

Promoting revegetation and soil carbon sequestration on decommissioned forest roads in Colorado, USA: A comparative assessment of organic soil amendments



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ABSTRACT

Forest roads are commonly decommissioned and revegetated to decrease erosion, prevent weed encroachment, manage recreation and improve overall watershed condition on federal lands, but may also provide a complementary opportunity to sequester carbon (C) in soils. Soils on decommissioned roads are typically compacted with limited capacity for water retention, decreased mineral nitrogen (N) availability and low organic matter content, impairing revegetation and soil C sequestration efforts. We evaluated the effects of an organic fertilizer, wood strand mulch and a woody biochar on soil physical, chemical and biological processes to improve revegetation and C sequestration on decompacted forest roads. We monitored plant and soil responses to the treatments and their combinations over three growing seasons on four decommissioned road segments in northern Colorado. The organic fertilizer increased plant available mineral N for the first year of the study and resulted in a 21% increase in total plant cover and 67% increase in root biomass. The wood strand mulch increased total plant cover and root biomass to a similar extent, but had no effect on soil water content or mineral N availability. Instead, mulch stimulated soil microbial respiration and increased soil C content, two of the best predictors of plant cover and biomass. The woody biochar increased soil water content by 26% and elevated mineral N availability throughout the study, but did not improve plant cover, above- or belowground biomass. Mulch, biochar and their combined treatments sequestered C, but through distinct pathways. Microbial processing of wood strand mulch added C to the mineral soil fraction, whereas biochar added C directly to the coarse particulate fraction with no effect on mineral soil C or soil respiration. Restoration practitioners can utilize these results to inform management decisions and guide further research on different rates and combinations of organic amendments to revegetate and sequester C on decommissioned forest roads.

1. Introduction

Forest lands in the United States contain a vast network of unpaved roads that increase sediment delivery to streams (Reid and Dunne, 1984), alter hillslope hydrology (Eastaugh et al., 2008) and create habitat fragmentation (Robinson et al., 2010; Trombulak and Frissell, 2001). Road construction and maintenance activities are primary sources of sediment to forest streams (Megahan et al., 2004), and sediment delivery rates from road surfaces can equal those from severely burned hillslopes (MacDonald and Larsen, 2009). One strategy to minimize the watershed effects of unpaved roads is to decommission underutilized roads and those located on sensitive soils, riparian and habitat areas (Madej, 2001; Switalski et al., 2004). The US Forest

Service has widely adopted this strategy, decommissioning 2500–8000 km of forest roads each year (Coghlan and Sowa, 1998; Forest Service, 2010). Road decommissioning includes a wide variety of treatments such as gating and blocking roads, decompacting and obliterating road prisms, recontouring hillslopes and revegetating road corridors (Bagley, 1998). Revegetation of decommissioned roads benefits watershed conditions and native plant diversity where it minimizes soil erosion, improves native plant cover and reduces or prevents invasion of non-native plants (Elseroad et al., 2003; Swift, 1984; Switalski et al., 2004). Intensive treatments, such as decompaction and addition of amendments, may simultaneously enhance revegetation and soil C sequestration. However, organic soil amendments influence revegetation and C sequestration via numerous, interacting physical, chemical

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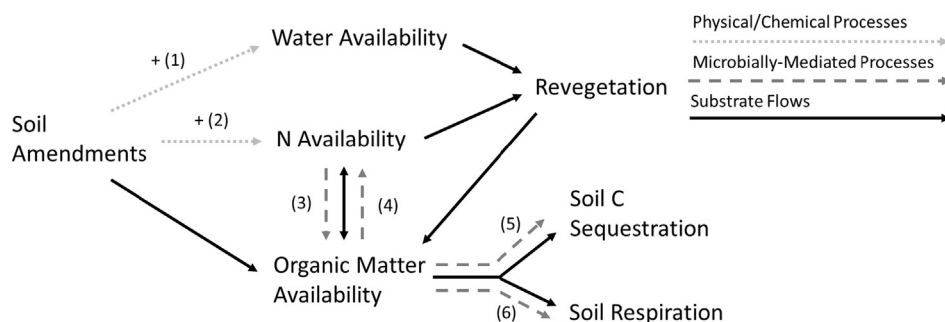


Fig. 1. Conceptual diagram depicting how organic soil amendments can impact revegetation and soil C sequestration through substrate flows of C, N and H₂O (black arrows), physical/chemical processes (light grey arrows) and microbially-mediated processes (grey arrows). Soil amendments can affect physical/chemical processes regulating soil water and N availability (with $\pm$ indicating the direction of the effect) by increasing soil water holding capacity or decreasing evaporation (1) and increasing the soil exchange capacity to retain mineral N (2). Amendments can also impact soil microbial activity by directly affecting mineral N availability through gross N immobilization (3) or N mineralization (4), directly supplying C which can be microbially-processed and stabilized to mineral surfaces to sequester C (5) or respired (6), or indirectly influencing soil moisture or temperature.

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and biological processes (Fig. 1), that must be evaluated to increase understanding of treatment efficacy and inform sound management decisions.

Plant recovery on closed forest roads is often limited by compacted soils, reduced seed bank, low soil organic matter (SOM) stocks, poor soil moisture water retention and decreased nutrient cycling (Elseroad et al., 2003). Low soil moisture can limit plant establishment, especially in arid and semi-arid ecosystems (Aronson et al., 1993). The low SOM on forest roads, reduces soil water retention, especially in rocky and coarse-textured forest soils (Rawls et al., 2003). Low SOM also results in limited substrates available to supply mineral N to plants through N mineralization (Booth et al., 2005). Soil structure is also important for revegetation with numerous studies demonstrating that alleviating compaction through mechanical ripping or biological activity enhance revegetation efforts (Alban et al., 1994; Ampoorter et al., 2011; Greacen and Sands, 1980). Decommissioned roads are typically seeded to facilitate rapid plant establishment to reduce erosion and invasion by non-native plants and provide habitat and forage for wildlife (Grant et al., 2011). However, after decompaction and seeding, SOM stocks may remain low relative to native soil (i.e., 30% lower (Viall et al., 2014)), suggesting that organic amendments that increase soil water, N and C content may more quickly or effectively meet revegetation objectives.

Soil amendments that persist over longer timespans (i.e. wood strand mulch or biochar), can improve soil water availability by increasing soil porosity, increasing the amount of water held at field capacity and decreasing soil evaporative losses. Mulching is commonly used to reduce surface erosion (Foltz, 2012; Sosa-Pérez and MacDonald, 2017), but may also benefit plant establishment by improving soil water retention (Benigno et al., 2013). Woody mulches made from forest residues have been shown to increase soil water content in post-fire rehabilitation treatments while utilizing non-merchantable materials and reducing surface fuels (Rhoades et al., 2015, 2017). Biochar, the product of biomass pyrolysis, may provide an alternative restoration treatment that can also be sourced from forest residues. Biochar has been shown to improve soil water retention by increasing soil porosity and surface area (Omondi et al., 2016), with numerous studies in forest soil revealing increased soil water-holding capacity after biochar application (Li et al., 2018).

Most forest ecosystems are N limited, and the low SOM content typical of decommissioned roads is likely to depress soil N availability and plant growth. Organic soil amendments can supply mineral N to enhance plant growth, but N in excess of plant demands can increase N losses to nearby waterways (Venner et al., 2009) or encourage competition from invasive plants (Davis et al., 2000). Organic fertilizer with low C:N ratios gradually release mineral N through mineralization. Compared to inorganic fertilizers, these organic amendments slowly deliver N over time. However, compared to recalcitrant organic amendments, the N supply from organic fertilizers is relatively short-lived. Recalcitrant organic amendments with high C:N ratios (e.g., wood strand mulch and biochar) are more likely to immobilize soil

mineral N (Bulmer, 2000) and temporarily reduce plant available N before releasing mineral N via mineralization (Rhoades et al., 2012; Rhoades et al., 2017). Amendments can also affect soil chemical properties. Biochar's high surface area and ion exchange capacity is known to increase soil N retention (Biederman and Harpole, 2013). Combining labile and recalcitrant organic amendments may provide water retention benefits and slow N release from high C:N material, but also ensure sufficient plant available N to support rapid revegetation of recently-decommissioned roads.

Organic soil amendments also contribute organic matter to fuel microbially-mediated processes and sequester soil C. As organic amendments decompose, a fraction of the C input is utilized by soil microbes to produce secondary compounds that can associations with soil minerals and persist in soils for decades to centuries (Grandy and Neff, 2008). In contrast, biochar amendments add highly-recalcitrant condensed polyaromatic C compounds to soil that resist microbial processing (Cross and Sohi, 2011), retain their porous structure and sequester C in soils for centuries (Spokas, 2010; Wang et al., 2016).

Organic fertilizers and mulches are commonly used to support revegetation of decommissioned forest roads, yet little is known about how their effects on chemical, physical and microbial soil processes may determine treatment efficacy. In this study, we examine organic fertilizer and wood strand mulch applications currently used to restore forest roads in Northern Colorado, as well as a woody biochar treatment, and combinations of the amendments. We expect soil amendments to have the greatest positive effect on revegetation and soil C sequestration of decommissioned roads when they reduce soil water, mineral N and SOM limitations (Fig. 1). We hypothesize:

- (1) Soil amendments, applied at rates commonly used in restoration practices, will impact revegetation and soil C sequestration on decommissioned forest roads through different processes:

Organic fertilizer can support revegetation by providing a short-term increase in N availability through N mineralization, but with no impact on soil moisture. Low C:N fertilizers will not significantly increase SOM content, thus will not sequester C.

Wood strand mulch can support revegetation primarily by increasing soil water content with mixed effects on mineral N; limiting N availability in the short-term due to its high C:N ratio leading to N immobilization, but supplying N through N mineralization in the long-term. Mulch can supply organic matter to support microbially-mediated process resulting in some soil C sequestration, but also increased soil respiration.

Woody biochar can support revegetation by increasing soil water content through increased porosity and increasing mineral N availability through enhanced soil ion exchange capacity. Biochar will sequester C in the particulate form and will not affect soil respiration or significantly increase the mineral soil C.

Combination treatments can support revegetation through synergistic effects of biochar and mulch improving soil moisture and the

addition of fertilizer decreasing short-term N limitation in the mulch and biochar treatments. Fertilized biochar treatments are expected to retain the most fertilizer N in the short term. The combined biochar and mulch treatment will have the greatest soil C sequestration potential from biochar's recalcitrance and contributions of mulch C to the mineral soil fraction.

- (2) Soil amendments will facilitate sustained soil restoration if they support the physical, chemical or biological processes that improve water and/or N availability to plants. Amendments that only contribute substrates to the soil will only provide short-term benefits.

The results of this study can have important implications for understanding how a variety of organic soil amendments and their combinations applied at standard rates alter soil processes that influence initial revegetation and C sequestration on recently-decommissioned forest roads.

2. Materials and methods

2.1. Study site and experimental design

To evaluate how various organic soil amendments impact revegetation and soil C sequestration on decommissioned forest road, we selected four (50–80 m long) road sections on the Arapahoe-Roosevelt National Forest near Red Feather Lakes, CO. Within the study area, average temperature ranged from 3.7 to 5.6 °C, with 429 to 491 mm rainfall (30-year normal, 1981–2010; [PRISM Climate Group, 2017](#)), and soils are characterized as Eutroboralfs, Argiborolls or Haploborolls ([NRCS, 2017](#)) with a gravelly to very gravelly loam texture (33–41% gravel, 27–30% sand, 26–31% silt, 4–12% clay). Roads were decompacted in October 2014 to a 30 cm depth using a three-tined mechanical ripper. Each road segment contained seven 3 m × 5 m plots with a 1 m buffer between plots. Within each road segment we randomly assign each of the seven treatments (an unamended control, organic fertilizer, wood strand mulch, woody biochar and their pairwise combinations). Each plot contained two 1 m² squares reserved for plant cover and biomass sampling, and eight designated locations for destructive soil sampling > 0.5 m from the plot edge. On Oct 16th, 2014, woody biochar, where applicable, was applied at a rate of 25 Mg ha⁻¹ (equivalent to a 2.1% by mass application rate) on the soil surface, and organic fertilizer, where applicable, was broadcast across the plot delivering 16 kg of N ha⁻¹. All plots were turned over to a 15 cm depth using hand tools to incorporate biochar, where applicable, and break up soil clods. Plots were then seeded with 1.5 g m⁻² of a native seed mix of grasses, forbs and shrubs and the seeds and fertilizer, where applicable, were covered with soil by tamping the soil surface with leaf rakes. Wood strand mulch, where applicable, was applied Oct 18th, 2014 at a rate of 12.3 Mg ha⁻¹ to achieve 50–70% coverage.

2.2. Materials

All the amendments used in this study are commercially available.

Table 1
Organic amendment physical and chemical properties.

Description	Organic fertilizer	Wood-Strand Mulch	Biochar
Feedstock	Soybean and cottonseed meal	Beetle-killed lodgepole pine	Beetle-killed lodgepole pine
Production	Fermented media for fungal growth, dried	Biomass shredded, baled and air-dried	Slow pyrolysis, 550 °C max temp
Size	2–4 mm granules	6–16 × 0.5 × 0.25 cm strands	Sieved to > 3 mm
Application rate	16 kg N ha ⁻¹	12.3 Mg ha ⁻¹	25 Mg ha ⁻¹
Organic C (%)	35	52	86
H:C _{org} (molar ratio)	–	–	0.35
C:N (mass ratio)	5	556.0	478.8
pH	6.1	4.8	8.5

The organic fertilizer, Biosol Forte®, was produced from a fermented soy media used in penicillin production ([Table 1](#)). The woody biochar was produced by Biochar Now (Berthoud, CO) from a beetle-killed lodgepole pine feedstock, and was analyzed by Hazen Research, Inc. (Golden, CO) in accordance with International Biochar Initiative protocols ([Table 1](#)). Wood-strand mulch was sourced from beetle-killed lodgepole pine feedstocks and supplied by Mountain Pine Manufacturing ([Table 1](#)).

2.3. Cover and biomass

Plant revegetation was evaluated using plant canopy cover and above- and belowground biomass. Cover was assessed in July of 2015, 2016 and 2017 by measuring plant canopy cover using a gridded point-intercept method in 1 m² sample quadrats, identifying plant species by functional groups; graminoids, forbs and shrubs. We clipped aboveground biomass on 0.5 m² sections of one of the quadrats in July of 2016 and 2017, dried samples at 60 °C for 48 h and separated samples into aforementioned plant functional groups. We sampled belowground biomass in the 0–15 cm and 15–30 cm soil depths from soil pit sampling described below ([Section 2.7](#)), where coarse root biomass was physically separated on a 4 mm sieve, rinsed in distilled water then dried at 110 °C for 24 h.

2.4. Soil moisture

Soil moisture content was evaluated across treatments by sampling soil volumetric water content (VWC) 7–12 times throughout the growing season each year of the study using a hand-held time domain reflectometry probe (CD 620, HydroSense Campbell Scientific, Logan, UT). Average VWC was composited from 5 measurements per plot taken in the 0–10 cm depth. Periodically (n = 129), we collected soil samples in conjunction with VMC measurements and determined gravimetric water content (GWC) to develop a relation that allowed conversion of VMC measurements to GWC. Soils sampled for GWC analysis were collected from a 0–10 cm depth, transported to the lab in a cooler, and GWC was determined by oven drying a 15–20 g soil subsample at 110 °C for 24 h. Soil moisture measured as VWC explained most of the variability in GWC (R² = 0.89) across the range of soil moisture encountered (1–20%) in our study. When calculating annual average GWC, only measurements from months sampled in all three years were included in the calculation.

2.5. Mineral nitrogen sampling

To determine soil mineral N availability, we used a combination of time-integrated ion exchange resin (IER) bag sampling and soil mineral N extracts at discrete points in time. In each plot, two IER bags were buried at a 10 cm depth in mineral soil and deployed for the winter (Oct to May) of 2014–2015, 2015–2016 and 2016–2017, and summer (May to Oct) of 2015, 2016 and 2017. Resin bags contained a 1:1 mixture of cation (Sybron Ionic C-249, Type 1 Strong Acid, Na⁺ form, Gel Type) and anion (Sybron Ionic ASB-1P Type 1, Strong Base OH⁻ form, Gel

Type) exchange resin beads. IER bags were removed from the field and the recovered resins were extracted with 100 mL 2 M KCl, shaken for one hour, filtered and frozen until analysis (Binkley and Matson, 1983). Time point samples for soil mineral N were extracted in October each year from the initial soils used for net mineralization assays (described below). Nitrate and ammonium concentrations of the extracts were measured by spectrophotometry using a flow injection analyzer (Lachat Company, Loveland, CO) in 2015 and 2016. In 2017 extracts were analyzed colorimetrically for nitrate and ammonium (Alpkem Flow Solution IV Automated wet chemistry system, O.I. Analytical, College Station, TX).

2.6. Carbon and nitrogen mineralization assays and soil pH

A 14-day aerobic mineralization assay was conducted each year to evaluate soil microbial respiration, potential N mineralization and potential nitrification, along with soil pH, modified from Stanford and Smith (1972) as described below. In October of 2014, 2015, 2016 and 2017 a soil sample was collected from each plot to a 10 cm depth using a bulb corer and stored in a cooler during transport to the lab. After sieving to 2 mm, three soil subsamples were taken from each core: (1) a 20 g subsample for determining initial mineral N concentration; (2) a 20 g subsample for an aerobic C and N mineralization incubation; (3) a 10 g subsample for soil pH. The incubated subsample was wetted to 60% field capacity and incubated for 14 days at 25 °C in air-tight, quart-sized Mason jars with rubber septa for gas sampling. During the incubation, soils were periodically sampled for CO₂ concentration using an infrared gas analyzer (LI 6252, LI-COR, Lincoln, NE). Jar headspace was flushed and samples rewetted after seven days to prevent CO₂ from accumulating over 1%. Cumulative CO₂ was calculated by summing the changes in CO₂ concentration between sampling points over the 14-day incubation.

Initial and incubated soil subsamples were extracted with 2 M KCl at a 5:1 ratio (extract:sample), shaken for one hour, filtered, and analyzed colorimetrically for ammonium and nitrate (Alpkem Flow Solution IV Automated wet chemistry system, O.I. Analytical, College Station, TX). Potential N transformations were calculated as follows:

$$\text{Potential N Mineralization} = (\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N})_{\text{Final}} - (\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N})_{\text{Initial}} / \text{Days} \quad (1)$$

$$\text{Potential Nitrification} = (\text{NO}_3^- - \text{N})_{\text{Final}} - (\text{NO}_3^- - \text{N})_{\text{Initial}} / \text{Days} \quad (2)$$

Soil pH was determined on a 1:1 (deionized water:sample) solution shaken for one hour and analyzed using a pH electrode and stirrer bar (Orion EA940 Expandable ionAnalyzer; Orion Research, Jacksonville, FL).

2.7. Soil total carbon and nitrogen and bulk density

To assess treatment impacts on C and N stocks and bulk density immediately after application and over time, soils were sampled two weeks after incorporating the treatments in October 2014, after the first winter in June 2015 and two years later in August 2017. Soil samples were excavated from a 20 × 20 cm soil pit at designated soil sampling locations to a 0–15 and 15–30 cm depth and stored in a cooler during transport to the lab. To estimate bulk density, pit volume was estimated using the method of Boot et al. (2015), filling the excavated soil pit with sterilized millet seeds and measuring the millet volume. Field moist soils were weighed upon arrival to the lab and GWC determined by oven drying a 15–20 g soil subsample at 110 °C overnight. After air-drying, samples were sieved to the coarse (> 2 mm) and soil (< 2 mm) size fractions. Coarse fragments, including rocks, litter, roots, mulch and biochar particles, were physically separated to derive the proportional mass of the coarse fraction. Dry weights for coarse mulch and biochar particles were determined after oven drying at 110 °C

overnight. The C and N content of coarse biochar and mulch particles was determined from their C and N concentrations (Table 1) and dry weight. For bulk soil total C and N measurements, oven-dried 2 mm sieved soils were pulverized and analyzed on a LECO True-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). Total mineral soil C stock was calculated using the hybrid bulk density approach of Throop et al. (2012), based on the mass of the fine earth fraction (< 2 mm) and volume of the entire core.

2.8. Statistical analyses

Treatment effects were analyzed on the following response variables: plant canopy cover, aboveground biomass, belowground biomass, soil bulk density, soil total C and N content, GWC, mineral N from IER and soil extracts, soil respiration, potential N mineralization and nitrification, and soil pH. All statistics were performed in R version 3.3.2 (R Core Team, 2016) with significance accepted at $p = 0.10$. All response variables were modeled using a mixed effect model (lme4 R package) with treatment and year as fixed effects and road segment blocks (and date for GWC or season for IER mineral N analysis) as a random effect. To account for repeated measures in the plant cover measurements, within plot variability was treated as a random effect. Where model residuals were non-parametric, exponential or logarithmic transformations were applied to the response variables to achieve a normal distribution. Models were evaluated using Dunnett's test (multcomp R package) to determine significant treatment effects relative to the control. We examined overall fertilizer, mulch and biochar effects using one-way analysis of variance (stats R package) of the three treatments containing the relevant amendment relative to the comparable set of three treatments that did not receive the relevant amendment. To evaluate which soil factors had the greatest controls on revegetation, we used additive multiple linear regression models to evaluate the relationship between the measured soil properties, treatments and year to total plant cover, total plant aboveground biomass and belowground biomass. The best fit model was selected based on the Akaike information criterion (AIC) corrected for small sample sizes (MuMin R package) to determine the best soil predictors of plant response.

3. Results

3.1. Cover and biomass

Total plant canopy cover varied by year ($p < 0.01$), where, across treatments, 2015 experienced the greatest total canopy cover averaging 43% while the drier 2016 experienced much less cover at 24%, then cover rebounded to 37% in 2017. Fertilizer amended plots increased total plant cover by 21% ($p = 0.08$) relative to unamended treatments but mulch ($p = 0.12$) and biochar amended plots did not significantly affect cover (Fig. 2a). However, considering the individual treatments relative to the control, the fertilized mulch (43%, $p = 0.02$), fertilized (41%, $p = 0.04$) and mulch (44%, $p = 0.06$) treatments all significantly increased plant cover with no effect in any of the biochar treatments (Fig. 2d).

Graminoids averaged 27% cover (77% of total plant cover), followed by forbs at 7% cover (20% of total plant cover) and shrubs with 1% cover (3% of total plant cover), across years and treatments. Average graminoid cover across treatments varied between years ($p < 0.01$), while forb canopy cover steadily increased from 6% to 8%, and shrub cover did not change over time (Fig. S1). Most of the response to treatments and annual changes were explained by changes in graminoid cover, with increased graminoid cover relative to the control in the mulch (81%, $p < 0.01$), fertilized (73%, $p < 0.01$), fertilized mulch (58%, $p = 0.02$), and mulched biochar (57%, $p = 0.06$) treatments, and no significant treatment response in forb or shrub canopy cover (Fig. S1).

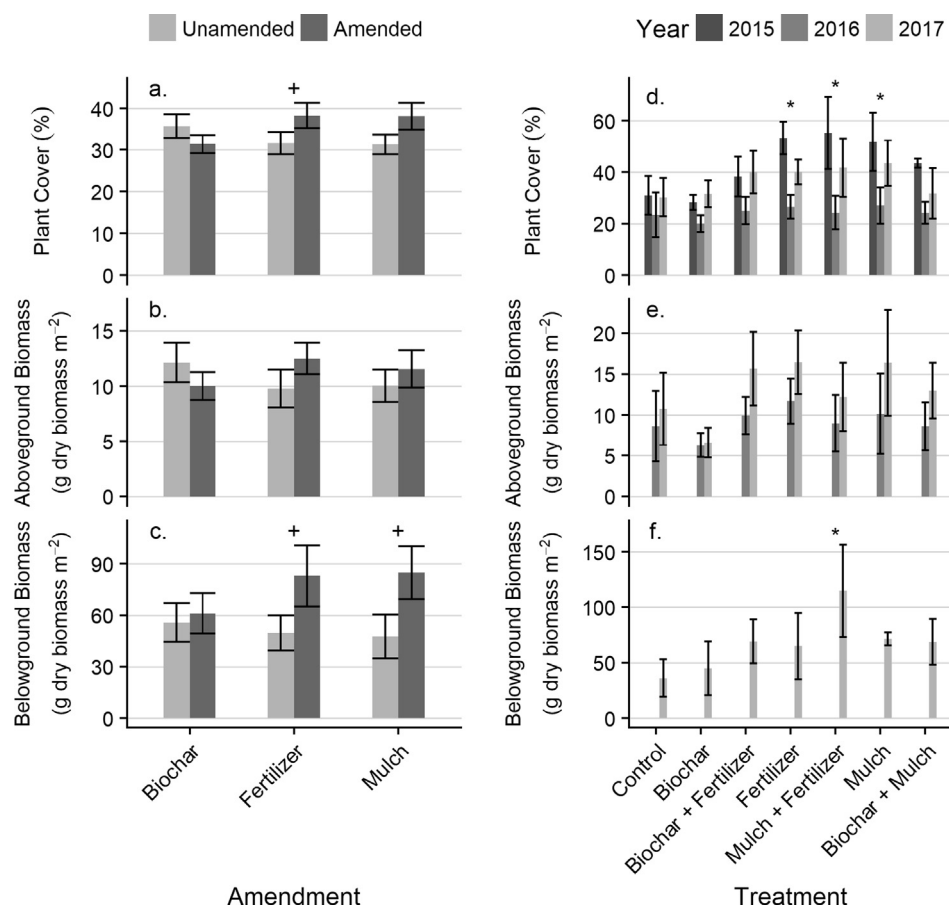


Fig. 2. Mean plant cover, aboveground biomass and belowground biomass for the amendments across years (a–c) and for each of the individual treatments by year (d–f). Amendments represent the three-year mean value of the 3 treatments containing the amendment vs the respective 3 unamended treatments. Error bars display one standard error, with significance effects across the three years denoted by + relative to the unamended plots and * relative to the control treatment.

Across treatments, average aboveground plant biomass increased from 2016 (9.2 g m^{-2}) to 2017 (12.9 g m^{-2} ; $p < 0.01$). While none of the effects were significant due to the high between site variability, aboveground biomass responded similarly to plant cover, where treatments receiving organic fertilizer and mulch tended to increase aboveground biomass relative to the unamended treatments, while biochar tended to slightly reduce plant biomass (Fig. 2b). Relative to the unamended plots, mulch (78%, $p = 0.02$) and fertilizer (67%, $p = 0.09$) amendments significantly increased root biomass, while biochar amendments had no significant effect (Fig. 2c).

3.2. Soil moisture

Across treatments, 2015 experienced higher average annual soil moisture (7.3% GWC, $p < 0.01$) than 2016 (5.5% GWC) and 2017 (5.7% GWC). Annual soil moisture patterns reflected precipitation inputs, where 2015 received 567 mm, 24% greater than the 30-year normal (456 mm), whereas 2016 received less at 426 mm and 2017 received 535 mm (PRISM Climate Group, 2017). Over the growing season, soil moisture content tended to decrease, reaching relatively dry values $< 5\%$ GWC by July except for after rain events (data not shown). Relative to the unamended plots, biochar (26%, $p < 0.01$) and fertilizer amendments (10%, $p < 0.01$) increased average GWC, while mulch had no significant effect (Fig. 3a). Over the three growing seasons among the individual treatments relative to the control, the mulched biochar treatment had the greatest increase in GWC (44%, $p < 0.01$) followed by the fertilized biochar treatment (27%, $p < 0.01$; Fig. 3b). The mulch only (-17% , $p < 0.01$), fertilized mulch (-26% , $p < 0.01$) and biochar only (-14% , $p = 0.09$) treatments all decreased average GWC relative to the control (Fig. 3b). Such treatment effects remained consistent across soil water contents (Fig. 4).

3.3. Mineral nitrogen availability

After organic fertilizer application, the fall 2014 soil extracts in the fertilized treatments contained twelve times the mineral N than the unfertilized treatments (Fig. 5b), equivalent to 22.8% of the N added as organic fertilizer. Mineral N availability remained elevated in the fertilized treatments with a 425% increase ($p < 0.01$) relative to the unfertilized treatments in the first winter IERs (Fig. 5c) and an 81% increase ($p = 0.07$) in the 2015 summer IER (Fig. 5a). After the first year, mineral N measurements showed no fertilization effect, whether measured by IER or soil extracts.

IER tended to recover more mineral N as nitrate ($\text{NO}_3^- \text{-N}$) than ammonium ($\text{NH}_4^+ \text{-N}$) with a greater $\text{NO}_3^- \text{-N}$ proportion in the winter months (70% $\text{NO}_3^- \text{-N}$) than the summer months (50% $\text{NO}_3^- \text{-N}$, $p < 0.01$) and an increasing $\text{NO}_3^- \text{-N}$ proportion over time ($p < 0.01$) from 49% $\text{NO}_3^- \text{-N}$ in 2015 to 72% $\text{NO}_3^- \text{-N}$ in 2015 (data not shown). Soil extracts contained much greater total mineral N than IER extracts (Fig. 5) and revealed a greater contribution from $\text{NH}_4^+ \text{-N}$ at an average 32% $\text{NO}_3^- \text{-N}$. Similar to IER, soil extracts also showed an increasing proportion of $\text{NO}_3^- \text{-N}$ over time ($p < 0.01$) increasing from 16% $\text{NO}_3^- \text{-N}$ in 2014 to 44% $\text{NO}_3^- \text{-N}$ in 2017 (data not shown).

Among the fertilized treatments, the fertilized biochar treatment contained the highest mineral N content relative to the fertilizer only treatments in fall 2014 soil extracts (229%, $p = 0.03$; Fig. 5e). Even after the initial mineral N pulse in year one, the fertilized biochar treatment continued to contain 30% more mineral N in soil extracts than the fertilizer only treatments ($p = 0.09$, Fig. 5e). The fertilized mulch treatments had no effect on mineral N from soil extracts or IER relative to the fertilizer only treatments (Fig. 5). There was no impact of biochar, mulch or their combination on mineral N from IER or soil extracts in unfertilized treatments relative to the control.

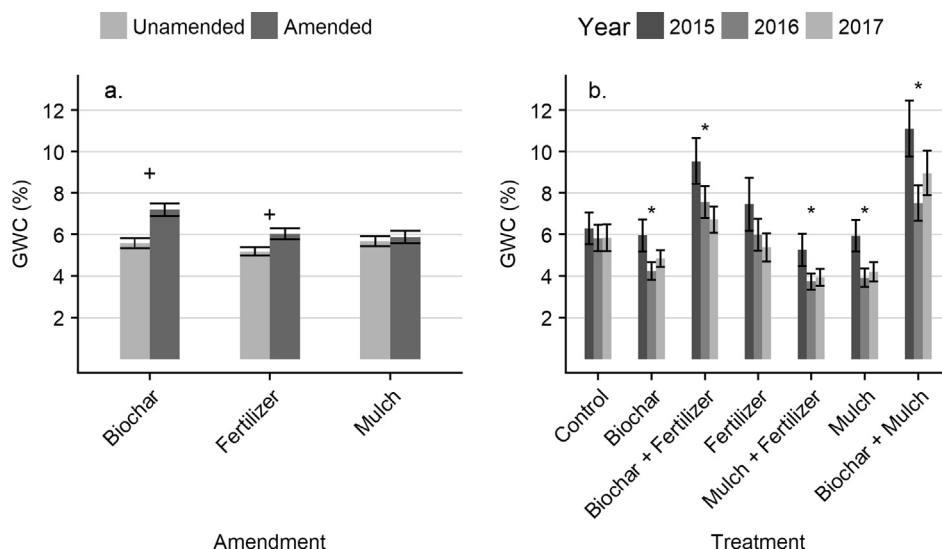


Fig. 3. Gravimetric water content (GWC) averaged over the growing season for the amendments across years (a) and for each of the individual treatments by year (b). Amendments represent the mean value of the 3 treatments containing the amendment vs the respective 3 unamended treatments. Error bars display one standard error, with significance effects across the three years denoted by + relative to the unamended plots and * relative to the control treatment.

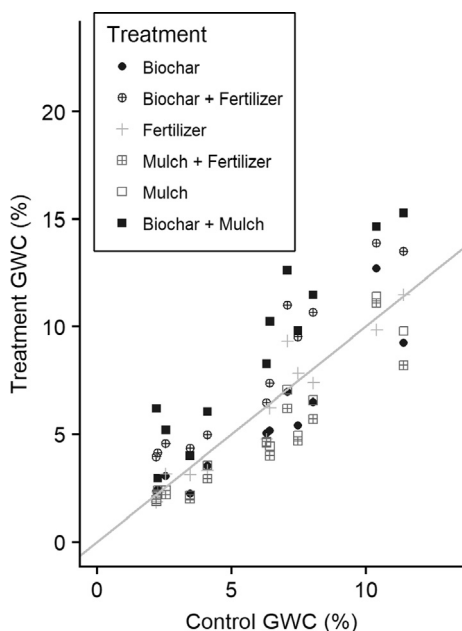


Fig. 4. Gravimetric water content (GWC) of the treatments relative to the GWC of the control plots sampled on the same date. The light gray line displays the 1:1 line.

3.4. Carbon and nitrogen mineralization assays and soil pH

Several of the fertilized treatments in the fall of 2014 had negative potential N mineralization estimates due to the high initial mineral N content, which was likely lost over the 14-day aerobic incubation to immobilization or N volatilization, yet soil respiration measurements indicated mineralization did occur. Removing these negative potential N mineralization estimates, the fertilizer amended plots showed increased potential N mineralization in 2014 (250%, $p < 0.01$) and 2016 (67%, $p = 0.02$), but did not significantly differ from the unamended plots in 2015 and 2017 (Fig. 6f). Similarly, fertilizer amended plots increased potential nitrification across years ($p = 0.06$) with interactions by year. In 2014 fertilizers increased nitrification by 241% ($p < 0.01$; Fig. 6g), but in later years shifted to having no effect to decreasing potential nitrification in 2017 (−57%, $p = 0.01$). Fertilization did not impact soil respiration (Fig. 6e) or pH (Fig. 6h).

Mulch amendments increased soil respiration relative to un-

mulched plots across years to an increasing degree, from 14.2% in 2014 to 57.9% in 2017 ($p < 0.01$; Fig. 6i). While mulch tended to increase N cycling across years, such effect was not significant across years for potential N mineralization (Fig. 6j) or nitrification (Fig. 6k). Mulch decreased soil pH relative to un-mulched plots across years ($p = 0.01$) and by 0.3 in 2017 ($p = 0.03$; Fig. 6l).

Despite the large C additions, biochar amendments did not impact soil respiration or potential N mineralization relative to unamended plots, but decreased potential nitrification by 44% ($p = 0.01$; Fig. 6c) and increased soil pH across years ($p = 0.01$), with a 0.2 pH increase in 2014 ($p = 0.08$; Fig. 6d).

3.5. Soil total carbon and nitrogen and bulk density

Total C, including the mineral soil C, mulch C and biochar C, showed high variance due to site differences in coarse fragments and mineral soil C content. Recovery of coarse biochar and mulch particles was variable and comprised the majority of the total C within treatments. After three field seasons, $23.1 \pm 7.1 \text{ Mg C ha}^{-1}$ was recovered in coarse biochar particles, which was within the variability of the initial application rates. In mulched treatments, $5.6 \pm 1.1 \text{ Mg C ha}^{-1}$ was recovered in coarse mulch particles indicating a loss of residual mulch C.

Mineral soil C averaged $7.5 \pm 1.0 \text{ kg C ha}^{-1}$ and $9.7 \pm 2.4 \text{ kg C ha}^{-1}$ in the 0–15 cm and 15–30 cm control treatments, with low soil C concentration ranging from 0.24% to 0.93% C. Mineral soil C did not change over the three-year study. Relative to unamended treatments, mulch amendments increased soil C by 3.6 kg C ha^{-1} ($p = 0.05$; Fig. 7a) and 5.2 kg C ha^{-1} ($p = 0.02$) for the 0–15 cm and 15–30 cm depths. In total this comprised 1% of the apparent loss in residual mulch C. Organic fertilizers and biochar amendments did not affect mineral soil C stocks at either depth. Total soil N did not change by depth, over time or by any of the amendments (Fig. 7b) or treatments (Fig. 7e). Over the three-year study, pit bulk density decreased from 1.61 to 1.14 g cm^{-3} in the 0–15 cm depth ($p < 0.01$) but remained at 1.77 g cm^{-3} at the 15–30 cm depth. At the 0–15 cm depth, pit bulk density decreased due to biochar (14%, $p = 0.05$) and mulch (17%, $p = 0.01$) amendments (Fig. 7c). Among the individual treatment relative to the control, the fertilized biochar treatment experienced an 18% decrease in pit bulk density ($p = 0.04$, Fig. 7f).

3.6. Soil factors and processes influencing revegetation

Indicators of soil microbial activity (soil respiration, potential N

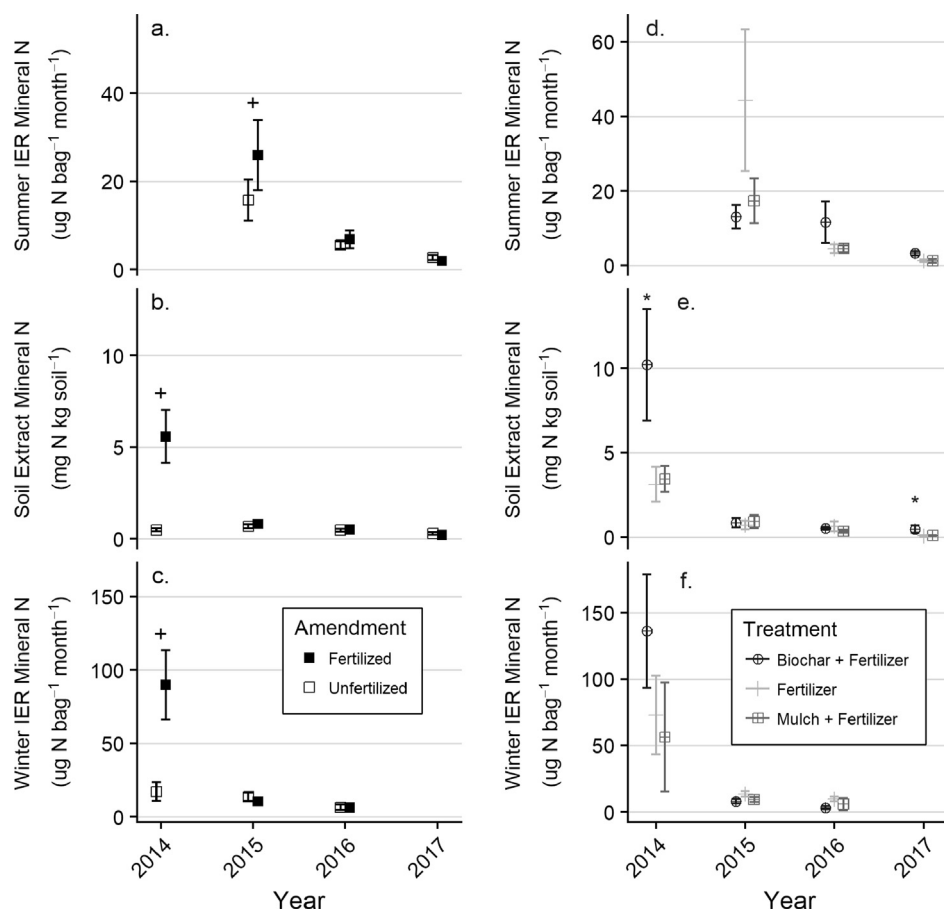


Fig. 5. Mean mineral N from soil extracts in the fall of each year (b, e) or ion exchange resin (IER) bags deployed in the winter (c, f) or summer (a, d) of each year for fertilized vs unfertilized amendments (a–c) or each of the fertilized treatments (d–f). Individual unfertilized treatments did not have a significant effect on mineral N availability relative to the unfertilized control. Error bars display one standard error, with significant effects denoted by + relative to the un-amended plots and * relative to the fertilizer only treatment.

mineralization and potential nitrification), soil C and N content and soil mineral N availability emerged as the best predictors of revegetation. Using all data available, total plant cover was best explained by a linear model containing year ($p < 0.01$), potential nitrification ($p < 0.01$), treatment ($p = 0.01$), and soil respiration ($p = 0.10$; $R^2 = 0.51$; Table 2). Summer IER mineral N was another important factor included in related models. In 2015 and 2017 where soil C and N content measurements were available, these properties emerged as the best predictors of plant cover ($R^2 = 0.62$, both $p < 0.01$) along with treatment, IER mineral N sampled during the summer months and potential nitrification (all $p < 0.01$).

Total aboveground biomass was best predicted by potential nitrification ($p < 0.01$), soil respiration ($p < 0.01$), year ($p = 0.02$) and treatment ($p = 0.06$; $R^2 = 0.58$; Table 2). When only looking at 2017 data where soil C and N content were available, N availability parameters including soil N content ($p < 0.01$), mineral N extracted from soil ($p = 0.02$), potential N mineralization ($p = 0.04$) and treatment ($p = 0.12$) best predicted aboveground biomass ($R^2 = 0.65$; Table 2). Root biomass was best explained by soil pH ($p < 0.01$, $R^2 = 0.35$; Table 2), with IER mineral N sampled during the summer months and GWC also contributing in related models. Single factor regressions between the best soil predictors of total plant cover (Fig. S2) and total aboveground biomass (Fig. S3) are provided for reference.

4. Discussion

4.1. Soil amendments' impact on revegetation

4.1.1. Organic fertilizer

The organic fertilizer used in this study increased total plant cover, comprised primarily of graminoids, the first year of the study. The same organic fertilizer, applied at a higher rate, increased total plant cover

along roadways in Mesa Verde National Park, in southwestern Colorado throughout a four year study (Paschke et al., 2000). The predominant effect of the fertilization we measured on graminoids agreed with a recent meta-analysis showing N additions are positively correlated to grass biomass in grassland ecosystems with neutral or negative effects on forb biomass (You et al., 2017). This may be due to grasses' increased height and cover outcompeting forbs for light, or branched root architecture that can better access soil mineral N (You et al., 2017). In our study, increased graminoid cover in fertilized plots resulted in greater root biomass three years after treatment, a factor critical for reducing sediment movement from road surfaces (Brooks et al., 2011). Organic fertilizers can also improve soil nutrient cycling by increasing N mineralization over time, with different amendments ranging from rapid N mineralization (fertilizers) to induced N immobilization (mulches) (Lashermes et al., 2010). The organic fertilizer applied in our study mineralized rapidly as evidenced by a 250% increase in potential N mineralization during laboratory mineralization assays from soils treated with organic fertilizers. Nearly a quarter of the N applied in the organic fertilizer was recovered within soil mineral N extracts within one month of application. Incubations of other commercial organic fertilizers reveal similar results with the majority of N mineralization occurring within the first month of application (Baldi and Toselli, 2014). Such improvements in potential N mineralization and nitrification and the resulting impacts on soil mineral N availability were no longer evident in later years of this study, indicating that a one-time fertilization did not sustain microbially-mediated nutrient cycling within these treatments. While the organic fertilizer used in this study did not support sustained mineral N provisioning in soils through gradual N mineralization, it may allow for direct mobilization of the initial mineral N pulse into root biomass.

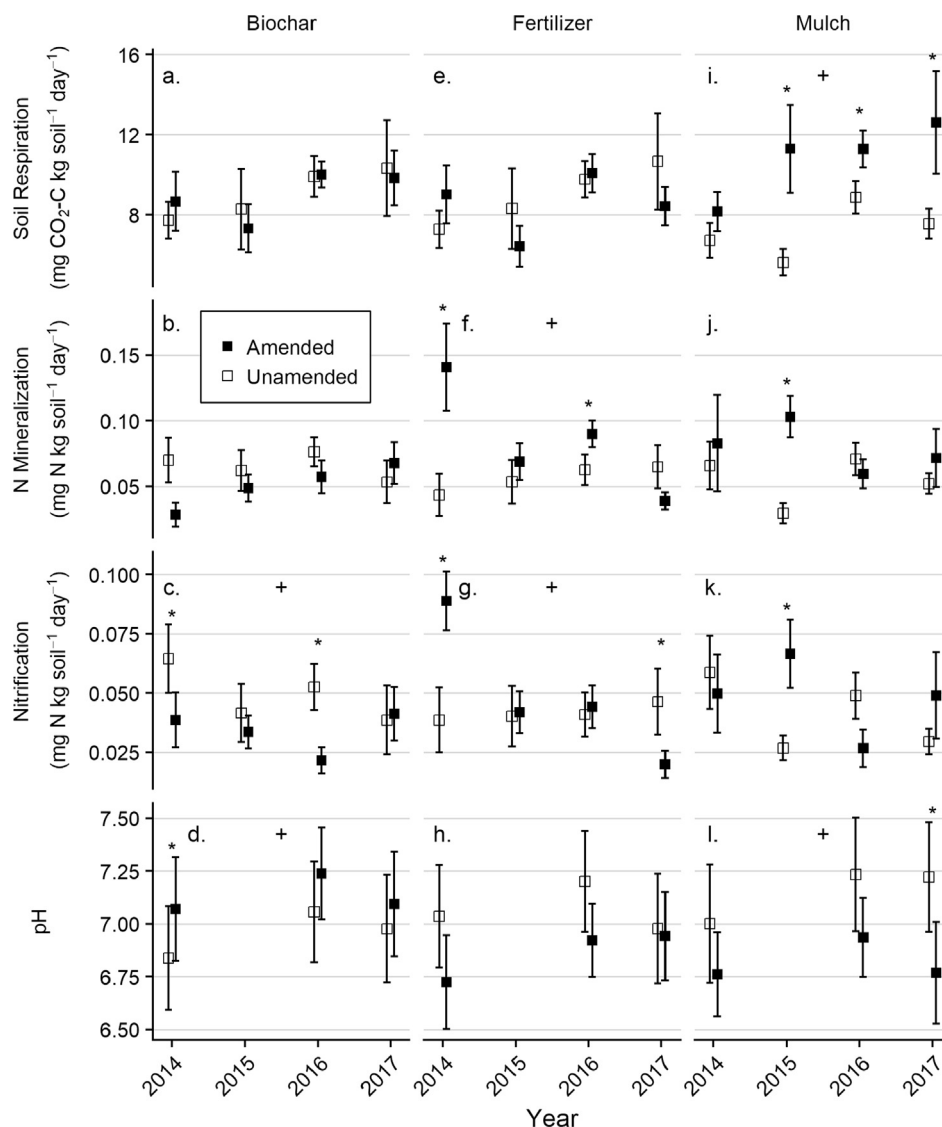


Fig. 6. Result of annual 14-day aerobic mineralization assays including cumulative soil respiration (a, e, i) potential N mineralization (b, f, j), potential nitrification (c, g, k) and pH (d, h, l) for the three amendments across all years sampled. Amendments represent the mean value of the 3 treatments containing the amendment vs the respective 3 unamended treatments. Error bars display one standard error, with significant effects by + across all years and * for individual years.

4.1.2. Wood strand mulch

Wood strand mulch increased graminoid cover the first year after application, but our results are not explained by mulch effects on soil moisture or mineral N availability. The effect of mulch application on plant recovery varies among studies based on mulch type, application rate and site conditions. For example, neither wood shred nor wood strand mulches (applied at ~50% cover) increased plant cover compared to untreated roads in the Northern Rockies (Foltz, 2012). On decommissioned roads near our study sites, the same wood strand mulch type (applied at a lower 6.2 Mg ha^{-1}) and organic N fertilizer treatment (21 kg N ha^{-1}) combination used in this study also increased basal plant cover (Sosa-Pérez and MacDonald, 2017). In contrast to most studies of organic mulches (Benigno et al., 2013; Goldin and Hutchinson, 2014; Rhoades et al., 2012, 2015; Roberts et al., 2005), we found no effect on soil moisture content beneath wood strand mulch, with some individual treatments showing a decrease in GWC relative to the control (Fig. 3). The effects of wood mulch on soil moisture increases with application rate and depth (Rhoades et al., 2012) and the 12.5 Mg ha^{-1} rate of this study is lower than most wood mulch studies. Mulch effects on soil moisture have also been shown to vary with seasonal soil moisture, with the greatest mulch effect under moderately

moist conditions (Rhoades et al., 2012, 2017). In this study, we found no evidence of increased soil moisture beneath mulch, even during relatively moist early-season conditions (Fig. 4). However, wood strand mulch increased total plant cover and thus soil water demand relative to the unamended control. Overall, the low wood strand mulch application rate would limit the potential effects of mulch on evaporative losses while the increased density and plant water use would have depleted soil moisture, especially during the drier years of this study. These possible plant feedbacks impacting mulch's ability to increase soil moisture contents, highlights the importance of considering above-ground-belowground linkages in restoration treatments (Kardol and Wardle, 2010).

The low wood strand mulch application rate did not increase soil moisture when applied alone, but when combined with biochar's capacity to retain soil moisture a synergistic effect led to the highest soil moisture contents (Fig. 3). Similar to the current study, application of mulch in combination with biochar is known to increase soil moisture content in degraded forest soils (Rhoades et al., 2017). However, on the severely-burned hillslopes of that study, both mulch added alone at 3-times the rate of the current study and biochar added at a comparable rate to this study increased soil moisture compared to untreated soils.

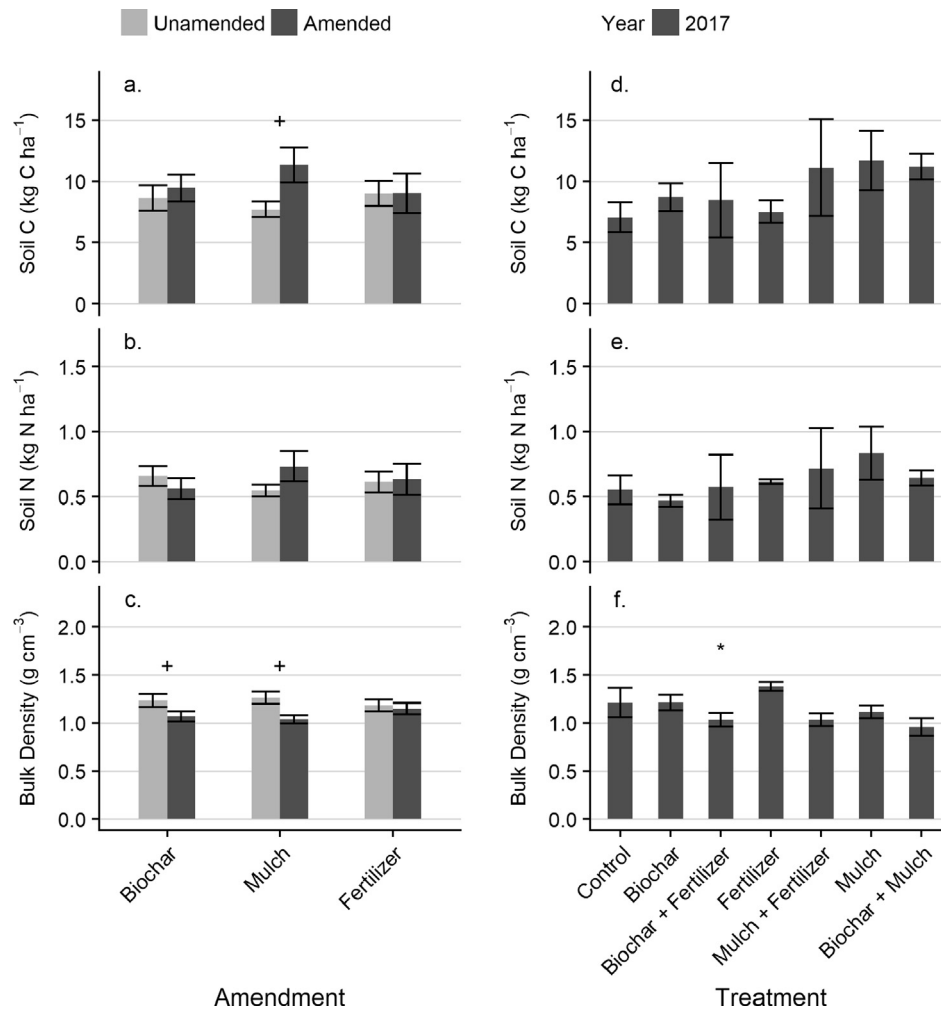


Fig. 7. Mineral soil C and N and bulk density measured at the 0–15 cm depth in the fall of 2017 for the amendments (a–c) and each of the individual treatments (d–f). Amendments represent the mean value of the 3 treatments containing the amendment vs the respective 3 unamended treatments. Error bars display one standard error, with significance effects denoted by + relative to the unamended plots and * relative to the control treatment.

Table 2

Statistical table of revegetation model selection using additive multiple linear regression models of soil variables to predict the given revegetation metric. Soil carbon (C) and nitrogen (N) emerged as a strong predictor but data was only available for 2015 and 2017. Thus, sample size (n) of the data modeled is provided. Model degrees of freedom (df), coefficient of determination (R^2), and Akaike information criterion (AIC) were used to assess model fit, where the best fit model (bold text) is represented by a Δ AIC score of zero. Predictor variables include year (Yr), treatment (Trt); soil respiration (CO_2), potential nitrification (Nit), potential N mineralization (NMin), pH (pH) and soil mineral N extracted from soil (NEx) from aerobic mineralization assay conducted in the fall of each year; summer ion exchange resin mineral N extracts (S.IER); mean gravimetric water content (GWC) for each year; and soil carbon C and N content from the 0–15 cm mineral soil depth.

Revegetation metric	Soil C & N	n	Model	df	R^2	AIC	Δ AIC
Total plant cover	Yes	52	S.IER + N + Nit + C + Trt	12	0.62	160.0	0
			Nit + N + C + Trt	11	0.60	160.3	0.36
			C + Nit + N + S.IER	6	0.51	161.0	0.99
	No	79	Yr + Nit + Trt + CO_2	12	0.51	258.8	0
			Yr + Nit + Trt + S.IER + CO_2	13	0.52	259.1	0.32
Total aboveground biomass	Yes	28	N + NEx + NMin + Trt	11	0.65	82.3	0
			N + NEx + Nit + Trt	11	0.65	82.4	0.12
			N + Nit + Trt + BD	11	0.65	82.6	0.34
	No	55	Nit + CO_2 + Yr + Trt	11	0.58	147.8	0
			Nit + CO_2 + Yr + Trt + GWC	12	0.59	148.0	0.18
Total belowground biomass	Yes	27	Nit + CO_2 + Yr + Trt + S.IER	12	0.58	148.9	1.13
			pH	3	0.35	134.6	0
			pH + S.IER	4	0.38	135.5	0.85
			pH + GWC	4	0.38	135.5	0.93

Such differences in mulch treatment effects may relate to low water holding capacity of these coarse-textured soil and the extremely low soil organic matter of our decommissioned roads (0.6% C) compared to the burned forest soils (1.7% C) (Saxton and Rawls, 2006). With the addition of biochar, the poor water retention of the road soil improved allowing for a synergistic effect with mulch's ability to decrease evaporative losses leading to improved soil moisture.

Mulches can also regulate soil N availability in restoration treatments, providing long-term N supply through gradual N mineralization but also short-term immobilization of excess soil mineral N to discourage competition from invasive plants (Perry et al., 2010; Vasquez et al., 2008). In this short-term study mulch had no significant impact on soil mineral N availability. Other studies have reported wood mulch increasing soil mineral N where it is used for erosion control on semi-arid lands (Bai et al., 2014), in poplar plantations (Fang et al., 2011) and in coniferous forest fuel reduction treatments (Rhoades et al., 2012). Changes in mulching effects on mineral N over time were observed in forest fuels reduction treatments where soil mineral N availability was not impacted initially, but increased after three years (Miller and Seastedt, 2009). In this study, the mineralization assays provided no clear trends of mulch immobilizing or supplying mineral N with variable potential N mineralization and nitrification results across years (Fig. 6). Over time, N mineralization of the mulch may supply some N to decommissioned road soils (Rhoades et al., 2012) but the limited amount of N supplied from mulch over time is not likely to provide a significant source of N to support early revegetation efforts (Laiho and Prescott, 2004).

4.1.3. Biochar

Despite indications of improved mineral N and water availability in biochar treatments, biochar did not improve revegetation efforts and in some years performed worse than the control (Fig. 2). This may indicate that mineral N retained on biochar particles was not readily accessible to plants and the increased water retention failed to alleviate plant water stress. Biochar's porous structure with high surface area allows for increased water retention through both its internal macroporosity and alteration of pore structure between soil particles (Liu et al., 2017). This increased porosity not only resulted in biochar increasing GWC but was also apparent through the decrease in bulk density in the fertilized biochar treatments. However, there was evidence that N limitation had a stronger control on plant establishment than water limitation, as the mulched biochar treatment, which had the greatest improvements in water retention, experienced less revegetation than the fertilized treatment.

The fertilized biochar treatment contained some of the highest mineral N availability as measured by both IER and soil extracts in the first year (Fig. 5), with this increased N availability sustained throughout the study for the soil extracts, although the magnitude of this effect was much smaller in later years. Woody biochar's high surface area with diverse functional groups increases the ion-exchange capacity of soils allowing for improved N retention (Gai et al., 2014). Woody biochar amendments to boreal forest soils showed a similar effect increasing NH_4^+ availability in soil but with no significant impact on vegetation (Gundale et al., 2016). Despite indications of improved N availability, neither plant cover nor biomass was enhanced by biochar suggesting that either this mineral N is not readily available to plants or not sufficient to overcome N limitation and significantly improve revegetation efforts.

4.2. Importance of biological soil processes for revegetation

While the fertilized treatments only provided a short-lived provisioning of N to soils, biochar, mulch and the combination treatments showed potential to restore soil physical, chemical and biological processes to improve revegetation. Restoration treatments that integrate improvements in soil physical, chemical and biological properties

provide the best opportunities to achieve sustained restoration of critical soil processes like nutrient cycling and C regulation (Heneghan et al., 2008). Such benefits were evident when considering mulch's ability to enhance plant cover and biomass. In these low SOM soils, mulch provided a sustained source of labile C to support microbial community structure and function resulting in improved plant nutrient uptake (Bai et al., 2014; Huang et al., 2008). This was evident in the mulch treatments which significantly increased soil respiration relative to unmulched treatments each year (Fig. 6). Indicators of improved microbial activity, such as soil respiration, potential N mineralization and potential nitrification, were also consistently the best predictors of both plant cover and biomass (Table 2). When soil C and N content data were available, these factors also emerged as the best predictors of plant cover and biomass (Table 2), highlighting the importance of improving SOM content to continually supply nutrients to meet plant demand. In the restoration of semi-arid shrublands, improved SOM content was correlated with improved microbial activity and nutrient cycling, with different organic amendments influencing the microbial community composition (Bastida et al., 2015).

Biochar also improved soil mineral N availability over the study, but likely by retaining mineral N on biochar surfaces via physical entrapment or ion exchange processes as opposed to increasing microbial N cycling. Similarly, improving physical properties to increase soil water content only emerged as an important predictor of revegetation in some models (Table 2). The greater revegetation response in the mulch treatments which supported microbial activity compared to no revegetation effect in the biochar treatments which improved soil chemical and physical properties, highlights the importance of promoting soil microbial communities in the restoration of decommissioned forest roads.

4.3. Soil amendments' impact on carbon sequestration

4.3.1. Wood strand mulch

Wood strand mulch's ability to promote microbial activity not only supported revegetation efforts but also provided broader environmental benefits through soil C sequestration. Mulch increased both soil C content and soil respiration, and recovery of mulch particles was decreased relative to initial application rates, indicating decomposition of residual mulch particles. In these soils with low C content, microbial processing of mulch C not only stimulated microbial activity and N mineralization, but the microbial byproducts may also contribute to SOM formation. Such microbially-processed C can become adsorbed to the silt and clay particles to help alleviate the C saturation deficit, the proportion of soil C relative to the maximum absorption on silt and clay particles (Grandy and Neff, 2008; Hassink, 1997). However, these rocky, loam soils with 4–12% clay content would have limited capacity for such matrix stabilization and eventually would reach C saturation (Stewart et al., 2007). In this study the increased soil C was roughly 1% of the apparent loss of residual mulch C. Therefore results indicate that mulch can stimulate microbial activity in these SOM depleted soils, but the total C sequestration potential will likely be limited by the capacity of the silt and clay fraction to retain such microbially processed C (Six et al., 2002). The remaining mulch C will likely be respired as CO_2 within a decadal timescale (Laiho and Prescott, 2004).

4.3.2. Biochar

Recovery of biochar particles was within the variation of the initial application rates and primarily consisted of the original coarse particle size fraction. Physical fragmentation of biochar can also be an important process that determines the longevity and effectiveness of biochar as a soil amendment (Spokas et al., 2014), but did not appear to be a significant factor in this system. The lack of a significant increase in the mineral soil C content and no effect on soil respiration indicates that in these forest soils biochar was neither highly fragmented nor contributed labile C to soils to prime C mineralization. This confirms

biochar's ability to contribute to long term soil C sequestration primarily through its inherent recalcitrance (Spokas, 2010; Wang et al., 2016), rather than actively contributing to SOM formation through the progressive decomposition of the organic matter and stabilization of the smaller biopolymers within aggregates or on mineral surfaces (Lehmann and Kleber, 2015). However, over time mineral stabilization of biochar C may play an important role, as pyrogenic C has been found to be mineral associated in soil historically amended with charcoal (Glaser et al., 2000) or in native grassland soil (Brodowski et al., 2006). In contrast to the mechanisms for soil C sequestration in the mulch treatments, biochar particles show evidence of biochemical protection allowing for long-term C accumulation above C saturation controlled by matrix stabilization (Lehmann et al., 2006; Lorenz and Lal, 2014).

4.4. Management implications

This short-term field study provides insights into the soil processes and properties affected by organic amendments that influence initial revegetation and C sequestration on decommissioned forest roads. Both organic fertilizer and wood strand mulch increased plant cover and root biomass, where the organic fertilizer increased soil N availability, and mulch increased soil C and microbial activity. Conversely, biochar had no effect on revegetation though it increased mineral N and soil moisture. When combined with organic fertilizer, the biochar treatment sustained higher mineral N availability throughout the three-year study, though surprisingly it had no effect on plant cover. Both the wood strand mulch and biochar applications increased C, though biochar alone and in combinations showed the greatest potential for C sequestration. The wood strand mulch application was the only treatment that enhanced both revegetation and C sequestration objectives on these decommissioned roads. Across all amendments, we found that microbially-mediated soil processes (i.e., C and N mineralization) were good general predictors of road revegetation; this highlights the importance of soil ecological knowledge to optimize restoration activities (Heneghan et al., 2008). Longer-term monitoring of these treatments and well-replicated evaluation of different application rates and combinations are needed to better understand the soil processes that regulate soil recovery and to optimize treatment design for revegetation and C sequestration on gravelly, coarse-texture, low SOM forest roads.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foreco.2018.05.059>.

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