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Effects of River Beads on Algal Nutrient Limitation Following Severe Wildfire

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ABSTRACT

1. Severe wildfires often increase inputs of nitrogen (N) and phosphorus (P) to streams, which can alter nutrient limitation and primary production of benthic algae. Changes in nutrient limitation, however, vary over space and time due to physical and biological factors. In particular, the low gradient, wide valley-bottom segments of montane rivers (hereafter 'beads') are zones of nutrient uptake and retention. Few studies, however, have evaluated the role of river beads in mediating algal nutrient limitation following wildfire in perennial streams.
2. We hypothesized that river beads would act as nutrient sinks and increase downstream N limitation following severe wildfire. We also expected that N limitation would be stronger during low flow conditions in autumn, when stream N concentrations are lower relative to high flows in spring.
3. To test effects of river beads across seasons on nutrient limitation, we deployed nutrient diffusing substrate treatments above and below beads in three perennial streams within the Cameron Peak Fire scar, which was the largest fire in Colorado state history. On average, the three focal catchments had 52% of their upstream area burned at moderate or high severity. Nutrient amendments included N and P in a 2×2 factorial design, which were deployed in high flows in spring and low flows in autumn. We quantified environmental factors at each location that were expected to mediate the strength of nutrient limitation, including macroinvertebrate grazers, canopy cover, water temperature, depth, stream nutrients, dissolved oxygen and flow velocity. We also characterized periphyton communities using 16S rRNA and 18S rRNA gene sequencing to determine if community structure varied across seasons or position relative to beads.
4. N addition increased algal Chl *a* concentrations downstream but not upstream of beads during low flow and low stream nutrient conditions. In contrast, during high flow, high nutrient conditions, benthic algae were co-limited by N and P, and the magnitude of response was higher upstream of beads than downstream. Macroinvertebrate grazer density had a negative effect on the magnitude of Chl *a* responses to N-containing treatments, which exceeded the relative importance of the other six environmental variables. Periphyton sequencing indicated the relative abundance of eukaryotic algae was lowest above beads in spring and below beads in autumn.
5. Taken together, these results confirm that river beads influenced periphyton nutrient limitation, with the spatial pattern of limitation changing across seasons. Because stream restoration efforts following wildfire often focus on enhancing the

functioning of degraded river beads, these findings also provide baseline information about how management interventions may influence nutrient limitation and retention in burned catchments.

1 | Introduction

Wildfire is increasing in frequency and severity in many regions globally (Dennison et al. 2014; Abatzoglou and Williams 2016; Jones et al. 2022; Richardson et al. 2022), resulting in widespread impacts on aquatic ecosystem structure and function (Betts and Jones Jr 2009; Klose et al. 2015; Rhea et al. 2021). Wildfire effects on perennial stream ecosystems are highly complex and can impact stream communities through multiple mechanisms (Bixby et al. 2015). Wildfire has the potential to elevate stream nutrient concentrations (Rhoades et al. 2011, 2019; Rust et al. 2018), reduce riparian canopy cover (Coble et al. 2023), increase sediment transport (Ryan et al. 2024), change community structure of macroinvertebrates and fishes (Verkaik et al. 2015; Whitney et al. 2016) and alter species interactions and energy fluxes in food webs (Spencer et al. 2003; Mihuc and Minshall 2005; Malison and Baxter 2010; Preston et al. 2023; Roon et al. 2025). Wildfires can pose complex management challenges, particularly in headwater streams where effects can cascade downstream (MacDonald and Coe 2007). In some regions, wildfires are burning at higher elevations and more frequently in forested headwaters, leading to effects on downstream water supply and water quality (Hallema et al. 2018). As a result, there is a growing need to understand both catchment-scale and local factors that may enhance resistance and resilience to physical and ecological changes following wildfire.

Wildfires often lead to increases in stream nutrient concentrations. Fire increases terrestrial to aquatic N transfer by killing vegetation and reducing plant and microbial N uptake, increasing soil N mineralization and allowing for increased leaching of excess mobile nitrate (NO_3-N) into streams (Wan et al. 2001; Certini 2005; Turner et al. 2007; Smithwick et al. 2009). Stream P concentrations frequently increase as well due to dissolution of ash, followed by direct deposition or overland flow during rainfall events (Earl and Blinn 2003; Rhoades et al. 2025). Increased N and P concentrations and exports following wildfire are a common short-term (< 5 years) response (Rust et al. 2018). Longer-term studies have shown that nutrient levels, especially for N, can remain elevated compared to pre-fire conditions in burned catchments for longer than 10 years (Rhoades et al. 2019). Increases in nutrient availability can affect primary producers via bottom-up effects by shifting or removing nutrient limitation, potentially influencing higher trophic levels in the aquatic food web (Silins et al. 2014).

Benthic algae, the autotrophic component of periphyton, are key primary producers in perennial streams (Minshall 1978) and are important drivers of in-stream nutrient dynamics through the uptake, assimilation and transformation of N and P (Borchardt 1996). Wildfire-driven nutrient inputs, especially N, can alleviate algal nutrient limitation, increasing benthic primary productivity (Betts and Jones Jr 2009; Klose et al. 2015; Rhea et al. 2021) and altering the composition of benthic algal communities (Carvalho et al. 2019; Bistarelli et al. 2021). In high NO_3-N post-fire environments, algal communities shifted from being primarily N-limited to N and P co-limited (Rhea et al. 2021). Shifts in limitation can affect both algal abundance and nutrient content, with impacts on secondary consumers and higher trophic levels (Atkinson et al. 2017). Variation in abiotic (e.g., geomorphology, hydrology) and biotic factors (e.g., grazer communities) can mediate the ways in which wildfire affects stream nutrients and periphyton over space and time, although the most relevant factors and underlying mechanisms are not always well understood.

Montane perennial streams generally vary in geomorphic characteristics from steep, confined and narrow channels to wider, unconfined floodplain areas. This variation in the geomorphic template within catchments can regulate aspects of aquatic ecosystem function (Bellmore and Baxter 2014, Wegener et al. 2018, Venarsky et al. 2018) and can control both nutrient and periphyton dynamics (Doyle and Stanley 2006; Wegener et al. 2018). Transitions between wide and narrow valley bottoms throughout catchments have been described as “beads on a string” (Stanford et al. 1996; Wohl et al. 2018). River beads, defined here as the low gradient, wide valley bottom channel segments of otherwise laterally confined montane rivers, may play an important role in N cycling and assimilation at the reach scale through controls on physical and biological conditions that enhance the potential for retention of sediment, water and organic matter (Wohl et al. 2018). The geomorphic complexity and valley width of river beads typically results in increased surface water area and subsurface storage, decreased velocity and increased residence time of water and nutrients relative to more confined reaches (Hall and Tank 1999; Ye et al. 2012). Longer residence times can lead to enhanced biogeochemical activity and uptake by primary producers, soil and hyporheic zones, collectively increasing in-stream nutrient processing and retention (Doyle and Stanley 2006; Bellmore and Baxter 2014). River beads can be areas of increased denitrification associated with saturated, anaerobic soils (Murray et al. 2023) and play significant roles in catchment-scale carbon storage (Wohl et al. 2012, 2018). Additionally, laterally unconfined beads can result in zones of flow heterogeneity and sediment deposition, both of which can facilitate nutrient retention (Hall and Tank 1999, Ye et al. 2012). As a result, the geomorphic template of streams not only mediates nutrient dynamics but can also shape benthic algal communities, although these interactions are not well understood in fire-affected perennial streams.

Nutrient diffusing substrates (NDS) are a standard method used to assess nutrient limitation of benthic periphyton in streams and rivers (Fairchild et al. 1985). NDS experiments frequently involve treatments that increase N and P in a factorial design, which can be used to infer whether algae are limited by N, P or a combination of both (Tank and Dodds 2003). NDS have been used to evaluate algal responses to nutrient amendments under a variety of conditions with varying bottom-up and top-down factors, including variations in light (Taulbee et al. 2005, Warren et al. 2017), velocity (Hoch 2008), temperature (Myrstener et al. 2018),

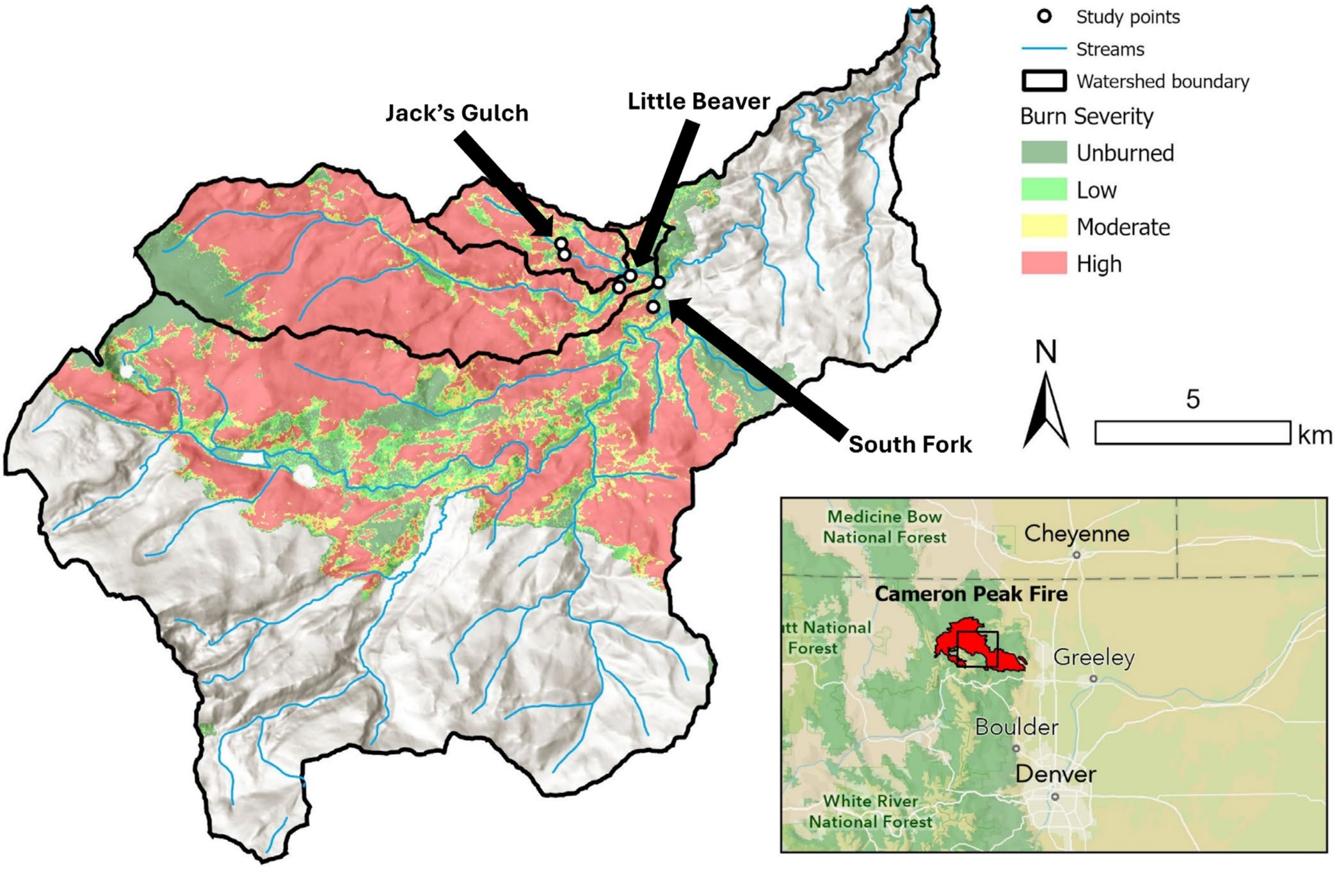

FIGURE 1 | The South Fork Cache la Poudre River basin with Little Beaver Creek and Jack's Gulch watershed boundaries. Cameron Peak Fire burn severity within the watersheds is denoted by red (high severity), yellow (moderate severity) and light green (low severity). The bottom right inset shows the general study location within the Cameron Peak Fire perimeter along the Colorado Front Range.

macroinvertebrate grazers (Rosemond et al. 1993, Wellnitz and Leroy Poff 2006, Beck et al. 2019) and nutrients (Rhea et al. 2021; Beck et al. 2021); however, little is known about how these environmental factors interact in combination with river beads and altered nutrient dynamics to influence algal responses in fire-affected streams.

Our objective was to evaluate the role of river beads in mediating stream nutrient limitation of benthic algae after severe wildfire in Colorado, USA. We conducted NDS experiments at the upstream and downstream ends of river beads in three streams within the Cameron Peak Fire scar during high flow, high NO_3-N conditions and low flow, low NO_3-N conditions. To help interpret NDS results, we collected data on environmental variables expected to influence the strength of algal responses to nutrient amendments to test whether other biological and physical variables could help explain spatial and temporal variation in N limitation under post-fire conditions. We also characterized benthic primary producer community structure using 16S and 18S rRNA gene sequencing to determine if periphyton composition changed above and below river beads or across seasons. We hypothesized that algal N limitation would be stronger downstream of beads than upstream due to downstream reductions in NO_3-N, especially during autumn low flow conditions when NO_3-N concentrations are lower. During high spring discharge, we predicted that higher NO_3-N concentrations, combined with colder temperatures and scouring flows, would decrease N limitation regardless of position relative to beads.

2 | Methods

2.1 | Study Area

We conducted the field experiment in the South Fork Cache la Poudre catchment, a mountainous drainage in the southern Rocky Mountains located northwest of Fort Collins, Colorado, USA (Figure 1). The South Fork Cache la Poudre is a major tributary of the Cache la Poudre River, the main source of drinking water for over 500,000 downstream users on the northern Colorado Front Range. The South Fork Cache la Poudre drainage lies between 1199 and 4079 m elevation. The hydrograph is dominated by spring snowmelt, which typically peaks in early June and subsides to baseflow conditions by late summer. Approximately 20% of the Cache la Poudre's contributing area was burned in 2020 during the Cameron Peak Fire, which burned a total of 84,544 ha (US Forest Service 2020), making it Colorado's largest wildfire in recorded history. The Cameron Peak Fire increased stream nitrate (NO_3-N) in catchments that experienced extensive high-severity wildfire (Rhoades et al. 2025). As reported after other wildfires occurring in

TABLE 1 | Site characteristics of the three study watersheds calculated for the contributing area of the downstream NDS deployment sites. Burn extent is the proportion of the watershed burned during the Cameron Peak Fire as calculated using Burned Area Reflectance Classification (United States Geological Service 2025) data.

			Burn extent by severity (%)				
Site	**Catchment area (ha)**	**Elevation range (m)**	**Total**	**Low**	**Moderate**	**High**	**Unburned**
Little Beaver Creek	4679	2403–3546	86	18	51	17	14
Jack's Gulch	767	2425–2969	97	35	58	4	3
SF Cache la Poudre	22,708	1199–4079	45	19	21	5	55

TABLE 2 | Mean nitrate-nitrogen (NO3-N), ammonium-nitrogen (NH4-N), total dissolved nitrogen (TDN), temperature, dissolved oxygen (DO), depth, current velocity, canopy cover and grazer density at each geomorphic position (upstream or downstream of beads) during high flows in spring and low flows in autumn.

Season	Position	NO3-N (mg/L)	NH4-N (mg/L)	TDN (mg/L)	Temp (°C)	DO (mg/L)	Depth (cm)	Vel. (m/s)	Canopy cover (%)	Grazer density (indiv./m^2)
Spring	Above	0.26	0.013	0.43	11	6.7	22	0.39	16.5	2743
	Below	0.25	0.010	0.39	11	7.4	19.7	0.38	30.3	3132
Autumn	Above	0.16	0.021	0.28	9.5	7.7	16.3	0.20	36.6	9378
	Below	0.13	0.020	0.23	9.7	7.8	16.6	0.19	29.1	5117

watersheds with snowmelt-dominated hydrology, the post-fire stream nitrate increase is most evident during spring runoff, and the response can persist for years to decades (Rhoades et al. 2019).

2.2 | Experimental Design

We selected three river bead reaches within the fire perimeter (Table 1). These sites included the main stem South Fork Cache la Poudre, Little Beaver Creek and Jack's Gulch (Figure 1). Nutrient diffusing substrates (NDS) were deployed at the upstream and downstream ends of the beads (see aerial imagery in Figure S1 for exact locations within each study reach). Sites were selected in an effort to minimize differences in forest canopy cover, stream velocity, depth and stream bed substrate. Herbaceous vegetation and woody shrubs within the bead floodplain had largely re-established at the time of the study, though tree regeneration in the surrounding area and uplands was sparse. We did not deploy NDS in unburned catchments because our research question was focused on the effects of river beads in burned catchments, rather than the effects of wildfire in general. Effects of beads on nutrient dynamics in unburned beads have also been previously studied (Wegener et al. 2018).

The nutrient diffusing substrate treatments included an unamended control, nitrogen amendment (N), phosphorus amendment (P) and a nitrogen and phosphorus amendment (NP). All treatments were replicated five times at each upstream and downstream bead location. We constructed NDS by filling 1 oz. polyethylene containers with unamended 2% laboratory grade agar for controls, agar with 0.5 M sodium nitrate ($NaNO_3$) for N treatments and agar with 0.5 M potassium phosphate dibasic (K_2HPO_4) + 0.5 M potassium phosphate monobasic (KH_2PO_4) for P treatments. These concentrations were based on recommendations from Tank et al. (2017) and are commonly used in NDS studies. The $N+P$ treatments contained both nutrients at the same concentrations described above. Containers were topped with fritted glass discs (5.7 cm^2, EA Consumables, Marleton, NJ) to promote diffusion and colonization on a uniform substrate. We attached five randomly placed replicates of each treatment to steel racks within a grid design and anchored them to the stream bed using rebar at each site. In 2023, NDS were incubated at the South Fork site for 20 days in low flow conditions from September 5th to September 25th. In 2024, NDS were incubated for 20 days at Little Beaver Creek and Jack's Gulch during high flow, high stream nutrient conditions from June 20th to July 10th and again at low flow, low nutrient conditions from August 30th to September 19th. NDS were not deployed at the South Fork in spring because this site had much higher discharge than the others, which would potentially destroy the experimental array. After the incubation periods, we collected fritted glass discs from containers in the field, placed them in plastic bags covered with tin foil to protect from chlorophyll-*a* (Chl *a*) degradation by sunlight during transportation and stored them in a dark freezer (−18°C) until further processing.

2.3 | Environmental Factors

We characterized physical and biological variables at each study site to evaluate their influence on algal responses to nutrients

(Table 2). Stream temperature (°C) and dissolved oxygen (mg/L) were recorded in 15-min intervals throughout the 20 day experiments at each site using miniDOT loggers (Precision Measurement Engineering, Vista, CA). We collected two water grab samples in acid-washed, stream-rinsed 1 L and 250 mL high-density polyethylene bottles weekly during NDS deployment in 2024 and once each during installation and takedown in 2023 to measure stream nitrate, ammonium (NH_4-N), phosphate (PO_4-P) and total dissolved N (TDN). We collected three replicate Surber samples (0.09 m^2 in area, 600 μm mesh) to quantify potential differences in grazing macroinvertebrates once during each NDS incubation period at each site. Samples were collected from riffle habitats adjacent to the NDS experiments, preserved in 80% ethanol and transported to the laboratory. Macroinvertebrate samples were subsampled to 200 individuals using a gridded tray with 150 μm mesh and then identified to family to assign functional feeding groups including predators, collectors, shredders and grazers (Merritt et al. 2019). We also measured the percentage of riparian canopy cover approximately above each NDS site as a proxy for light availability using a densiometer. Current velocity and depth were measured at each experimental location using a velocity meter and top-setting wading rod.

2.4 | Laboratory Sample Processing

Fluorometric analysis of Chl a was used to quantify autotrophic algae on fritted glass discs following Environmental Protection Agency (EPA) method 445.0 (Arar and Collins 1997). Each fritted glass disc was placed in a 0.5 oz. polypropylene jar with 10 mL of acetone and extracted for 24 h in a dark refrigerator. Extracts were run on a fluorometer to quantify Chl a (raw fluorescence unit, RFU) using a non-acidification module (Turner Designs, Sunnyvale, CA) to account for the presence of chlorophyll b and pheophytin *a* in the samples. Chl *a* concentration (μg/cm^2) was calculated by multiplying the difference between the fluorometer reading of the sample (R_b) and the average blank sample reading (blank) by the response factor ($F_s = 0.1724\,\mu g/L \times RFU - 1$) and the sample extract volume (V) divided by the area of the fritted glass disc (a) using the following equation (Arar and Collins 1997):

$$\text{Chl a} = \frac{\left(R_b - \text{blank}\right) \times F_s \times V}{\text{a}}$$

We calculated the response ratio of Chl *a* concentration in the treated versus control samples as a measure of the treatment effect (i.e., ln[mean treatment Chl *a* concentration/mean control Chl *a* concentration]). Positive response ratios indicate algal growth was enhanced in the amended treatments relative to controls whereas negative ratios indicate that the amendments suppressed growth. We inferred primary limitation if just the N or P treatment significantly enhanced growth, co-limitation if the N and P or $N+P$ treatments significantly enhanced growth and secondary limitation if N or P alone plus the $N+P$ treatment significantly enhanced growth (Tank and Dodds 2003).

Grab samples were sub-sampled and filtered through 0.7 μm pore-size glass fibre filters (Millipore Corp, Burlington, MA) and then analysed for TDN using a C/N analyser (Shimadzu Corporation, Columbia, MD). Sub-samples for anions and cations were filtered through 0.45 μm pore-size membrane filters (Millipore Durapore PVDF, Billerica, MA) and analysed using ion chromatography (Thermo Fisher, Waltham, MA). Samples were analysed for acid neutralizing capacity (ANC), pH and specific conductivity (SC) using an inMotion Pro automated titrator (Mettler Toledo, Columbus, Ohio, USA).

2.5 | Periphyton Community Composition

We attached three additional unamended fritted glass discs to a steel bar that was anchored to the stream bed as a uniform substrate to evaluate the community composition of benthic periphyton at each site during the 20 day NDS experiments. Sample surfaces of the fritted glass discs were brushed with sterile swabs and placed in 15-mL falcon tubes, transported to the lab and stored at −20°C until DNA extraction and amplification.

Swabs were bisected; one half was stored as a backup at −20°C while the other was immediately extracted. *Quick* DNA Faecal/Soil Microbe Microprep Kits (Zymo Research, Irvine, CA) were used to extract DNA following the manufacturer's protocol. The v4 region of the 16S rRNA gene was amplified using 515f and 806r Earth Microbiome Project primers with modification for Illumina adaptors (Thompson et al. 2017), while the v9 region of the 18S rRNA gene was amplified using the Euk_1391f and Euk_Br reverse primers (Amaral-Zettler et al. 2009). Amplicons were multiplexed using unique Access Array Barcodes (Fluidigm, San Francisco, CA) in a second PCR step and normalized using a SequalPrep Normalization Plate Kit (Applied Biosystems, Waltham, MA). Samples were sequenced on an Illumina MiSeq for 300 cycles at the Colorado State University Microbiome Core.

The resulting reads were processed in the QIIME2 environment (Bolyen et al. 2019) using the 2023.9 release. Samples were trimmed and denoised using DADA2 (Callahan et al. 2016). Two samples were removed from the 16S rRNA gene dataset due to having < 100 assembled sequences. Sequence counts in the remaining samples ranged from 671 to 172,166 16s rRNA gene sequences. For the 18S rRNA gene sequencing, sequence counts ranged from 1357 to 57,645 sequences, all of which were retained for analysis. Accumulation curves were generated for 16S and 18S amplicon sequence variants (ASVs) using the 'rarecurve' function from the R-package 'Vegan' (Oksanen et al. 2025) and all samples were observed to reach saturation. Both 16S and 18S ASVs were classified taxonomically using the Silva 138.1 database (Quast et al. 2013; Bokulich et al. 2018; Robeson et al. 2021). ASVs assigned as chloroplasts, mitochondria or unassigned were filtered from the 16S rRNA gene dataset while those assigned as Vertebra, Insecta, Arachnida, Bacteria, Archaea or unassigned were removed from the 18S rRNA gene dataset. Relative abundance was calculated after summing counts at the class level per sample. We focused our analyses on algal phyla per the authority of Guiry (2024) and the phylum Euglenozoa were subset to only contain the photosynthetic class Euglenophyceae (Guiry 2024).

2.6 | Data Analysis

We analysed changes in stream nutrient concentrations (NO_3-N, NH_4-N or TDN) using linear mixed effects models (LME) with bead position (upstream or downstream of beads) and seasonal flow conditions (high flow, low flow) as fixed effects and stream identity as a random effect to account for the paired upstream and downstream design. Stream nutrient concentrations were log-transformed to meet assumptions of normality. Stream PO_4-P concentrations were below the analytical detection limit (<0.01 mg/L) in all samples. We analysed results from the NDS experiments using a LME that included interactions between treatment type (C, N, P, NP), bead position, and season as the predictor variables, stream as a random effect and log-transformed Chl *a* concentration as the response variable. Following this LME, we used post hoc Dunnett's tests to assess whether Chl *a* concentrations on a given treatment were significantly different from controls at each position and season to identify nutrient limitation status during experiments. To test our hypothesis about whether algal N limitation was stronger downstream of beads seasonally, we used a LME with bead position, season and their interaction as fixed effects, stream identity as a random effect and response ratios of N-containing treatments (N, NP) as the response variable. To help interpret NDS results and evaluate how environmental factors influenced algal N limitation, we used a LME with NO_3-N, NH_4-N, temperature, DO, canopy cover, current velocity, depth and macroinvertebrate grazer density as predictor variables, a random effect of stream and N-containing amendment response ratios (N, NP) as the response variable. N-containing amendments were grouped for this analysis because those treatments responded similarly. Lastly, to test for differences in the abundance of eukaryotic algae and cyanobacteria between bead positions within each season, we used paired Wilcoxon signed-rank tests. All statistical analyses were completed in R statistical software (v4.4.2; R Core Team 2024) and we used 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017) for the linear models.

3 | Results

3.1 | Water Chemistry

Stream NO_3-N and TDN concentrations decreased significantly from spring to autumn (NO_3-N: $p=0.002$; TDN: $p=0.01$). The magnitude of the seasonal decrease was on average 63% for NO_3-N and 50% for TDN (Figure 2a,b). NO_3-N and TDN decreased 10%–25% from upstream to downstream of beads, but the differences were not statistically significant (NO_3-N: $p=0.69$; TDN: $p=0.94$, Figure 2a,b). We also did not observe a significant bead position-by-season interaction for NO_3-N or TDN (Table S1). NH_4-N concentrations did not differ significantly by season, bead position or their interaction (Figure 2c, Table S1), although concentrations were approximately 75% higher during autumn.

3.2 | Algal Responses and Nutrient Limitation Patterns

The effects of nutrient treatments on algal growth (i.e., Chl *a*) depended on both season and bead position, resulting in a significant three-way interaction (LME, treatment*season*position, F value = 2.8, $p=0.04$). In spring at the upstream sites, N treatments increased algal growth by 140% (LME, Dunnett's test, $p=0.008$, Table S2) and NP treatments increased algae growth by 275% ($p<0.0001$, Figure 3c, Table S2). The P amendment also significantly enhanced algal growth relative to controls by 111% upstream of beads in spring ($p=0.02$, Figure 3c, Table S2). Downstream of beads in spring, the NP amendment enhanced algal growth relative to the control by 116% (NP, $p=0.03$, Table S2). Significant, positive responses of Chl *a* to both N and P-containing amendments (i.e., positive response ratios) during spring indicated that N and P were co-limiting regardless of location (Figure 3a,c). During autumn, N and NP amendments significantly enhanced algal growth relative to controls by approximately 145% for N ($p=0.001$) and 190% for NP ($p=<0.0001$, Figure 3d) at the downstream location only, indicating primary N and secondary P limitation downstream of beads and no evidence for nutrient limitation upstream of beads at this time of year.

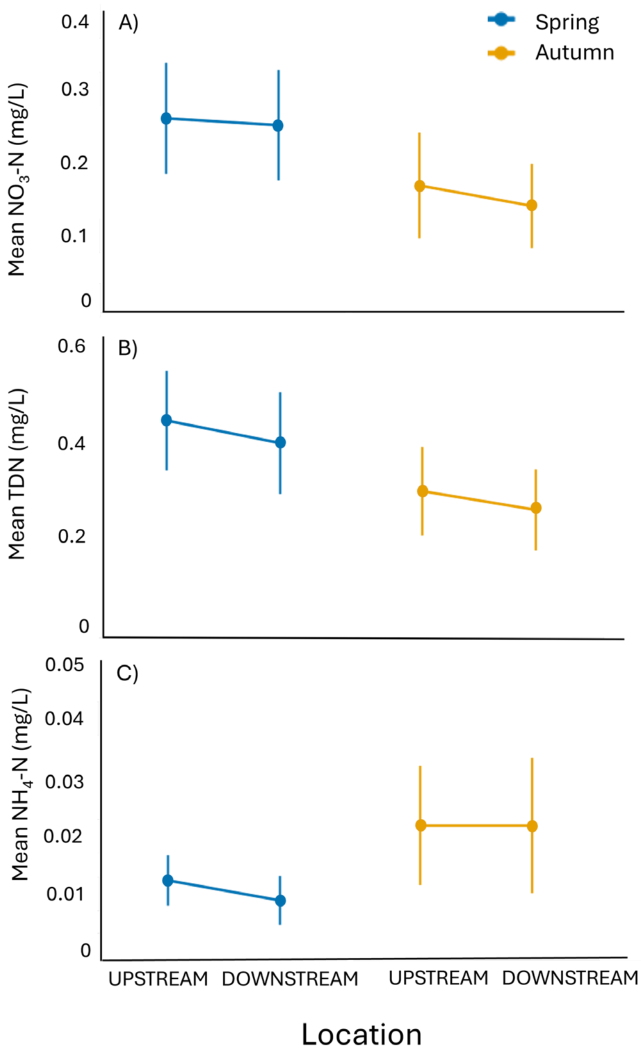

FIGURE 2 | Mean NO_3-N (A), TDN (B) and NH_4-N (C) concentrations upstream and downstream of beads during spring (blue) and autumn (orange) throughout the duration of NDS experiments. Bars represent standard errors of the mean nutrient concentration for each group.

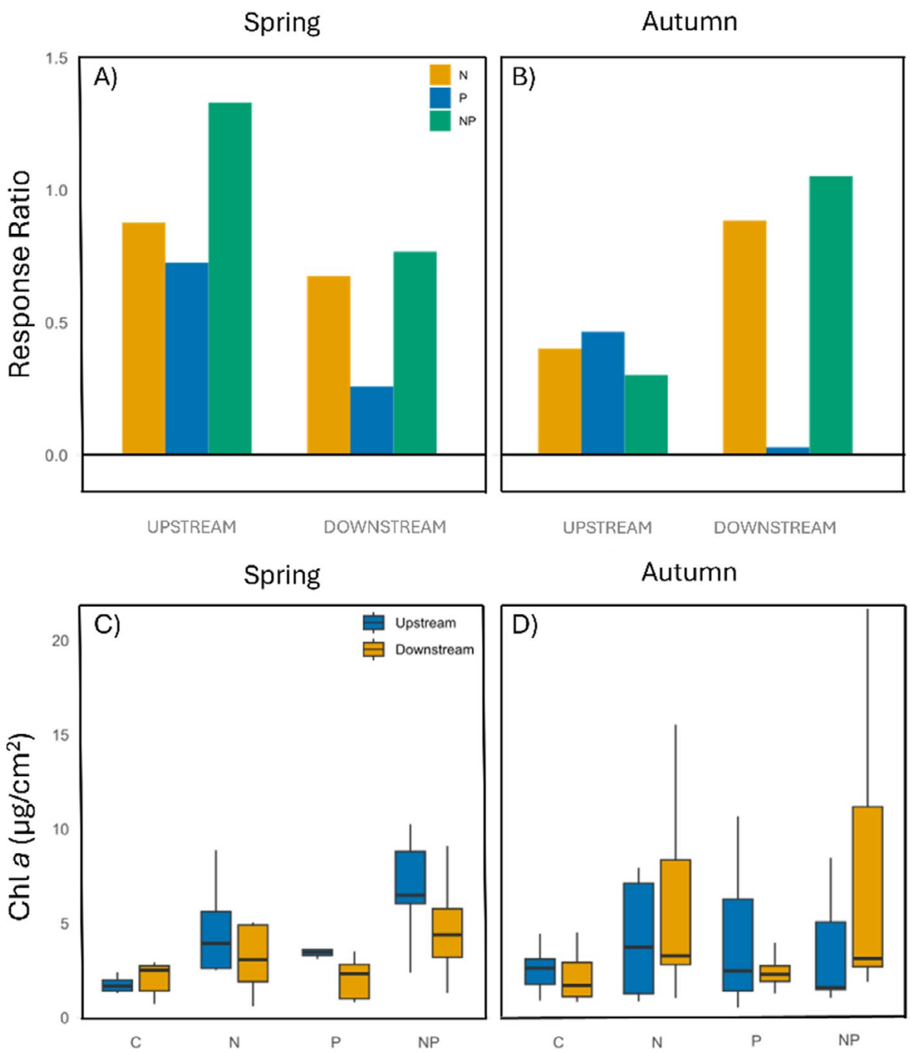

FIGURE 3 | Algal response to NDS nutrient treatments above and below river beads during spring and autumn. The top panel shows nutrient amendment response ratios (ln[mean treatment Chl *a*/mean control Chl *a*]) upstream and downstream of beads during spring (A) and autumn (B) (nitrogen, blue; nitrogen + phosphorous, orange; phosphorous, green). The bottom panel shows raw Chl *a* of controls and nutrient treatments upstream (blue) and downstream (orange) of beads during spring (C) and autumn (D). The bold horizontal lines in each boxplot indicate medians, the height of the box shows the extent of the interquartile range, and the whiskers extend 1.5 times the interquartile range.

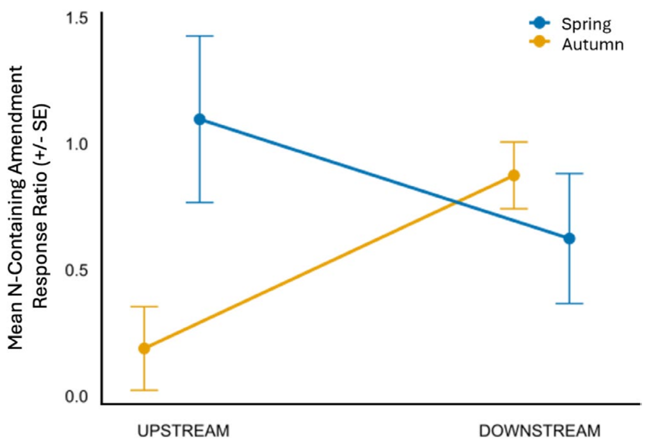

FIGURE 4 | Mean response ratios of N-containing (N, NP) nutrient treatments upstream and downstream of river beads during spring (blue) and autumn (orange). Bars represent standard errors of the mean N-containing amendment response ratios.

Response ratios for treatments containing N were dependent on both season and bead position, resulting in a significant two-way interaction (LME, season*location, F value = 12.56, p = 0.003, Figure 4; see Table S1 for all LME test statistics). During spring, N-containing amendment response ratios were larger upstream of beads than downstream by approximately 52% (Figure 3a). This pattern was opposite to what was observed during autumn, where N-containing amendment response ratios were larger downstream of beads than upstream (Figure 3b,d). Downstream response ratios of the N and NP amendments were 120% and 250% higher, respectively, than upstream in autumn (Figure 3b).

3.3 | Environmental Influences

Several of the environmental variables were significantly correlated with N-containing amendment response ratios. Grazer density had the strongest effect, negatively impacting

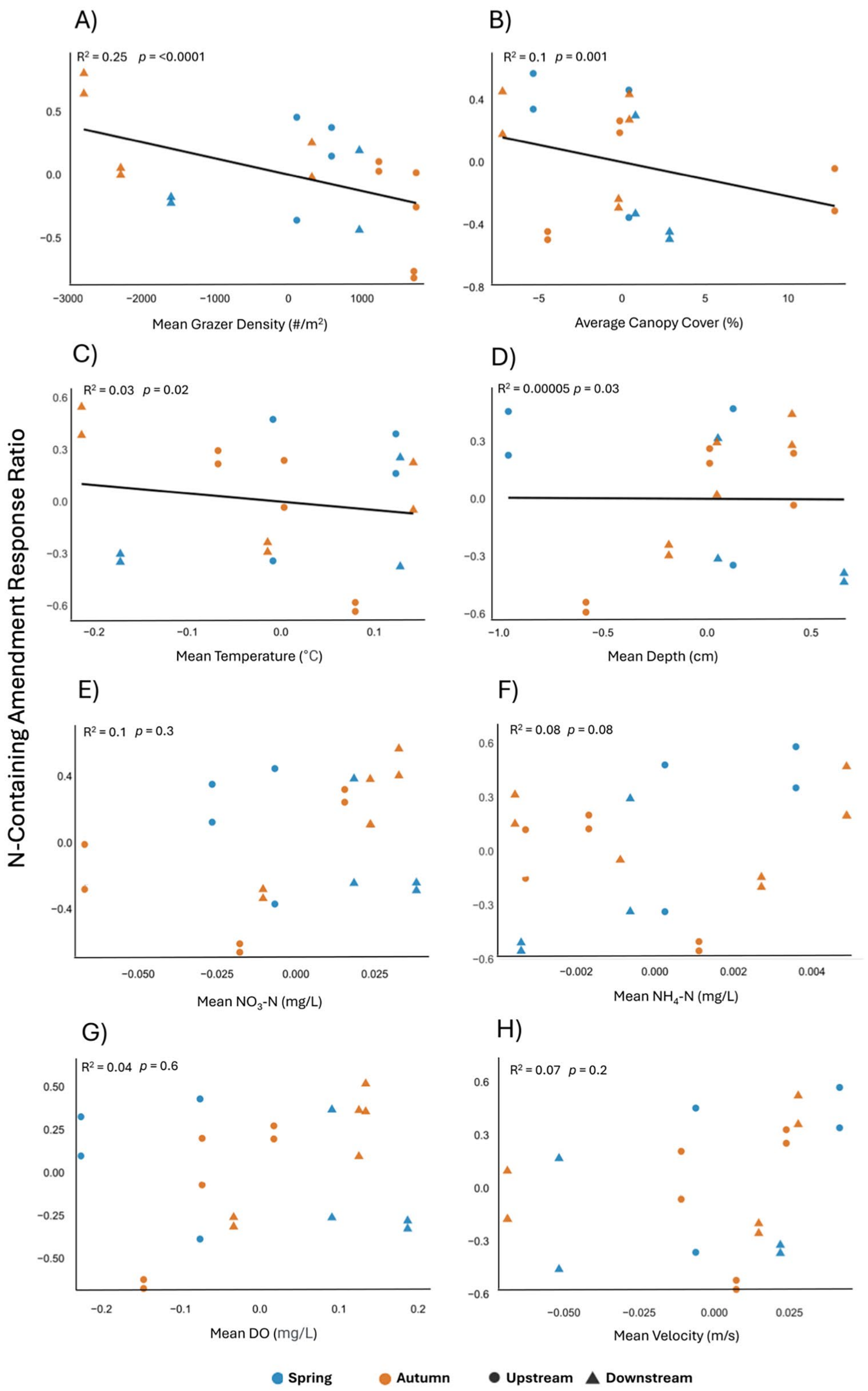

FIGURE 5 | Partial regression plots showing relationships between N-containing amendment response ratios and mean grazer density (A), average canopy cover (B), mean temperature (C), mean stream depth (D), mean NO_3-N (E), mean NH_4-N (F), mean dissolved oxygen (G) and mean current velocity (H). The *x*-axis displays residuals of the predictor variable after accounting for all other predictors in the model, and the *y*-axis shows residuals of the N-containing amendment response ratios after accounting for the remaining predictors in the model (not including the one on the *x*-axis). Each point represents an individual sample symbolized by location (upstream, circle; downstream, triangle) and season (spring, blue; autumn, orange). The solid black line indicates the linear relationship between the N-containing amendment response ratios and the predictor variable.

N-containing amendment response ratios (LME, partial $R^2 = 0.25$, $\beta = -0.0003$, $p < 0.001$, Figure 5a). Canopy cover (partial $R^2 = 0.097$, $\beta = -0.07$, $p = 0.001$, Figure 5b), temperature (partial $R^2 = 0.025$, $\beta = -4.18$, $p = 0.02$, Figure 5c) and stream depth (partial $R^2 = 0.00005$, $\beta = -0.3$, $p = 0.03$, Figure 5d) were also negatively associated with N-containing amendment

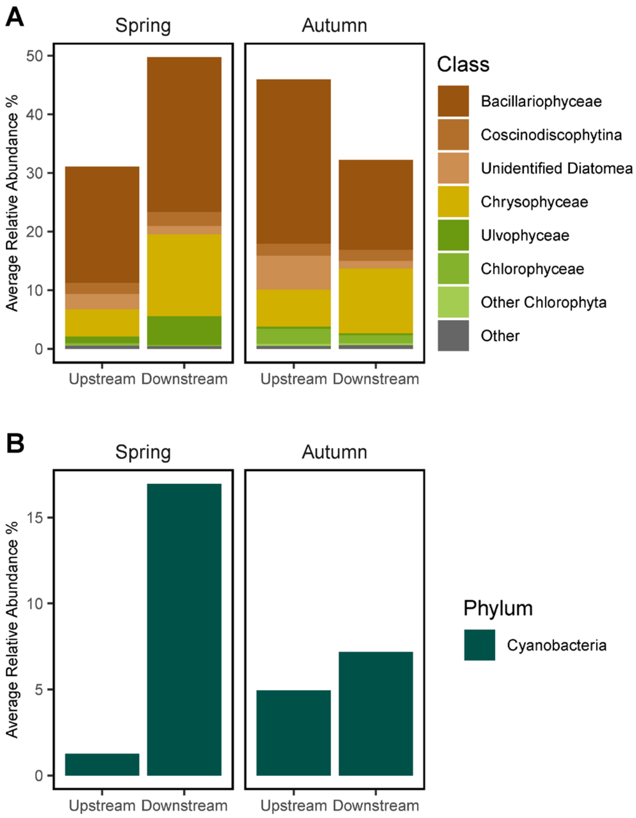

FIGURE 6 | Average upstream and downstream relative abundances of eukaryotic algae (A) and Cyanobacteria (B) during spring and autumn. Stacked bars for Eukaryotic algae are coloured by their broad classification as diatoms/brown algae in brown, golden algae/Ochrophyta in yellow and Chlorophyta/green algae in green.

response ratios, although these variables explained relatively little variation in the model. Stream nutrients were not as influential: stream NO_3-N and NH_4-N explained 8%–10% of the model variation (NO_3-N, partial $R^2=0.1$, $\beta=14.05$, $p=0.3$; NH_4-N, partial $R^2=0.08$, $\beta=-75.3$, $p=0.08$, Figure 5e,f). Dissolved oxygen (DO, partial $R^2=0.04$, $\beta=0.2$, $p=0.6$) and velocity (partial $R^2=0.07$, $\beta=13$, $p=0.2$) were not significant predictors of N-containing amendment response ratios (Figure 5g,h). Mean values for environmental variables are provided in Table 2.

3.4 | Periphyton Composition

Bead position and season influenced the relative abundance and membership of algal populations. In spring, eukaryotic algae were more abundant below beads than above them (Wilcoxon, $W=3$, $p=0.007$, Figure 6a), while the inverse pattern of greater relative abundance of eukaryotic algae above beads was observed, but not significant, in autumn (Wilcoxon, $W=21$, $p=0.35$, Figure 6a). Select phyla also exhibited distinct patterns; in autumn, *Ochrophyta* (i.e., golden algae) were more abundant below beads compared to above (Wilcoxon, $W=7$, $p=0.046$). There was also a shift in Chlorophyta membership seasonally, being dominated by *Ulvophyceae* in spring and *Chlorophyceae* in autumn (Figure 6a). Lastly, cyanobacteria had greater relative abundance below beads than above, especially in spring (Figure 6b).

4 | Discussion

The nutrient diffusing substrate experiments indicated that algal N limitation varied in relation to river bead position in fire-affected, high elevation streams. Consistent with our hypothesis, algal responses to N were stronger downstream of beads than upstream in lower stream NO_3-N conditions during low flows. At this time of year, algae downstream of beads were primarily limited by N, while we found no evidence of nutrient limitation upstream of beads. We observed the opposite pattern during high flow, high stream NO_3-N conditions, where algal responses to N were stronger upstream than downstream. Our findings also showed that seasonally shifting environmental variables, especially macroinvertebrate grazer density, influenced algal responses to N in fire-affected streams. These results highlight how physical features of rivers, such as the presence of beads, can interact with biological factors, such as grazing, to influence algal responses to nutrient amendments.

To better understand the mechanisms driving spatial and temporal variation in N limitation, we evaluated stream chemistry above and below river beads and across seasons. In-stream NO_3-N and TDN concentrations changed significantly by season but less so in relation to bead position, although we did generally observe small downstream nutrient reductions. Significant seasonal declines in stream NO_3-N and TDN concentrations aligned with our expectations that spring snowmelt transports terrestrial NO_3-N to the aquatic environment, increasing in-stream concentrations during spring and early summer, with decreasing concentrations during low flows in late summer and autumn. While spatial differences in NO_3-N and TDN were not significant, downstream reductions in NO_3-N were most pronounced during low flows in autumn. During base flows, lower stream velocities and longer water residence times can enhance biological processing and nutrient removal (Kaushal et al. 2008; Klocker et al. 2009; Cunha et al. 2018). Seasonal changes in discharge, however, are also likely to drive temporal changes in floodplain connectivity, which should influence N uptake. A previous study in an unburned catchment in the Colorado Rockies found that an unconfined valley segment in a stream acted as a NO_3-N sink during peak flows and a NO_3-N source during low flows (Wegener et al. 2018). Notably, NO_3-N concentrations in our study streams were more than double the reported NO_3-N concentrations in the previous study (Wegener et al. 2018), which was on unburned catchments. The river beads in Wegener et al. (2018) were also larger, with more extensive side channels and off-channel ponds, which likely increased floodplain connectivity and N retention at those sites during spring snowmelt. The beads in our study had relatively little side-channel aquatic habitat and likely less floodplain connectivity at the time of our study. This may explain the relatively modest changes in nutrient concentrations from above to below beads at our study sites.

The NDS results indicated a shift from primary N limitation of algae during low flows to N and P co-limitation during high flows. The significant, positive response of Chl *a* to the N and NP amendments indicated a primary N limitation with secondary P limitation downstream of beads in autumn under low stream NO_3-N conditions (Tank and Dodds 2003). In contrast, in spring the significant, positive response of Chl *a* to the N, P and NP amendments upstream of beads, as well as to the NP amendments downstream of beads, indicated co-limitation of N and P (Tank and Dodds 2003) under high stream NO_3-N conditions. Our findings during high flows were consistent with a previous study in the Colorado Front Range that found streams draining burned catchments were N and P co-limited, likely due to elevated post-fire NO_3-N concentrations (Rhea et al. 2021). That said, when compared to other environmental covariates, nutrient concentrations alone were relatively weak predictors of N-amendment response ratios in our study. Other studies using NDS under high N conditions have found that N amendments can promote algal growth due to differences between stream concentrations and bioavailability (Earl et al. 2006; O'Brien et al. 2007), which may decouple algal nutrient limitation from water column nutrients. Indeed, ambient stream nutrient concentrations are often a relatively weak predictor of algal nutrient limitation status, with other covariates sometimes playing an equal or more important role (Wold and Hershey 1999; Beck et al. 2017).

Among environmental variables, macroinvertebrate grazer density was most strongly correlated with N amendment response ratios. Grazers exert strong top-down controls on periphyton by consuming algal biomass and altering community structure (Rosemond et al. 1993; Feminella and Hawkins 1995), especially under high grazer densities (Hillebrand 2009; Beck et al. 2019). Additionally, a meta-analysis of 85 experiments found that negative effects of macroinvertebrate grazers on periphyton biomass were stronger than the positive effects of nutrient additions (Hillebrand 2002). In our study, we found the highest grazer densities composed primarily of mayflies (families *Baetidae* and *Heptageniidae*) at upstream bead locations during autumn, coinciding with the absence of nutrient limitation at this site. Beck et al. (2019) found that grazing effects on periphyton in the South Fork Cache la Poudre upstream of our study sites were strongest in late summer when grazer densities, particularly of heptageniid mayflies, were highest. While upstream and downstream sites were selected for similar canopy cover, velocity and depth, the elevated grazer densities observed at upstream sites may reflect favourable microhabitat conditions associated with the transition from steeper, high-velocity confined reaches into broader, low-gradient unconfined segments. These transitional areas can accumulate organic matter from the upstream transport reach, supporting algal growth and providing food resources at the base of the food web (Rice et al. 2001). It is possible that the high grazer densities in these areas are a response to favourable conditions for algae. Experimental manipulations of grazers, alongside nutrient amendments, would be needed to elucidate the strength of top-down versus bottom-up control in relation to bead position. Future work on spatial variation in macroinvertebrate communities in relation to river beads would also be useful to test the generality of that finding.

Several of the other environmental factors we evaluated were shown to be predictors of N-containing amendment response ratios. Average canopy cover, stream depth and mean temperature were significantly negatively associated with N-containing amendment response ratios. Stream periphyton can be nutrient limited under low canopy cover, high light conditions and light limited under high canopy, low light conditions (Warren et al. 2017). Other work has shown that nutrients were more limiting during seasons with higher light availability (Rosemond et al. 2000). In our study, canopy cover was nearly 1.5 times higher during autumn than spring, suggesting that differences in canopy cover may have modulated the strength of N limitation. Similarly, stream depth is associated with light attenuation, as deeper water may inhibit light availability to the benthos (Morgan et al. 2006). Stream depth was similar between bead positions but varied slightly between study streams and was deeper during high flows; however, it explained relatively little variation in response ratios and probably was not deep enough to cause light limitation. Mean temperature was also a significant predictor of N response ratios, with temperatures being higher in spring, but not varying strongly with bead position. The warmer temperatures in spring and early summer seem consistent with prior work in this catchment and may reflect warmer air temperatures at this time of year relative to fall (Shaw et al. 2017; Beck et al. 2019). Other studies have found that temperature is an important factor in algal response to nutrients, with warmer temperatures increasing growth and uptake rates (Cross et al. 2015) as well as biomass (Francoeur et al. 1999; Myrstener et al. 2018). Counter to expectations, current velocity was not a strong predictor of N-containing amendment response ratios, though it has been shown to both increase algal nutrient availability (Hiatt et al. 2019) and decrease algal growth rates (Biggs 1998).

Microbial sequencing indicated subtle shifts in periphyton composition from above to below beads and across seasons. NDS experiments typically focus on Chl *a* or algae biomass, with few studies examining composition. Interestingly, the relative abundance of eukaryotic algae was higher above beads in autumn and below beads in spring. These locations correspond with the bead locations that had the lowest levels of nutrient limitation based on response ratios. While many factors control algal composition, it is possible that the relative abundance of eukaryotic algae in the community was changing in response to spatial differences in nutrient limitation. The eukaryotic algae were dominated by diatoms (Bacillariophyceae), which can reflect changes in nutrient status of streams (Lobo et al. 2016; Lavoie et al. 2008). One group—the golden algae (Chrysophyceae) had higher relative abundance below beads. This group has a variety of functional strategies (Bock et al. 2022), making it challenging to link this shift to a specific mechanism relative to nutrient limitation. In general, algal richness and composition are expected to influence rates of nitrogen assimilation, and communities with greater proportions of nitrogen fixers should have lower rates of N uptake from stream water and have a competitive advantage when N is limiting (Baker et al. 2009). However, we found cyanobacteria relative abundance to be highest downstream of beads in spring, which was not among the most N-limited locations based on the NDS results. Future studies to link periphyton composition to stream nutrient status following wildfire would be useful to more mechanistically understand these linkages.

The influence of the beads in our study on nutrient dynamics should be considered within the historical context of stream ecosystem changes in the study area. The river beads had indications of degradation such as channel incision and straightening, and generally lacked the full complexity and hydrologic connectivity typically associated with unconfined stream-floodplain environments. Historically, the study beads may have supported greater nutrient, sediment and water retention facilitated by beaver dams and large wood (Wohl et al. 2018). Long-term effects of historic logging and beaver removal have reduced the biotic drivers of stream complexity in the study area (Polvi and Wohl 2013), potentially dampening the capacity of beads to function as zones of nutrient retention. This loss of complexity may partially explain why observed downstream reductions in N were lower than expected. Additionally, increasing N deposition has been observed on the Colorado Front Range for decades (Lewis and Grant 1980), potentially influencing biological uptake of N as streams become saturated (Wegener et al. 2018). Together, fire-driven increases in nutrient inputs, degraded bead functioning and chronic N deposition may interact to reduce the sink potential of stream reaches that might otherwise serve as key biogeochemical hotspots in the stream network (McClain et al. 2003).

Our findings have implications for stream restoration in post-fire catchments. This study included two stream reaches prior to restoration using low-tech-process-based restoration (LTPBR) to mitigate post-fire impacts, presenting an opportunity for studies that evaluate how LTPBR affects nutrient retention and algal dynamics in burned catchments. Restoring geomorphic complexity to degraded river beads may improve the capacity of unconfined channel segments to retain nutrients (Bukaveckas 2007) and regulate benthic primary production following fire. Increasing stream-floodplain connectivity and enhancing structural heterogeneity may support more resilient nutrient uptake and retention dynamics in streams with elevated N, helping to mitigate effects on water quality in areas that supply water for human needs. As both stream restoration efforts and wildfires increase, studies that link geomorphology and ecological processes will be critical for advancing our understanding of stream resilience in the face of growing disturbance.

Author Contributions

Conceptualisation: A.M.G., T.S.F., A.E.R., C.C.R., D.L.P. Developing methods, conducting the research and data interpretation: A.M.G., T.S.F., A.E.R., W.J., C.C.R., M.J.W., D.L.P. Data analysis: A.M.G., A.E.R., D.L.P. Preparation of figures and tables: A.M.G. Writing: all authors.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data from this work are available from the corresponding author upon request. 16S and 18S rRNA gene amplicon sequences are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the bioproject number PRJNA1311598. The biosamples utilized in this project (SAMN) were 50683598–50683622 and 50683634–50683657.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Study site locations upstream and downstream of river beads in South Fork (A), Little Beaver Creek (B) and Jack's Gulch (C). See Table 1 for watershed characteristics of each stream. **Table S1:** Results of the linear mixed effects models (LME) for each response variable and associated predictor variables. **Table S2:** Post hoc Dunnett's test following the Chl *a* response LME (see Table S1 for LME results) *p*-values comparing each nutrient treatment with the control within locations during high flow in spring and low flow in autumn.