Powell River Project Annual Report (2013 – 2014)

Reforestation and Ecosystem Services:
If you build it, will they come?

Brian D. Strahm and Bethany N. Avera
Department of Forest Resources and Environmental Conservation
Virginia Tech

Project Summary

This report provides an update on a newly established project that will contribute to the legacy of reforestation research at the Powell River Project and provide benefit to the larger Appalachian Coal Region. The overarching question driving this research is to determine how quickly, and to what extent, forest ecosystem services are restored to the post-mining landscape through reforestation. During a plenary address at the American Society of Mining and Reclamation annual meeting in 2010, Dr. Jim Burger challenged reclamation professionals to advance the Forestry Reclamation Approach (FRA) beyond simply recreating forest structure (e.g., stem density, species composition) and to more explicitly work toward what he termed the "Forest Ecosystem Reclamation Approach" where carbon and nutrient cycling processes; water quality and quantity regulation; and greenhouse gas sink and source dynamics are considered as critical components of functioning forest ecosystems. This proposal seeks support to identify a chronosequence (a group of sites that differ primarily in time since establishment) of reforested mined sites to evaluate the rate at which these valuable ecosystem services are developed. The ultimate goal is to use this information to relate forest ecosystem structure and function in reclaimed mined lands and better inform reclamation practitioners on strategies to successfully return a functional forest ecosystem to the landscape as efficiently as possible.

Scope of Work

Introduction:

The temporal aspects of successful reclamation and reforestation were well stated in a text on land restoration by Bradshaw and Chadwick (1980):

"Rome was not built in a day; neither can a self-sustaining soil/plant ecosystem..."

The disturbance of natural ecosystems through processes like surface mining temporarily and abruptly reduce both the structure (e.g., percent cover, species diversity, stem density) and function (e.g., carbon and nutrient accrual and cycling, regulation of water quality and quantity) of forest ecosystems (Fig. 1; Point 1 → 2). Over time, natural processes (e.g., succession) can restore both structure and function (Fig. 1; Point 2 → 1), but the timescale of the processes driving that restoration can range up to 10,000 years (Dobson et al., 1997). One of the goals of successful reclamation is to accelerate these natural processes and more quickly return the landscape to an acceptable level of function, particularly where the provisioning of ecosystem services are concerned.

Developed from decades of work largely centered at the Powell River Project, the Forestry Reclamation Approach (FRA) is a five-step process that is capable of accelerating the return of the structural element of a forest ecosystem (Fig. 1; Point 2 → 3), specifically species composition, that would otherwise take centuries to achieve through natural successional pathways. Thus, the FRA has been identified as a desirable method to support forested land uses on reclaimed mined land by the Appalachian Regional Reforestation Initiative (ARRI), the US Office of Surface Mining and many state mining agencies, including the Virginia Department of Mines, Minerals and Energy, and is an increasingly feasible and appealing option for reclaiming post-mining landscapes throughout the Appalachian region.

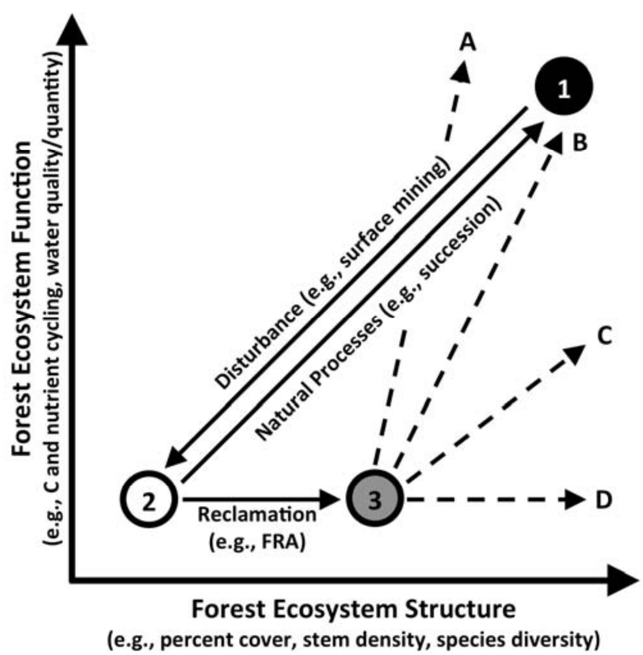


Figure 1. Responses of forest ecosystems in terms of both structure and function as affected by disturbance, succession

Far less is known, however, about the relationships between this accelerated return of forest structure and the ecosystem services desired as outcomes of successful reclamation. A number of possible outcomes include (Fig. 1; A - D): A) a more rapid restoration of ecosystem functions relative to the structure of the original system; B) a return to the previous ecosystem condition in terms of both structure and function; C) the restoration of structure with a decreased functional capacity; or D) little to no restoration of ecosystem function. Although some of these scenarios are certainly more likely than others, the fact is that we have very little information to guide reforestation research regarding how rapidly key ecosystem services/functions are returned to the post mining landscape.

In the same way the FRA strives to "fast forward" vegetative succession and return high value hardwoods to the post-mining landscape, there is a need to rapidly reestablish the ecosystem services and functions associated with the native pre-mining forested landscape. This is ecologically important in sustaining forests after seedling establishment, but is also critical in addressing the increasing social and regulatory pressures regarding environmental quality following mining operations. During a plenary address at the American Society of Mining and Reclamation annual meeting in 2010, Dr. Jim Burger challenged reclamation professionals to advance the FRA beyond simply recreating forest structure and to more explicitly work toward what he termed the "Forest Ecosystem Reclamation Approach" where carbon and nutrient cycling processes; water quality and quantity regulation; and greenhouse gas sink and source dynamics are considered as critical components of functioning forest ecosystems. This proposal seeks support to identify a chronosequence (a group of sites that differ primarily in time since establishment) of reforested mined sites to evaluate the rate at which these valuable ecosystem services are developed. The ultimate goal is to use this information to relate forest ecosystem structure and function in reclaimed mined lands and better inform reclamation practitioners on strategies to successfully return a functional forest ecosystem to the landscape as efficiently as possible.

Objectives:

The initial objectives of this proposal were to:

1. Identify a chronosequence of reclaimed mined sites at the Powell River Project that can be used as the basis for long-term research on the rate at which key forest ecosystem services are restored.

2. Characterize structural components of the reclaimed ecosystems, including biotic (e.g., forest, microbial) and abiotic (e.g., soil physical properties) components.

3. Quantify key ecosystem functions (e.g., productivity, mineralization, soluble and gas fluxes) for the reclaimed ecosystems.

4. Relate ecosystem structure to ecosystem function and assess current reclamation strategies with specific regard to the rate at which forest structure and function are returned to the post mining landscape relative to natural successional processes.

Methods and Procedures:

Research Sites

We have established a chronosequence, investigating four sites that were previously surface mined for coal that have since been reclaimed by intentional planting of hardwood tree species. The sites are 5, 11, 21 and 30 years post tree planting at the time of sampling. Three nearby, un-mined sites with mixed hardwood forest will be used as an unmined comparison. All sites are located in Wise County in Southwestern Virginia. The 5, 11 and 21-year-old sites are located on the Powell River Project and the 30-year-old site is along the Roaring Fork River at Kent Junction, VA.

In choosing sites, the priority was to find a site at each age cohort that had been intentionally reforested with hardwoods. All sites are assumed to have had fertilizer applied at rates consistent with standard reclamation practices. Parent material will be variable between the sites and even within each of the sites as mine soil is made from blasted overburden [*Sencindiver and Ammons*, 2000]. All sites are within the Pennsylvania Age Wise Formation, characterized by sandstone and siltstone. Therefore, the mine soils of the chosen sites are a mix of weathered and unweathered sandstone and siltstone, with shale and coal fragments. In accordance with the Surface Mine Control and Reclamation Act of 1977 (SMCRA), all sites have steep slopes to achieve approximate original contour (AOC). Remaining variables were constrained to the best of our ability, however aspects do vary. Site characteristics of the mined sites are summarized in Table 1. Three un-mined sites will be selected within the Powell River Project that closely mimic the chronosequence sites in terms of aspect (S-SW) and slope. One plot will be installed on each un-mined site for a total of three un-mined plots, in a manner that mimics plot selection and size of the previously mined sites.

The 5-year-old site was originally part of Chris Fields-Johnson's Master's thesis (2011). It was hydroseeded in the fall of 2007 and planted with trees mid-January 2008. We are using Block 1 of the original experiment. It has a south aspect and about a 60% slope. All subplots for our experiment will be within the loose graded, conventional seed mix treatment. Planted hardwood species included white ash, white oak, sugar maple, black cherry, red oak, chestnut oak, black oak and yellow poplar [*Fields-Johnson*, 2011].

The 11-year-old site was reclaimed in summer 2001 using a loosely graded mix of sandstone and shale and was hydroseeded with a conventional ground cover. In 2002, the following season, it was planted with mixed hardwoods; white ash, red oak, white oak, chestnut oak, sugar maple and tulip poplar. Additionally, dogwood, crab apple, white pine and bristly locust were planted for their value as nurse and wildlife tree species [*Burger et al.*, 2005; 2008]. We are using the control plot of each of the original three blocks, which had no herbicides to control the herbaceous growth.

The 21-year-old site was studied by Burger and Fannon (2009) as a part of Amy Gail Fannon's graduate research. The site was mined in 1990 and originally reclaimed in 1991 by the conventional method of smooth tracking and was sown with conventional erosion control grasses. Hardwood trees were then planted in March of 1992 as part of a species trial by Burger and Fannon (2009). Single species plots of 24 trees were planted with northern red oak, white oak, white ash, black walnut, cottonwood, American sycamore and yellow poplar. This experiment will use Blocks 1 and 2 of the original experiment that have a southern and western aspect, respectively.

The 30-year-old site is located along the Roaring Fork River at Kent Junction, VA. This site was mined and recontoured in 1979 then hydroseeded with a conventional seed mix in 1980. Tree planting followed in 1981. It has northwestern aspect and a slope of about 39%. Tree species planted include black locust, European black alder, black walnut, chestnut oak, Chinese chestnut, yellow poplar, sycamore, cottonwood, red oak, loblolly pine, scotch pine, shortleaf pine, Virginia pine and eastern white pine. Fifteen trees of each species were planted in rows in four replicate blocks [*Torbert et al.*, 1985].

The tree species on the 5 and 11-year-old sites were planted the season following initial reclamation and hydroseeding. The tree species on the 21-year-old site were planted one year after reclamation and planting with erosion control grasses and the 30-year-old was planted within two years of initially being recontoured. It is important to note that all four sites were sown with a conventional seed mix. The intention of a conventional seed mix is to establish a quick and hardy cover. However the specific species that comprised this seed mix for each of the age cohorts varied. The 5-year-old site was sown with rye grain (*Secale cereale*), orchard grass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), Korean lespedeza (*Lespedeza cuneata*), birdsfoot trefoil (*Lotus corniculatus*), white (Ladino) clover (*Trifolium repens*), redtop (*Agrostis gigantea*), and weeping lovegrass (*Eragrostis curvula*). The 11-year-old site was sown with orchard grass, timothy grass (*Phleum pratense*), birdsfoot trefoil and red clover (*Trifolium pratense* L.). The 21-year-old site was sown with tall fescue, orchard grass, red top, perennial ryegrass, red clover, and Serecia lespedeza. The 30-year-old site was sown with Kentucky-31 tall fescue, birdsfoot trefoil, redtop, white (Ladino) clover, and annual rye. There are no species that are constant through all four age classes, however there are several species that are common between at least two age classes. Amongst these species, tall fescue and certain clovers (*Trifolium* sp.) are recognized as tree-competitive species. While redtop, timothy, birdsfoot trefoil and white clover are all among the species listed that are recognized as more tree-compatible [*Zipper et al.*, 2011].

Table 1: Chronosequence Selected Site Summary

Site Age	*Aspect*	*Slope*	*Treatment*
5 y.o.	South	~60%	Loose-graded, conventional seed mix
11 y.o.	SW, SE & NE	(to be recorded)	Loose-graded, conventional seed mix (no herbicide)
21 y.o.	S & W	39-50%	Conventional smooth tracking, erosion control grasses
30 y.o.	NW	~39%	Conventional seed mix, smooth tracking assumed

Sampling Plan and Laboratory Analysis

Three sampling plots were established on each site on January 7th, 2013. A systematic approach was used to choose plot locations, as space is very limited in three of the four age cohorts. All plots will have a 5m radius (10m diameter) for an area of 78.54m^2. Each plot center is located approximately 10m, keeping the plot edge 5m, from the edge of any abnormal landscape feature and atypical vegetation. Every effort was made to install the plots the maximum distance from each other. Exclusion criteria were used to avoid areas that were not representative of the site as a whole. Areas excluded included those that had atypical canopy cover, noticeably different slope, and uncharacteristic understory vegetation within the site. Site to site canopy cover, slope and understory vegetation changed, thus exclusion criteria were unique to each site.

Triplicate soil samples by method of quantitative soil pits were collected from each plot, prior to leaf-out. All pits were located 120° apart from each other along the circumference of the plot. The first pit of each plot will be at 0° N, and the second two at 120° ESE and 240° WSW (Figure 2). Soils will be sampled using a quarter-meter-square (25cm x 25cm) frame lain on top of the soil; all soil within the frame to a depth of 25cm will be excavated and collected. Each sample will be collected in four layers: forest floor litter, 0-5, 5-10, and 10-25 cm of mineral soil. Samples were returned to the lab, air-dried and sieved to 2-mm.

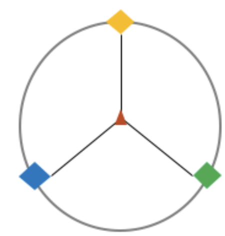


Figure 2: Quantitative Soil Pit Sampling Scheme

= plot boundaries
= plot center
= 1st pit at 0°N
= 2nd pit at 120°ESE
= 3rd pit at 240°WSW

In the coming year, sample processing will be completed and physical and chemical analysis will begin. Volumetric density (VD) of the fine fraction will be calculated for each depth increment by dividing the mass of the fine fraction and coarse fragments by the total volume of the quadrat in order to allow for extrapolation to the landscape scale. Other analyses will be performed on a plot level by compositing each depth increment for a particular plot. Particle-size analysis will be done using the pipette method [*Gee and Bauder*, 1986]. Soil pH will be determined using the aqueous extract from a 1:1 water to soil slurry using a Thermo Orion 3 Star benchtop pH meter with a Ross Ultra Sure-Flow Combination pH electrode. Additionally, a characterization of the belowground nutrient pools of each age cohort will be determined. Total C and N will be quantified using dry combustion on an elementar vario MAX CNS elemental analyzer (elementar vario MAX, Elementar, Hanau, Germany). Available K, Ca and Mg will also be quantified using an ammonium acetate extraction [*Simard*, 1993]. Available P will be extracted using a 0.5 M solution of sodium bicarbonate ($NaHCO_3$) and analyzed with inductively coupled plasma atomic emission spectrophotometry (ICP-AES).

Previous use of the Walkley-Black method for soil organic matter and organic C fractions as well as the Kjeldahl method to determine organic N fraction of mine soils have met with many challenges due to the presence of geogenic C and N contamination in the form of coal fragments. Therefore, we will plan to use stable isotope ratio mass spectrometry (IRMS) to determine the fractionation of the C and N pool and to correct for any coal contamination [*Acton et al.*, 2011]. Collected coal fragments from each site, will be used to determine the geogenic signature of C and N at each site. Prior to running the coal fragments on the IRMS, they will be reacted with hydrochloric acid (HCl) to remove any remaining carbonates that would otherwise contaminate the organic C signature. Coal fragments will be crushed with a mortar and pestle and then further homogenized using a ball-mill. The IRMS signature for each plot will then be determined and corrected using the coal signature to determine the size of the available C and N pools. To determine pedogenic C, we will use the following equation:

$$[C_T]\times\delta^{13}C_T = [C_G]\times\delta^{13}C_G + [C_P]\times\delta^{13}C_P$$

where C_T is total C, C_G is geogenic C (i.e., coal) and C_P is pedogenic C. Pedogenic N will be determined similarly. Alternatively, if samples remain from the overburden material used to make the mine soil for each site are available then these samples can be used to determine the geogenic signatures in place of coal.

Soil microbes and their associated process are expected to be dynamic over time. Thus, many parameters are being measured at monthly intervals throughout the growing season to characterize these changes. Sampling of microbial biomass and activity, greenhouse gas [i.e., carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)] fluxes, and available soil nutrients are underway for the current growing season. Soils are being routinely collected for both active and total microbial biomass, using the substrate-induced respiration (SIR) [*Bailey et al.*, 2002; *Beare et al.*, 1990; *Lin and Brookes*, 1999; *Parkinson and Paul*, 1982] and chloroform fumigation [*Parkinson and Paul*, 1982] methods, respectively. Soil greenhouse gas fluxes are being collected using static vented gas chambers and analyzed using gas chromatography [*Holland et al.*, 1999]. Two PVC collars will be installed in each plot for two subsamples of each plot. The collars were installed at the time of the quantitative soil pit to allowed to re-equilibrate following the disturbance for at least two weeks prior to any measurements. Anion and cation exchange membranes will be utilized to get an integrated index of the flux of available ions, diffusing through the soil profile. Ion exchange membranes (IEMs) are two-dimensional membranes that are made of cross-linked copolymer reinforcing fabric, embedded with quaternary ammonium anion exchange groups [*Cheesman et al.*, 2010; *Cooperband and Logan*, 1994; *Subler et al.*, 1995]. IEMs are similar in concept to resin beads, but surpass resins *in situ* because they can be inserted with minimal disturbance, achieve greater contact with the soil and are able to capture diffusion and therefore allow determination of rates. These membranes are being replicated three times within each plot and installed when the microbial biomass sampling begins and exchanged on a similar schedule. To install the IEMs, we cut a vertical slit in the soil, insert the IEM and then gently press the soil back together to seal the IEM within the soil. IEMs are saturated with a solution such as potassium chloride (KCl) prior to installation in field to saturate all the exchange sites with the exchangeable ion (Cl^-) [*Subler et al.*, 1995]. Once in the field, ions are redistributed from the soil solution to the IEM by exchange with the ion on the resin [*Skogley and Dobermann*, 1996].

Additionally, to complete the characterization of the development of each cohort required for this study, there will be a coarse characterization of aboveground vegetation. This characterization for the under-story will include: percent ground cover in each season, stem count and species identity. For the over-story, characterization will include: stem density of tree species and species composition.

Analysis of Data

For all statistical analysis we will have to make the following two assumptions, 1) each of the age plots is representative of all reclaimed forests of that age class and 2) that outliers in the data are a result of site effects and not age effects.

Two options exist for the initial analysis of data to determine if there are differences in a given variable across the four age groups and the control, one-way analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA). Using ANOVA, all dependent variables will be analyzed independently to identify differences in the means between age groups. However, ANOVA is limited by lack of ability to analyze interactions between dependent variables and the potential for increase in Type 1 error. Therefore, MANOVA that is designed to analyze more than one response or dependent variable at a time has the potential to produce more informative results. MANOVA addresses questions about the interaction among the independent variables, the importance of the dependent variables and the strength of association between dependent variables. Assumptions of MANOVA include normal distribution of dependent variables, linearity between all pairs of dependent variable and homogeneity of variances in all variables. For either method, ANOVA or MANOVA, multiple comparisons using a method such as Tukey's HSD and regression analysis will be used to further evaluate relationships of statistically significant variables.

Results:

Results to date include soil chemical (i.e., C and N concentrations and ratios, pH, available N), physical (i.e., volumetric density, temperature, moisture), and biological (i.e., microbial biomass and activity) parameters, component and total ecosystem C pools, biosphere-atmosphere exchange of greenhouse gasses (CO_2, CH_4, and N_2O). Both soil C and N concentrations generally increase with time since reclamation (Fig. 1). The rate of increase is especially rapid early in succession and plateaus after a few decades, especially in the surface (0-5 cm; Fig. 1). In addition, soil pH changes over the first few decades, initially increasing over the first decade or two, then decreasing as it approaches 30 years old. At age 30, there are no differences between reclaimed and unmined soil pH (Table 2). Volumetric density has no significant patterns over time, and is in fact, not very different than the unmined plots (Table 2). With respect to ecosystem C pools, the 5 year old has the least amount of C for tree, mineral soil, and total ecosystem C pools (Table 3). For all ecosystem components, the general trend is that age 5 has the least, the other mined plots are not significantly different, and then unmined has the greatest (Table 3). Forest floor and root C did not have any significant patterns or trends (Table 3). Carbon sequestration rates are generally highest early in ecosystem succession (Table 3).

Many measurements were made with higher frequency (e.g., monthly) in order to capture the potential affect of reforestation age on the annual dynamics of the flux or measurement. Soil temperature is consistently highest in the 5-year-old age cohort, likely due to lack of a closed forest canopy (Fig. 2). Soil moisture patterns showed no significant trends and are likely more dramatically affected by weather than stand development (Fig. 2). Perhaps most interesting was the seemingly rapid reestablishment of a functioning N cycle. This is evidenced by a lack of significant differences in available N and N_2O fluxes (Figs. 3 and 5) tha may be due to the apparent rapid restablishment of the microbial community (Fig. 4). Although the reforested sites did not have the same total or active microbial biomass, there were few differences among the age classes. The one functional attribute that did not appear to restablish with reforestation was methane consumption (Fig. 5). Upland forest soils are one of the most significant global CH_4 sinks. Despite the observed microbial community dynamics (Fig. 4), methanotrophs do not appear to have reestablished at any of the observed reforestation ages based on the complete lack of CH_4 consumption (Fig. 5).

In order to understand the relationship between forest structure and the ecosystem functions upon which society depends, we related measured structural and functional attributes (Figs. 6 and 7). In general, there were positive correlations between ecosystem C accrual and microbial biomass and activity (Fig 6). Further, every component of ecosystem C accumulation has a strong, positive, significant correlation with total soil N (Fig. 7).

Thus, quite simply, N allows for the development of biomass, or C. As C increases in the system, so does microbial biomass and activity. These actors are largely responsible for many of the observed ecosystem services from forest (e.g., biosphere-atmosphere exchange of greenhouse gasses). So this study suggests that reforested ecosystems rapidly return many important ecosystem services, these can be optimized by working to increase rates of ecosystem N accrual.

Deliverables:

Bethany Avera is preparing to defend her M.S. thesis based on this research during fall 2014. The support Bethany has received from the Powell River Project has almost exclusively supported her research and development. Bethany has represented herself, this project, and Virginia Tech well, earning the H.E. Burkhart Outstanding Masters Student distinction in the Department of Forest Resources and Environmental Conservation, and the award of “Best Paper of Session” at the 2013 Soil Science Society of America International Annual Meeting in Tampa, FL. In addition, we have been invited to present this work at the upcoming 2nd IUFRO Restoring Forests conference which will result in the publication of her thesis work in an accompanying issue of *New Forests*.

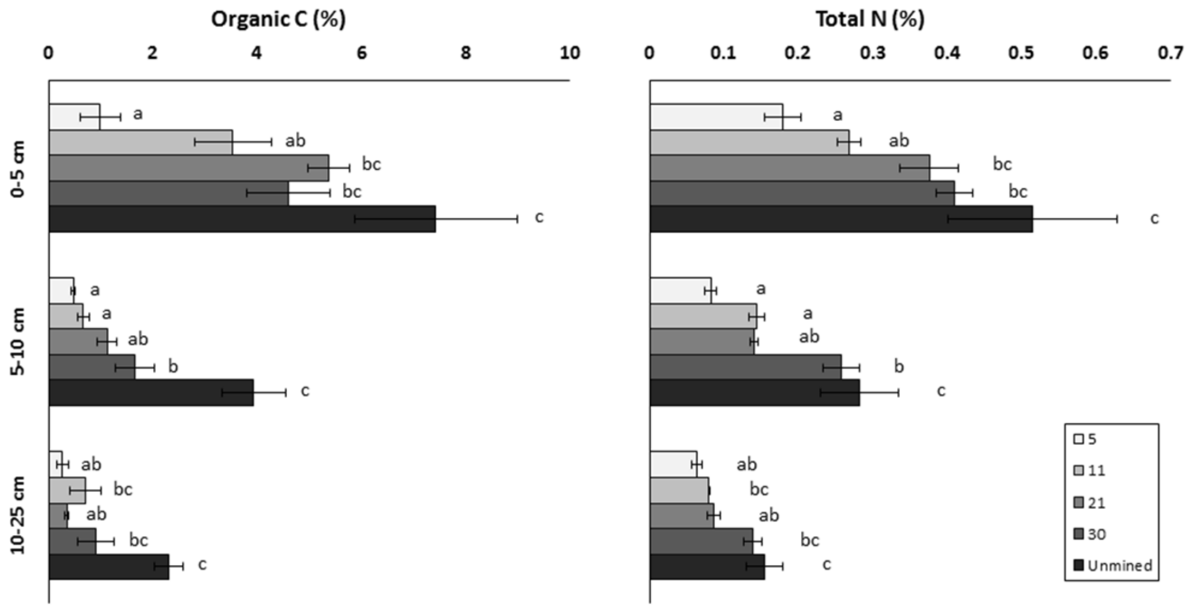


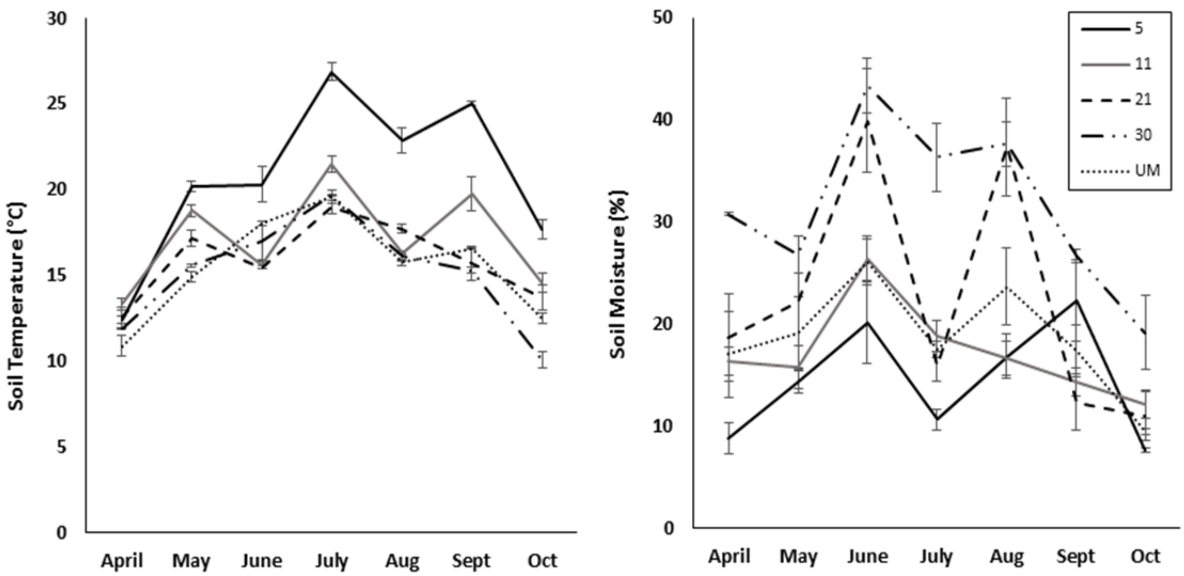
Figure 1. Concentration (%) of organic C and total N within each age cohort by depth, 0-5, 5-10, and 10-25 cm. Standard error bars (n=3) of treatment mean for each depth are displayed. Letter groups indicate significant differences between age cohorts within each depth (p<0.10).

Table 2. Mean and standard error (n=3) of soil pH, volumetric density (g cm-3), and C:N ratio by age and depth. Within each depth significant differences among the age cohorts are represented by different letters (p<0.10).

Age	5		11		21		30		Unmined	
Depth					pH					
0-5 cm	5.78 ± 0.199	(ab)	6.08 ± 0.198	(a)	6.12 ± 0.413	(a)	5.44 ± 0.226	(ab)	5.26 ± 0.500	(b)
5-10 cm	5.68 ± 0.280	(a)	5.92 ± 0.287	(a)	5.71 ± 0.548	(a)	5.21 ± 0.285	(ab)	4.85 ± 0.323	(b)
10-25 cm	5.84 ± 0.245	(ab)	6.36 ± 0.339	(a)	5.73 ± 0.332	(b)	4.89 ± 0.152	(c)	4.60 ± 0.062	(c)
					Volumetric Density (g cm^{-3})					
0-5 cm	0.317 ± 0.025	(a)	0.267 ± 0.010	(ab)	0.289 ± 0.022	(ab)	0.206 ± 0.007	(b)	0.346 ± 0.066	(a)
5-10 cm	0.369 ± 0.050		0.400 ± 0.039		0.526 ± 0.0002		0.381 ± 0.077		0.467 ± 0.057	
10-25 cm	0.292 ± 0.029	(a)	0.414 ± 0.025	(ab)	0.364 ± 0.056	(ab)	0.421 ± 0.021	(ab)	0.478 ± 0.067	(b)
					C:N					
0-5 cm	6.05 ± 2.78	(a)	13.97 ± 1.83	(b)	14.62 ± 1.03	(b)	10.26 ± 1.69	(ab)	14.47 ± 0.51	(b)
5-10 cm	5.46 ± 1.25	(a)	4.62 ± 0.65	(a)	7.81 ± 1.07	(a)	5.80 ± 0.67	(a)	14.16 ± 0.67	(b)
10-25 cm	4.54 ± 1.28	(a)	5.65 ± 2.80	(a)	4.72 ± 1.00	(a)	4.90 ± 2.16	(a)	15.02 ± 0.63	(b)

Table 3. Mean and standard error (n=3) of ecosystem C pools and C sequestration rates for tree, forest floor, root, mineral soil, and the total ecosystem components by age cohort. Within each component significant differences among age cohorts are represented by different letters (p<0.10).

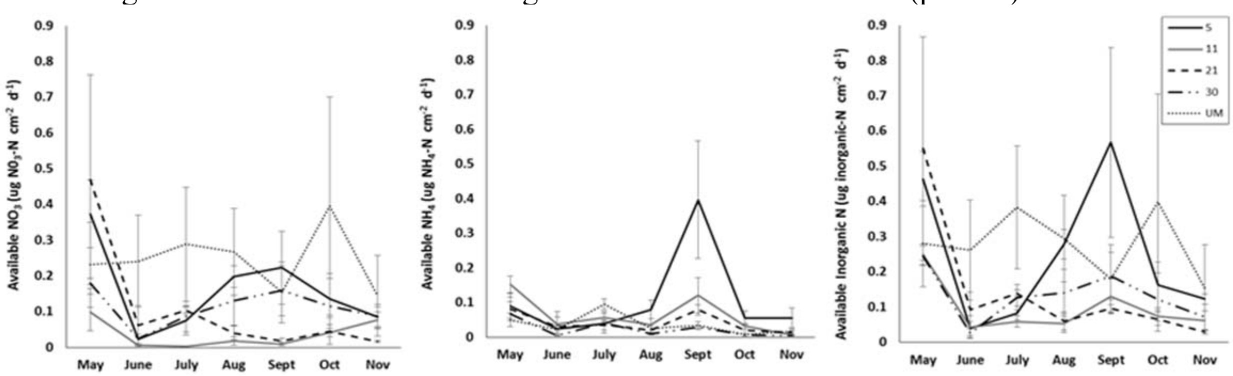
Age	5		11		21		30		Unmined	
Component					Ecosystem C Pools (Mg ha^{-1})					
Tree	0.27 ± 0.06	(a)	53.80 ± 8.28	(bc)	48.86 ± 7.20	(b)	64.27 ± 13.40	(bc)	125.71 ± 37.93	(c)
Forest Floor	4.57 ± 0.48	(a)	5.85 ± 0.41	(ab)	9.11 ± 1.05	(b)	6.86 ± 1.40	(ab)	6.53 ± 1.39	(ab)
Mineral Soil	2.45 ± 1.16	(a)	7.67 ± 0.72	(b)	12.24 ± 1.38	(b)	9.76 ± 1.75	(b)	36.32 ± 0.88	(c)
Root	2.22 ± 0.19	(a)	1.63 ± 0.24	(a)	5.24 ± 0.49	(b)	2.64 ± 0.51	(a)	10.38 ± 2.92	(b)
Total Ecosystem	*9.51 ± 1.74*	*(a)*	*68.95 ± 8.15*	*(b)*	*75.46 ± 5.07*	*(b)*	*83.53 ± 16.33*	*(b)*	*178.89 ± 39.71*	*(c)*
					C Sequestration Rate (Mg ha^{-1} yr^{-1})					
Tree	0.05 ± 0.01	(a)	4.89 ± 0.75	(b)	2.33 ± 0.35	(c)	2.14 ± 0.45	(c)	-	
Forest Floor	0.91 ± 0.10	(a)	0.53 ± 0.04	(b)	0.43 ± 0.05	(bc)	0.23 ± 0.05	(c)	-	
Mineral Soil	0.49 ± 0.23	(a)	0.70 ± 0.07	(a)	0.58 ± 0.07	(a)	0.33 ± 0.06	(a)	-	
Roots	0.44 ± 0.04	(a)	0.15 ± 0.02	(bc)	0.25 ± 0.02	(b)	0.09 ± 0.02	(c)	-	
Total Ecosystem	*1.90 ± 0.35*	*(a)*	*6.27 ± 0.74*	*(b)*	*3.59 ± 0.24*	*(a)*	*2.78 ± 0.54*	*(a)*	-	



	April	May	June	July	Aug	Sept	Oct
5	ab	a	a	a	a	a	a
11	a	ab	b	b	bc	b	b
21	ab	bc	b	c	b	c	bc
30	bc	cd	bc	bc	bc	c	d
UM	c	c	c	bc	c	c	c

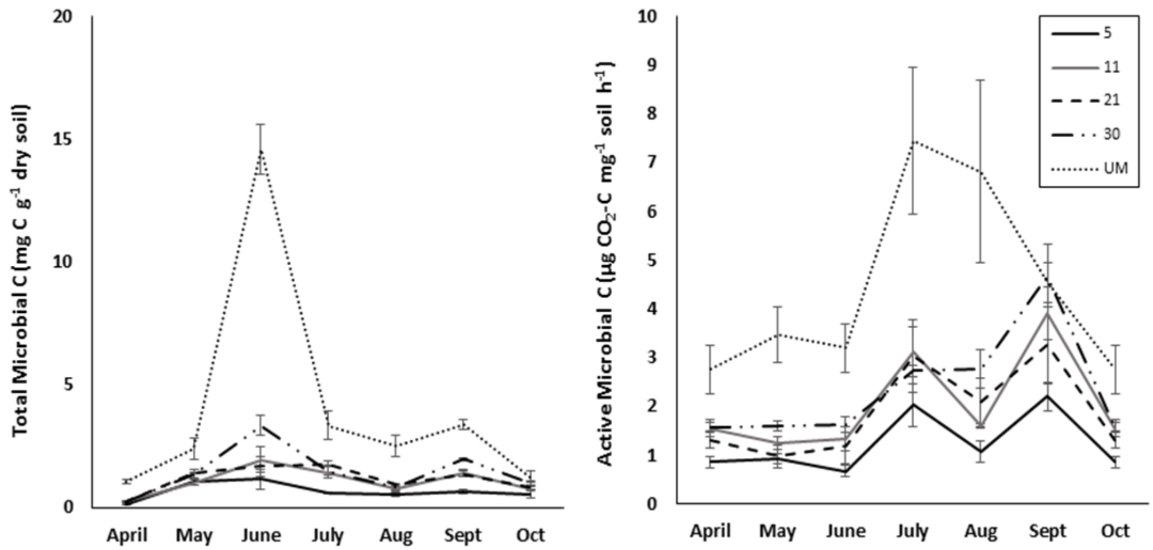
	April	May	June	July	Aug	Sept	Oct
5	a	a	a	a	a	ab	a
11	b	a	ac	c	a	c	bc
21	b	ab	bc	ac	b	c	ac
30	c	b	b	b	b	a	b
UM	b	ab	ac	c	a	bc	ac

Figure 2. Mean and standard error (n=3) of soil temperature (^{0}C) and soil moisture (%) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month (p<0.10).



5	ab	ab	a	*5*	a	*5*	a	a
11	b	b	b	*11*	ab	*11*	a	b
21	ab	a	ab	*21*	ab	*21*	ab	b
30	ab	a	ab	*30*	ab	*30*	a	ab
UM	a	a	ab	*UM*	ab	*UM*	b	ab

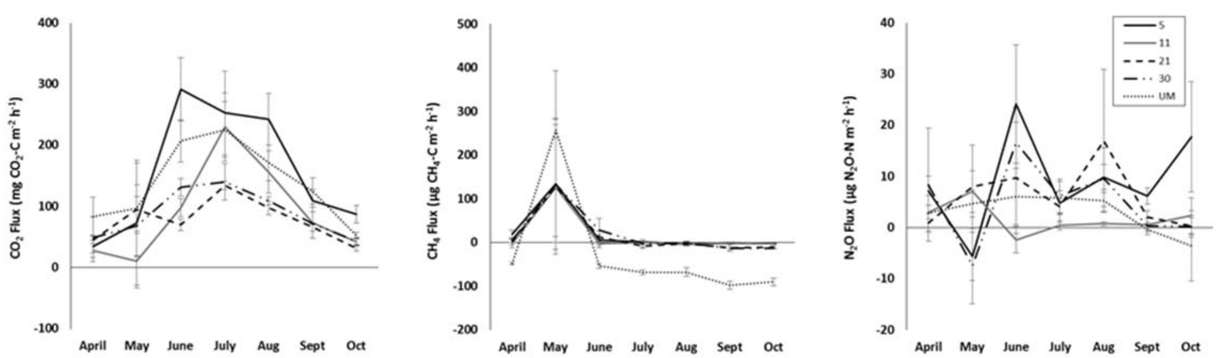
Figure 3. Mean and standard error (n=3) of nitrate (NO_3^-), ammonium (NH_4^+) and inorganic N (NO_3^- and NH_4^+) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month (p<0.10).



	April	May	June	July	Aug	Sept	Oct
5	a	a	a	a	a	a	a
11	b	a	b	b	ab	b	ab
21	b	ab	b	b	b	b	ab
30	b	a	c	b	ab	b	b
UM	c	b	d	c	c	c	b

	April	May	June	July	Aug	Sept	Oct
5	a	a	a	a	a	a	a
11	b	ab	b	a	ab	ab	bc
21	ab	ab	ab	a	b	ab	ab
30	bc	b	b	a	b	b	bc
UM	c	c	c	b	c	b	b

Figure 4. Mean and standard error (n=3) of total microbial biomass C (mg C g^{-1} dry soil) and active microbial biomass C (µg CO_2-C mg^{-1} soil h^{-1}) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.10$).



	April	May	June
5	ab	a	a
11	a	b	ab
21	ab	ab	b
30	ab	ab	ab
UM	b	ab	ab

	April	July	Aug	Sept	Oct
5	ab	a	a	a	a
11	ab	a	a	a	a
21	ab	a	a	a	a
30	a	a	a	ab	ab
UM	b	b	b	b	b

	Aug
5	ab
11	b
21	ab
30	b
UM	ab

Figure 5. Mean and standard error (n=3) of CO2 flux (mg CO_2-C m^{-2} h^{-1}), CH4 flux (µg CH_4-C m^{-2} h^{-1}) and N2O flux (µg N_2O-N m^{-2} h^{-1}) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.10$).

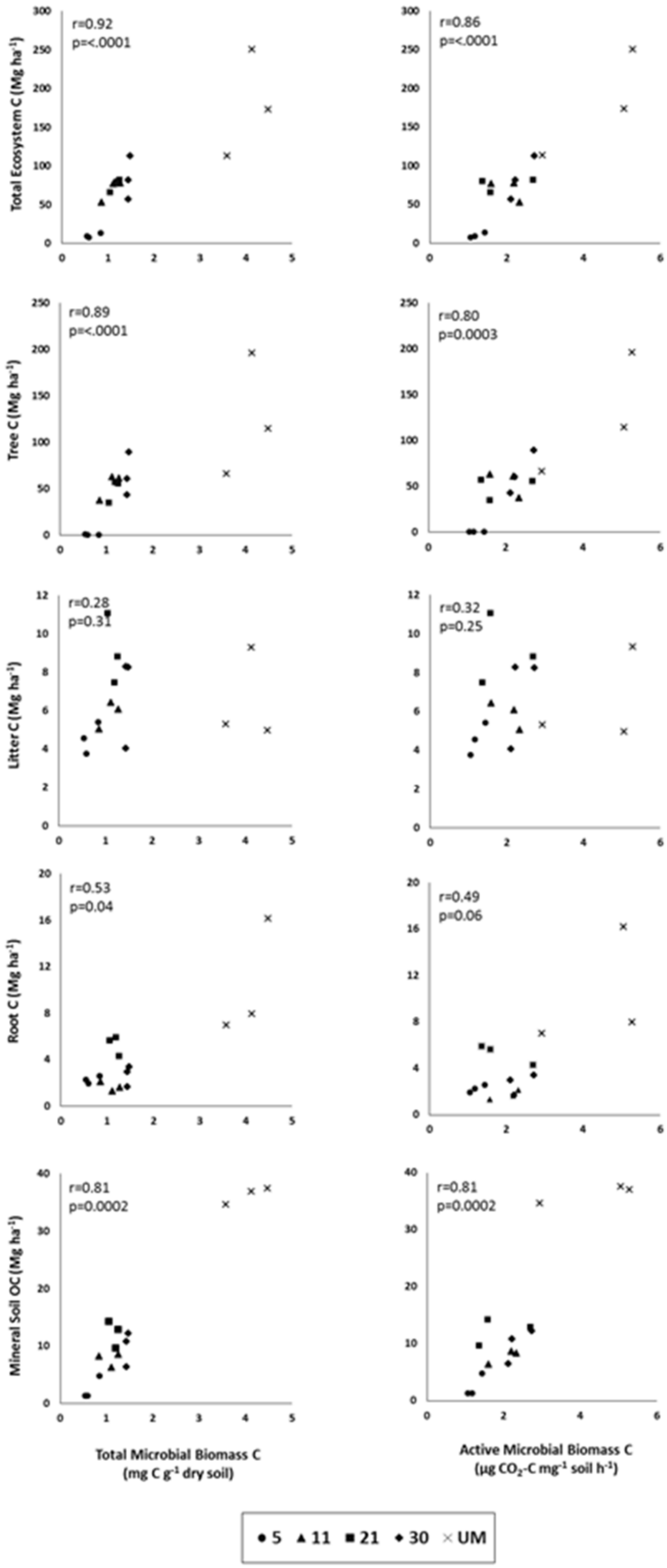


Figure 6. Mean (n=3) of total microbial biomass C (mg C g^{-1} dry soil) and active microbial biomass C (µg CO_2-C mg^{-1} soil h^{-1}) correlated with ecosystem C components. Displayed on the graph are Pearson's Correlation Coefficients (r) and significance values (p).

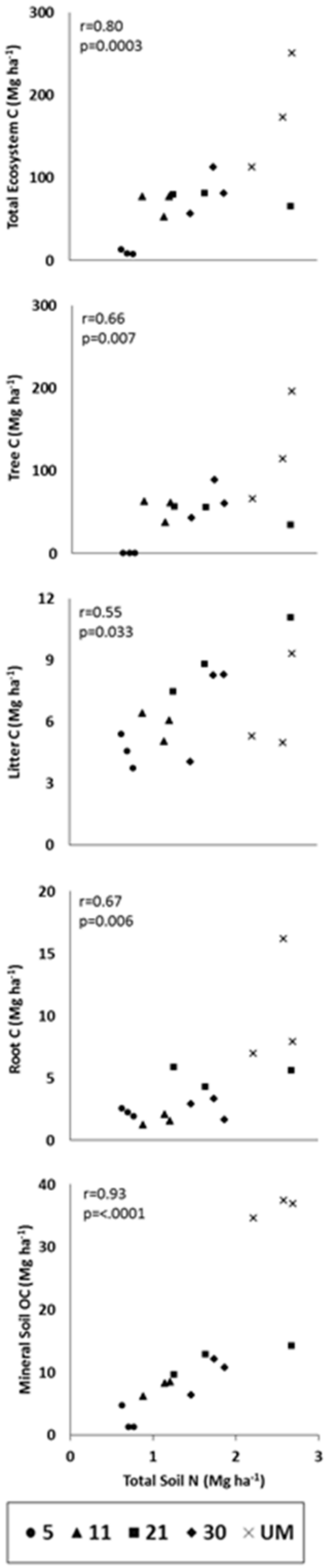


Figure 7. Mean (n=3) of total soil N (Mg ha^{-1}) correlated with ecosystem C components. Displayed on the graph are Pearson's Correlation Coefficients (r) and significance values (p).