm and dry season (August), and the cool and dry season (November). Error bars represent ± one standard deviation from the mean.

Table 5. Repeated measures analysis of variance (RMANOVA) tested whether pools of C, N, and P in surface mineral soil (0-10 cm) significantly varied by treatment as a function of season. Table includes degrees of freedom (*df*), sum of squares (SS), mean of squares (MS), F values, and *p*-values using an alpha of 0.05 to determine significance.

DOC	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	7.764	3.882	11.39	<0.001
	Error	61	20.797	0.341		
	Within subjects					
	Season	2	1.714	0.8571	4.855	0.00936
	Season x treatment	4	3.183	0.7958	4.508	0.00197
	Error	122	21.538	0.1765		
NH_4^+	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	2.989	1.4944	8.708	<0.001
	Error	61	10.486	0.1716		
	Within subjects					
	Season	2	27.961	13.981	158.721	<0.001
	Season x treatment	4	0.336	0.084	0.954	0.436
	Error	122	10.746	0.088		
NO_3^-	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	28.15	14.073	34.02	<0.001
	Error	61	25.23	0.414		
	Within subjects					
	Season	2	4.315	2.1574	12.733	<0.001
	Season x treatment	4	6.598	1.6496	9.736	<0.001
	Error	122	20.671	0.1694		
Inorganic N	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	10.45	5.224	11.63	<0.001
	Error	61	27.40	0.449		
	Within subjects					
	Season	2	6.248	3.1241	15.015	<0.001
	Season x treatment	4	7.234	1.8085	8.692	<0.001
	Error	122	25.385	0.2081		
PO_4^{3-}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	14.99	7.498	17.98	<0.001
	Error	61	25.43	0.417		
	Within subjects					
	Season	2	19.860	9.93	44.53	<0.001
	Season x treatment	4	4.407	1.102	4.94	0.001
	Error	122	27.207	0.223		
C_{mic}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	44896	22448	7.045	0.00177
	Error	61	194368	3186		
	Within subjects					
	Season	2	21758	10879	4.575	0.0121
	Season x treatment	4	14258	3564	1.499	0.2067
	Error	122	290108	2378		

Table 6. Repeated measures analysis of variance (RMANOVA) tested whether seven-day antecedent soil temperature and soil volumetric water content (VWC) varied by treatment as a function of season. Table includes degrees of freedom (*df*), sum of squares (SS), mean of squares (MS), F values, and *p*-values using an alpha of 0.05 to determine significance.

VWC	Source	*df*	SS	MS	F	p-value
	Between subjects					
	Treatment	2	468.3	234.13	11.66	<0.001
	Error	61	1224.7	20.08		
	Within subjects					
	Season	2	5801	2900.3	274.915	<0.001
	Season x treatment	4	69	17.1	1.624	0.172
	Error	122	1287	10.5		
Temperature	Source	*df*	SS	MS	F	p-value
	Between subjects					
	Treatment	2	112.1	56.03	6.044	0.00403
	Error	61	565.5	9.27		
	Within subjects					
	Season	2	16699	8350	1291.117	<0.001
	Season x treatment	4	151	38	5.841	<0.001
	Error	122	789	6		

Table 7. Repeated measures analysis of variance (RMANOVA) tested whether fluxes of C in surface mineral soil (0-10 cm) significantly varied by treatment as a function of season. Table includes degrees of freedom (*df*), sum of squares (SS), mean of squares (MS), F values, and *p*-values using an alpha of 0.05 to determine significance.

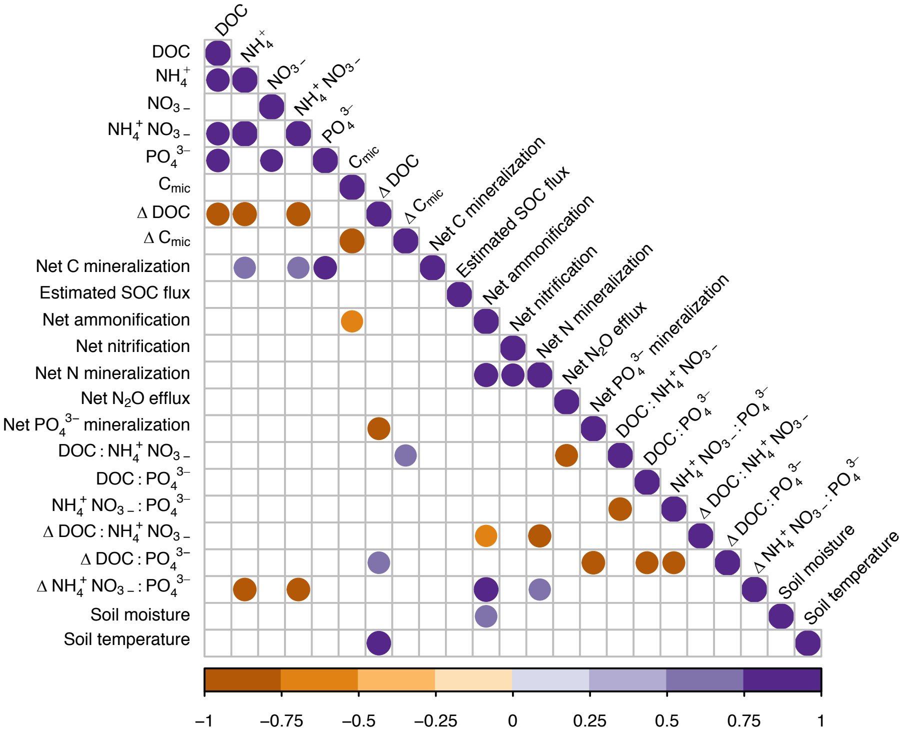
ΔDOC	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	520	260	0.592	0.556
	Error	61	26782	439		
	Within subjects					
	Season	2	3650	1824.9	5.157	<0.001
	Season x treatment	4	2344	586.1	1.656	0.16458
	Error	122	43172	353.9		
ΔC_{mic}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	44096	22048	5.16	0.0085
	Error	61	260636	4273		
	Within subjects					
	Season	2	237965	118982	35.935	<0.001
	Season x treatment	4	59552	14888	4.496	0.00201
	Error	122	403952	3311		
Net C mineralization	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	0.86	0.4282	0.564	0.572
	Error	61	46.32	0.7594		
	Within subjects					
	Season	2	119.79	59.89	182.33	<0.001
	Season x treatment	4	7.61	1.9	5.788	<0.001
	Error	122	40.08	0.33		
Estimated soil organic C loss	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	39830	19915	4.579	0.014
	Error	61	265326	4350		
	Within subjects					
	Season	2	464429	232214	57.047	< 0.001
	Season x treatment	4	103903	25976	6.381	< 0.001
	Error	122	496614	4071		

Table 8. Repeated measures analysis of variance (RMANOVA) tested whether fluxes of N and P in surface mineral soil (0-10 cm) significantly varied by treatment as a function of season. Table includes degrees of freedom (*df*), sum of squares (SS), mean of squares (MS), F values, and *p*-values using an alpha of 0.05 to determine significance.

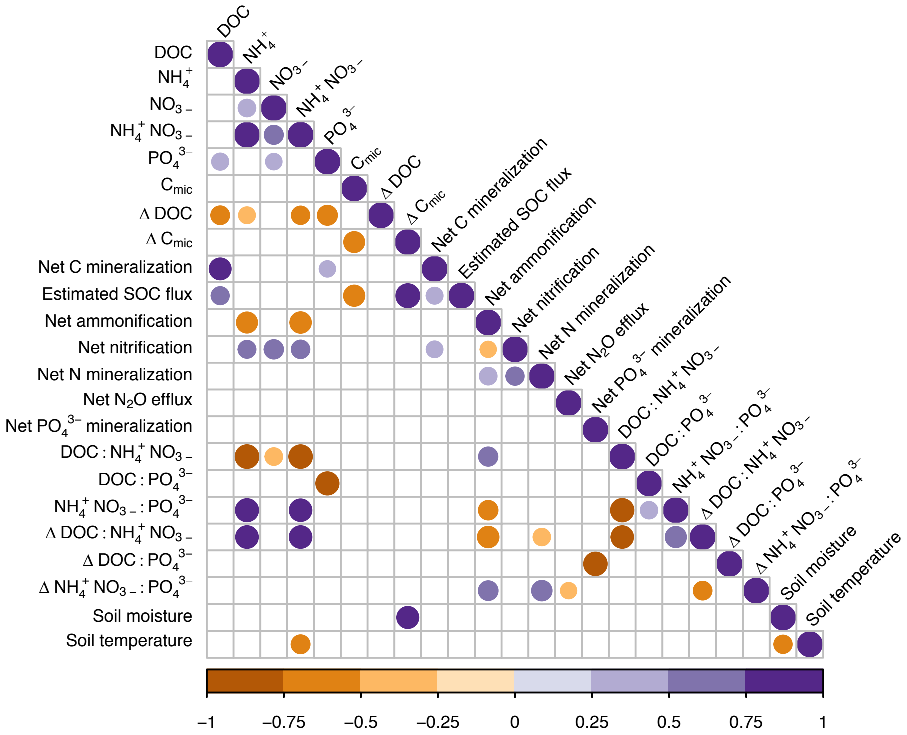
Net ammonification	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	0.187	0.09337	0.582	0.562
	Error	61	9.784	0.16039		
	Within subjects					
	Season	2	1.491	0.7454	6.443	0.00219
	Season x treatment	4	0.075	0.0187	0.162	0.957
	Error	122	14.114	0.1157		
Net nitrification	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	18.1	9.027	1.067	0.35
	Error	61	516.2	8.463		
	Within subjects					
	Season	2	271.7	135.83	11.14	<0.001
	Season x treatment	4	49.7	12.43	1.019	0.4
	Error	122	1487.5	12.19		
Net N mineralization	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	30.2	15.11	0.91	0.408
	Error	61	1012.5	16.60		
	Within subjects					
	Season	2	190.2	95.09	6.25	0.0026
	Season x treatment	4	117.8	29.45	1.936	0.109
	Error	122	1856.2	15.21		
Net N_2O flux	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	0.00015	7.4e-05	2.758	0.0713
	Error	61	0.0016	2.7e-05		
	Within subjects					
	Season	2	0.0023	0.00113	40.991	<0.001
	Season x treatment	4	0.00028	0.00007	2.573	0.0411
	Error	122	0.0034	0.000028		
Net PO_4^{3-} mineralization	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	457	228.6	1.601	0.21
	Error	61	8710	142.8		
	Within subjects					
	Season	2	1005	502.3	4.062	0.0196
	Season x treatment	4	227	56.7	0.458	0.7663
	Error	122	15085	123.6		

Table 9. Repeated measures analysis of variance (RMANOVA) tested whether ratios of C, N, and P pools and fluxes in surface mineral soil (0-10 cm) significantly varied by treatment as a function of season. Table includes degrees of freedom (*df*), sum of squares (SS), mean of squares (MS), F values, and *p*-values using an alpha of 0.05 to determine significance.

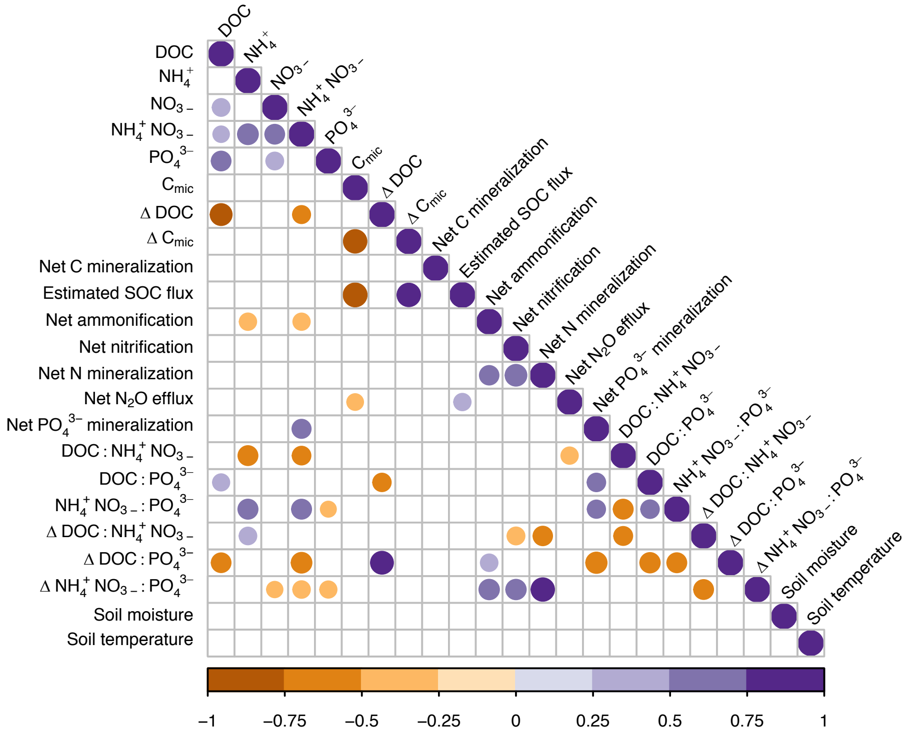
DOC:available N	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	18.49	9.243	9.052	<0.001
	Error	61	62.29	1.021		
	Within subjects					
	Season	2	24.12	12.058	15.43	<0.001
	Season x treatment	4	33.51	8.378	10.72	<0.001
	Error	122	95.32	0.781		
DOC:PO_4^{3-}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	5.99	2.998	5.44	0.0067
	Error	61	33.61	0.551		
	Within subjects					
	Season	2	16.85	8.425	22.209	<0.001
	Season x treatment	4	2.53	0.632	1.666	0.162
	Error	122	46.28	0.379		
Available N:PO_4^{3-}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	3.97	1.983	1.641	0.202
	Error	61	73.72	1.209		
	Within subjects					
	Season	2	3.61	1.807	2.150	0.121
	Season x treatment	4	27.19	6.798	8.089	<0.001
	Error	122	102.54	0.841		
ΔDOC:available N	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	0.576	0.2878	0.842	0.436
	Error	61	20.863	0.3420		
	Within subjects					
	Season	2	0.49	0.2446	0.711	0.493
	Season x treatment	4	1.26	0.3154	0.916	0.457
	Error	122	42.0	0.3442		
ΔDOC:PO_4^{3-}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	216	108.03	1.523	0.226
	Error	61	4327	70.93		
	Within subjects					
	Season	2	47	23.51	0.403	0.669
	Season x treatment	4	374	93.6	1.605	0.177
	Error	122	58.32	0.7131		
ΔAvailable N: PO_4^{3-}	Source	*df*	SS	MS	F	*p*-value
	Between subjects					
	Treatment	2	0.021	0.0106	0.098	0.907
	Error	61	6.612	0.1084		
	Within subjects					
	Season	2	0.041	0.02068	0.179	0.837
	Season x treatment	4	0.154	0.03846	0.332	0.856
	Error	122	14.123	0.11576		



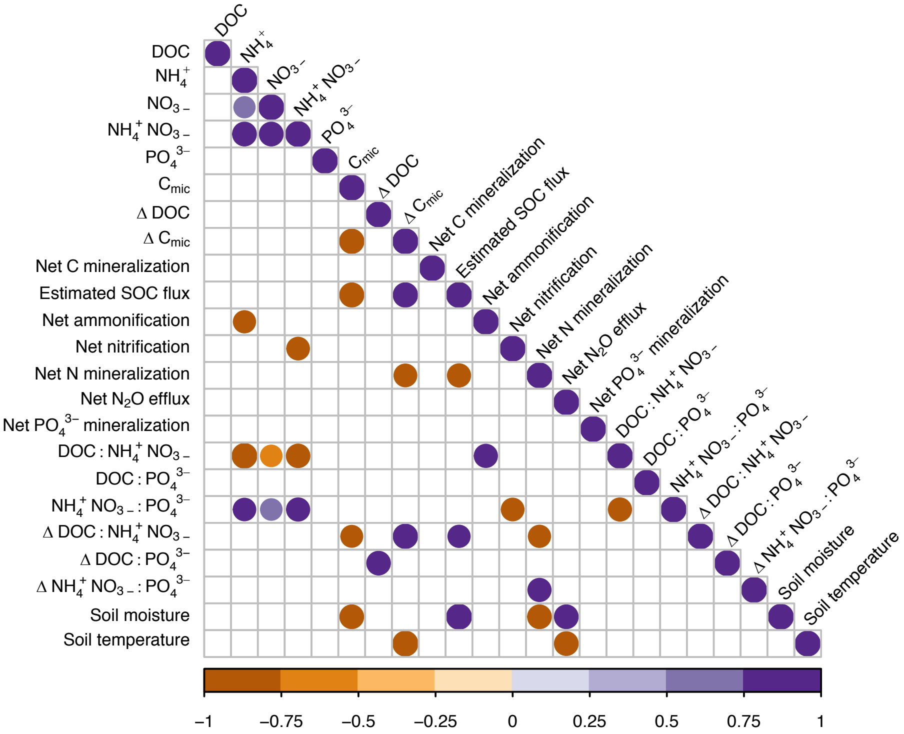
Supplementary Figure 1. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in control plots in June.



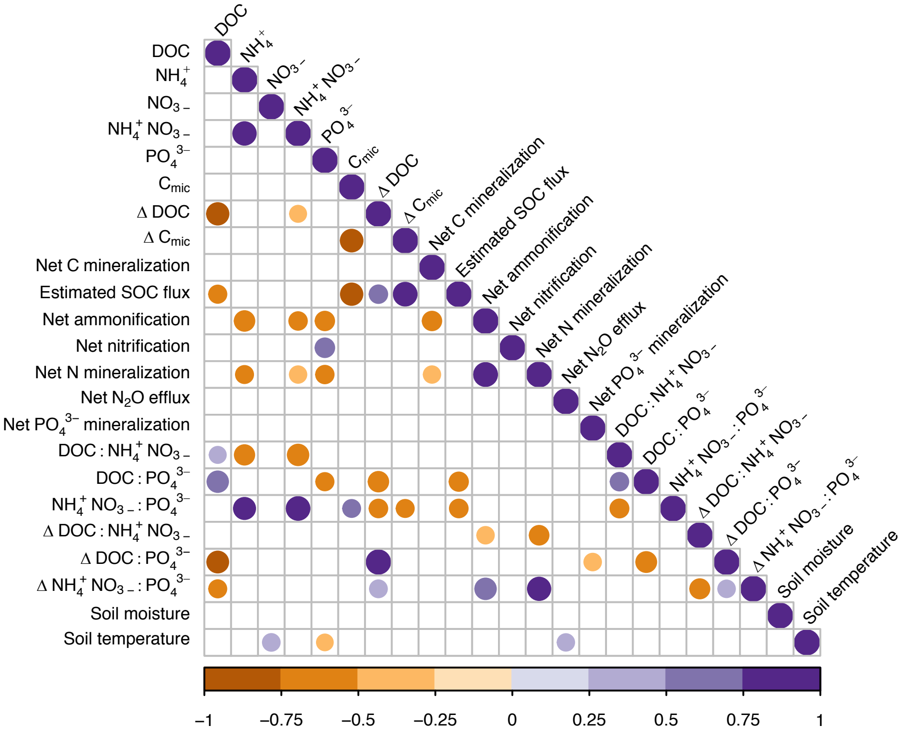
Supplementary Figure 2. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in lop plots in June.



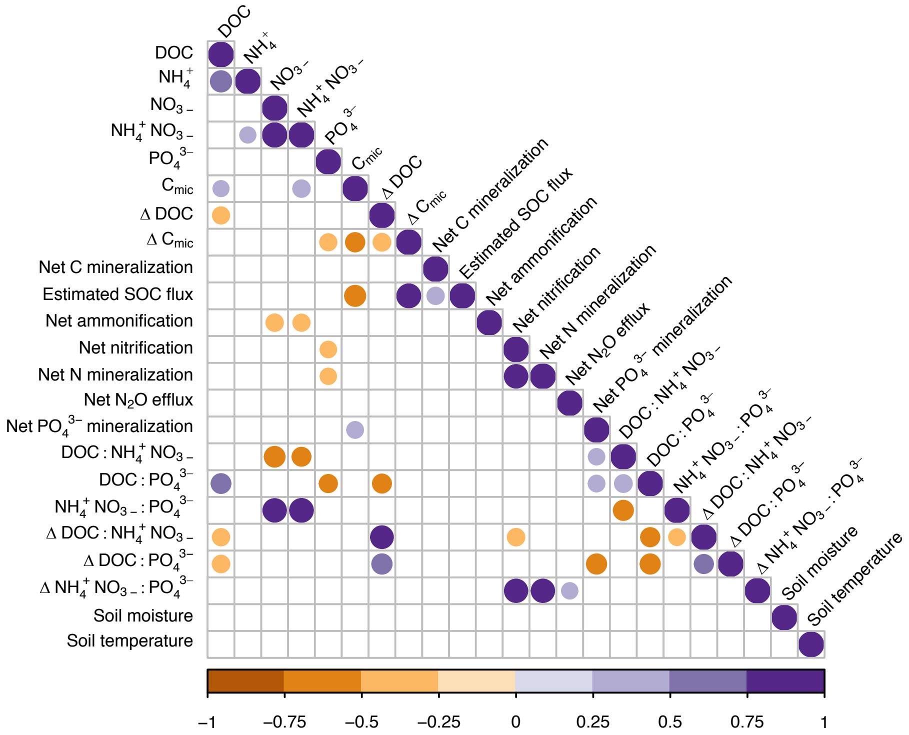
Supplementary Figure 3. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in mastication plots in June.



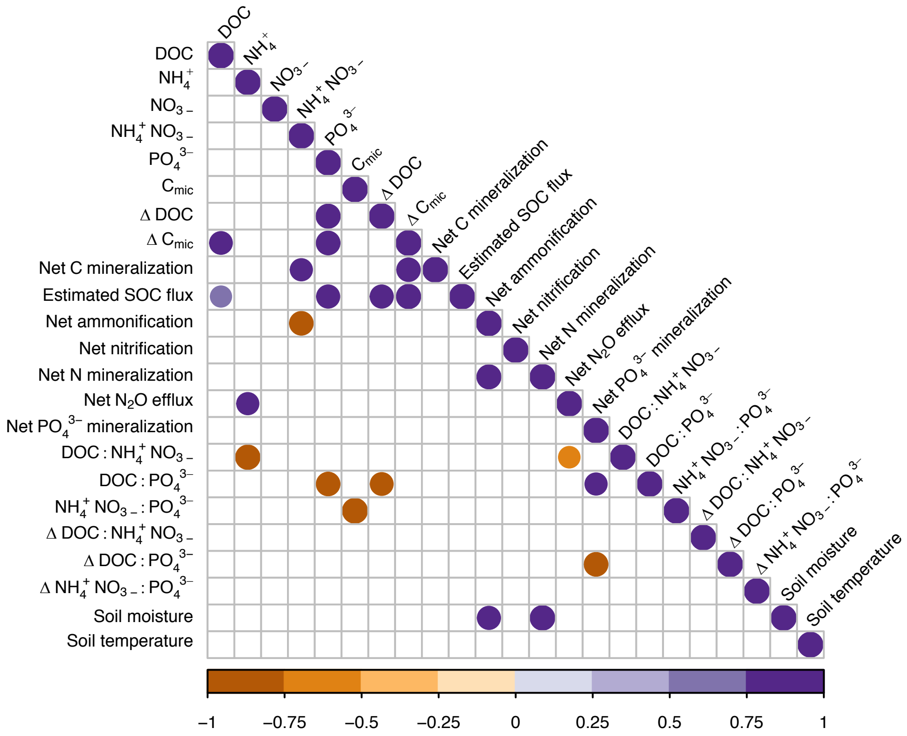
Supplementary Figure 4. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in control plots in August.



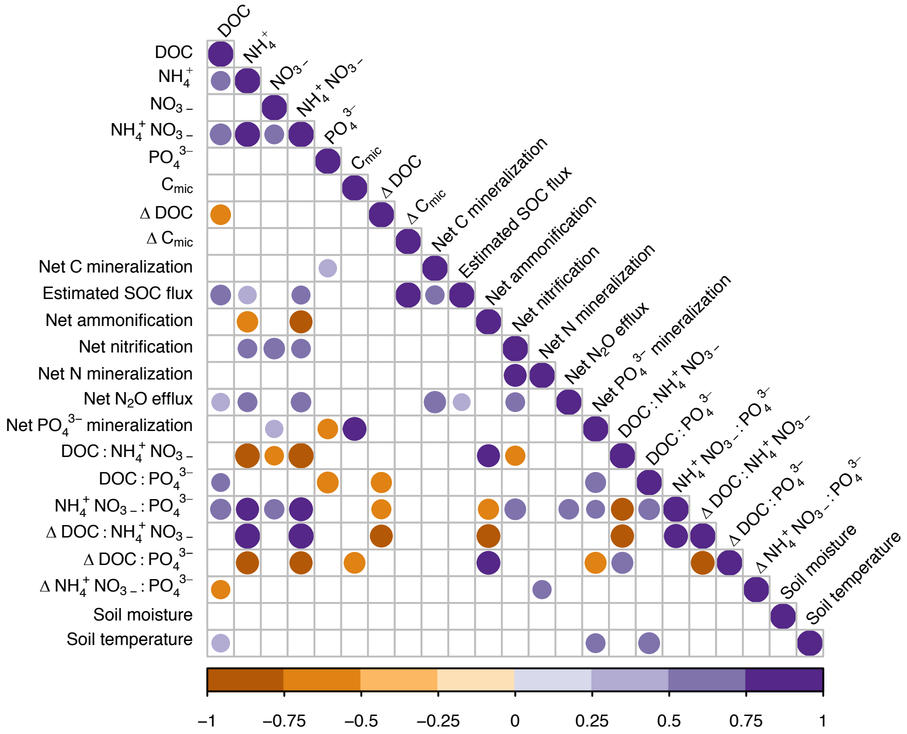
Supplementary Figure 5. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in lop plots in August.



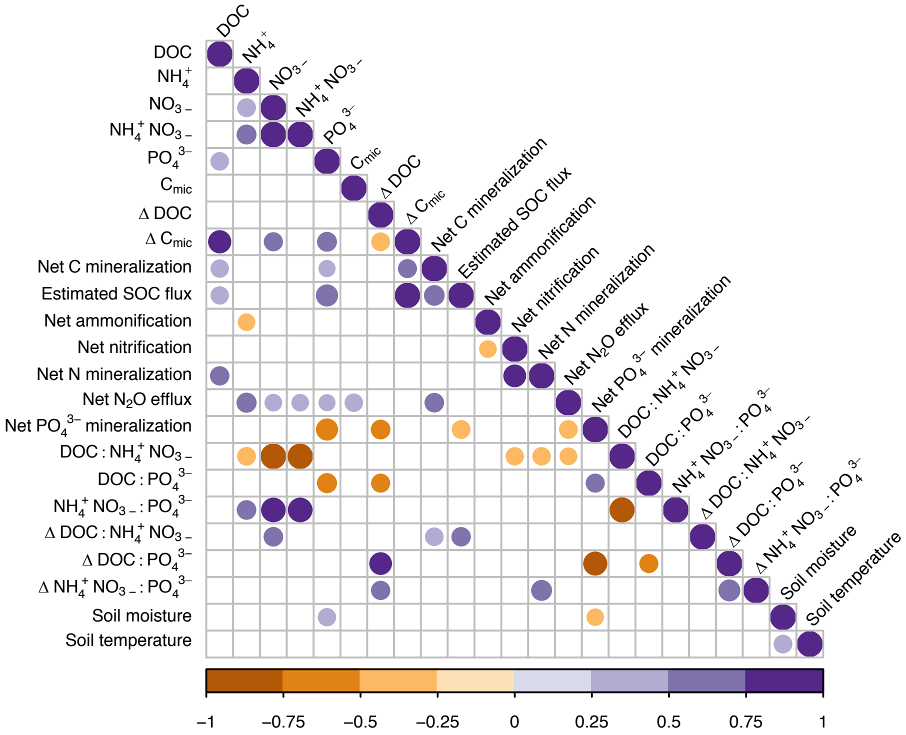
Supplementary Figure 6. Shown are significant ($P < 0.05$) Spearman’s rank correlation coefficients for pools and fluxes of C, N, and P in mastication plots in August.



Supplementary Figure 7. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in control plots in November.



Supplementary Figure 8. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in lop plots in November.



Supplementary Figure 9. Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for pools and fluxes of C, N, and P in mastication plots in November.

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