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Functional Role of the Herbaceous Layer in Eastern Deciduous Forest Ecosystems

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Abstract

The importance of the herbaceous layer in regulating ecosystem processes in deciduous forests is generally unknown. We use a manipulative study in a rich, mesophytic cove forest in the southern Appalachians to test the following hypotheses: (i) the herbaceous functional group (HFG) in mesophytic coves accelerates carbon and nutrient cycling, (ii) high litter quality input and rapid nutrient turnover associated with HFG will have a positive effect on overstory tree growth, and (iii) the HFG regulates tree regeneration with negative effects on seedling establishment due to competition for resources. We established treatment plots in a mesic, cove-hardwoods forest and removed the herbaceous flora (HR, removed twice per year) or added herbaceous organic material (OMA, once per year) for comparison to a no removal (NR) reference for a total of 14 years. The OMA treatment stimulated soil N-mineralization and increased litterfall mass and N content. OMA N-mineralization rates were more than two times greater than both the NR and HR treatments; however, we did not detect significant differences in soil CO_2 efflux among treatments. Higher overstory litterfall mass and N in the OMA treatment plots indicated that overstory trees were benefiting from the enhanced soil N-mineralization. Higher overstory leaf mass and N suggests an important linkage between HR and aboveground net primary production even though this did not translate into greater tree basal area increment. We found an increase in regeneration of all tree species with HFG removal, and the response was particularly evident for *Acer rubrum* seedlings.

Key words: functional group; mesophytic cove; tree growth; litterfall; forest floor; soil CO_2 efflux; N-mineralization.

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Author contributions KJE and JMV conceived the study, designed the experiment, and wrote the paper. KJE performed the analyses. KJE, JMV, JDK, BDC, and BDK performed the research. KJE, JMV, and JDK contributed to discussions and editing.

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Introduction

Ecologists in the 1960s proposed several hypotheses regarding the importance of diversity in maintaining ecosystem integrity (MacArthur and Wilson 1967; Margalef 1968; May 1973); and more recently research has been conducted to evaluate the functional importance of diversity for regulating ecosystem processes (Díaz and others 2004; Cadotte

and others 2011; Cardinale and others 2011; Mouillot and others 2013). Some researchers suggest that ecosystem processes are determined primarily by the functional characteristics of component organisms or groups rather than species diversity per se (Wardle 1997; Hooper and Vitousek 1997; Gilliam 2007; Eisenhauer and others 2011). Others suggest that many species are needed, specifically in grassland ecosystems, to maintain function at multiple temporal and spatial scales (see Isabel and others 2011; Hooper and others 2012), which supports the hypothesis that diversity stabilizes ecosystem processes.

In the eastern U.S., most plant diversity in forest ecosystems is found in the herbaceous layer (Roberts 2004; Whigham 2004; Gilliam 2014), which may contain 70–90% of the plant species in temperate deciduous forests. In a recent review, Gilliam (2007) argued that the herbaceous layer also plays an important role in the structure and function of forest ecosystems and whereas it represents less than 1% of the aboveground forest biomass, it can contribute up to 20% of the foliar litter to the forest floor (Gilliam 2007; Muller 2014). High litter quality, high nutrient concentrations, and low concentrations of lignin and cellulose (Melillo and others 1989), could make herbaceous flora more important to the function of forest ecosystems than would be suggested by its biomass contribution. To our knowledge, no studies have experimentally examined the functional role of this group of species (hereafter HFG, herbaceous functional group) that turnover annually and decompose faster than woody or evergreen plant species (Muller 2014) in eastern deciduous forests.

Much of the information and theory on plant functional diversity has been derived from studies in grassland ecosystems (for example, Fornara and Tilman 2008; Grigulis and others 2013; Polley and others 2013; Pillar and others 2013) with fewer studies in temperate forest systems (for example, Nadrowski and others 2010; Eisenhauer and others 2011). Almost all manipulative biodiversity experiments carried out so far have used fast-growing and small model systems, mostly semi-natural or early successional grasslands, and aquatic or terrestrial microcosms (see Allan and others 2013). Thus, longer term studies in forested ecosystems are needed to provide additional information on the role of functional diversity or functional groups of species.

The experimental removal of a functional group of species, such as the HFG, from an ecosystem can provide information on its contribution to ecosystem functioning. The relative contribution of functional groups can change over time as species composition and stand structure change or can vary spatially due to edaphic conditions that support varying amounts and types of functional groups. For example, in the southern Appalachians, the species composition in late successional acidic mesophytic coves often includes an increasing proportion of evergreen overstory (*Tsuga canadensis* (L.) Carr.) and shrub species (*Rhododendron maximum* L.) with recalcitrant litter and slow decomposition rates. The result is a scarce HFG in both density and diversity however, where *Rhododendron* is scarce, the HFG is highly diverse and abundant (Elliott and others 2014). In either case, the HFG may play an important role in regulating ecosystem processes such as biogeochemical cycling, net primary productivity, and tree growth and regeneration.

To examine the importance of the HFG in regulating ecosystem processes, we chose a mesophytic cove forest with a rich and diverse herbaceous flora. Our experimental approach was to completely eliminate the herbaceous layer. We hypothesized that (i) the high quality (relatively high nutrient concentrations, low lignin concentration, and high decomposition rate) HFG litter in mesophytic coves accelerates carbon and nutrient cycling, (ii) increased nutrient cycling will have a positive effect on overstory tree growth, and (iii) the HFG regulates tree regeneration with negative effects on seedling establishment due to competition for resources. To test these hypotheses and isolate causal factors, we used a long-term (14 years) experiment consisting of two treatments: (1) manual removal of all herbaceous layer flora, (2) annual addition of an equivalent amount of herbaceous leaf and stem material to the existing herbaceous flora and an untreated reference.

Materials and Methods

Study Area

We chose a rich, cove forest in the Coweeta Hydrologic Laboratory, western North Carolina (35°02′N latitude, 83°27′W longitude) as the site for our experimental manipulation. Rich, cove communities are described as mesic sites at moderate elevation (1,065–1,220 m), with rich and generally deep soils, and primarily broad coves in lower slope positions (Schafale and Weakley 1990). Soils at our site are Cullasaja–Tuckasegee complex, loamy-skeletal or coarse-loamy, mixed, mesic Typic Haplumbrepts, with depth to bedrock ranging from 80 to 180 cm (Thomas 1996). Mean annual pre-

cipitation is 1,800 mm with most months receiving at least 100 mm (Figure 1). Mean annual temperature is 13°C, and average temperatures are 6.7°C in the dormant season and 18.5°C in the growing season (Laseter and others 2012; Figure 1).

The forest has a dense canopy with mesophytic tree species, including *Liriodendron tulipifera* L., *Tilia americana* Miller, *Aesculus flava* Aiton ex Hope, *Betula lenta* L., *Magnolia acuminata* L., *Prunus serotina* Ehrhart, and *Fraxinus americana* L. and a lush and diverse herb layer (Appendix 1 in Online publication). *T. canadensis* mortality has been widespread in the southern Appalachians due to hemlock woolly adelgid (*Adelges tsugae* Annand; HWA) infestation (Vose and others 2013); however, *T. canadensis* represented only a small component (5.8 ± 1.5%) of the overstory in our study site and was equally distributed among plots (Appendix 2 in Online publication). Hence, we do not expect that HWA influenced the results that we observed, based on studies where hemlock was at least 50% of the overstory (Knoepp and others 2011; Ford and others 2012). The cove also had a minimal evergreen shrub (*R. maximum*) component and absence of known keystone species (such as, nitrogen fixers, calcium accumulators) in the her-

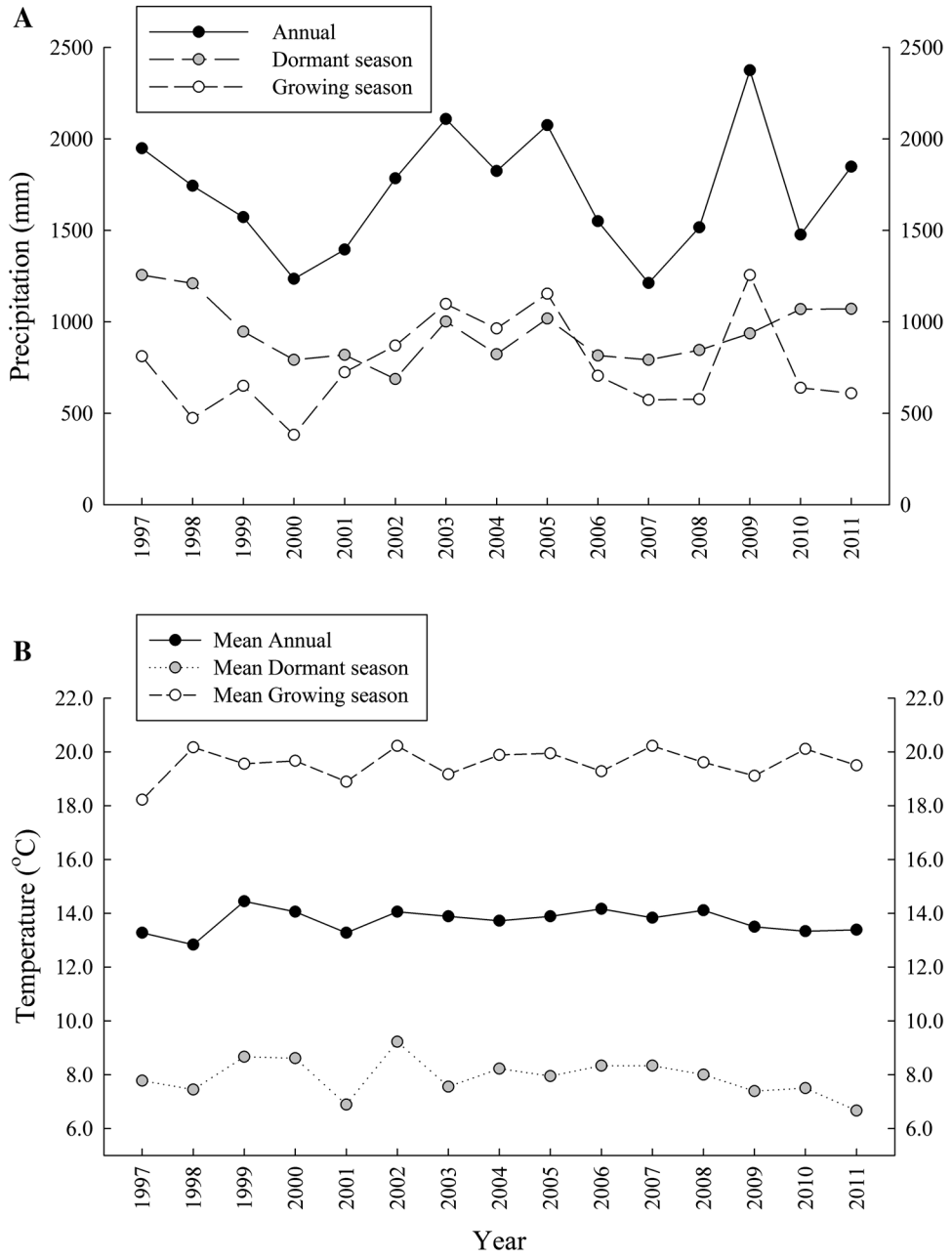

Figure 1. Annual, growing season, and dormant season climate for years 1997–2011: **A** precipitation and **B** temperature. These data were collected at Coweeta Hydrologic Laboratory CS01, main climate station, less than 2.0 km from the study plots.

baceous layer, which avoids possible erroneous interpretations (sensu Huston1997).

Experimental Design

Eighteen plots—six replicates (20-m × 20-m plots) of herbaceous removal (HR), six replicates (10-m × 20-m plots) of organic matter amendment (OMA), and six replicates (10-m × 20-m plots) of no removal (NR, reference)—were installed in summer 1998. The OMA treatment was designed to increase the potential influence of herbaceous aboveground leaf and stem material on nutrient and C cycling processes by doubling the input. Plots were permanently marked and vegetation was measured in each plot. Percent cover of herbaceous species was estimated using the line-intercept method along four 20-m length transects for each plot. Initial biomass was estimated by clipping all herbaceous + deciduous shrubs in six 1.0 m^2 quadrats randomly placed in each plot. In mid-summer 1998 (after plot establishment), herbaceous species were removed by hand (stems of large herbs were clipped, and young or newly emerging herbs were pulled minimizing soil disturbance), small shrubs (for example, *Gaylussacia ursina* (M.A. Curtis) Torr. & Gray and *Vaccinium pallidum* Aiton) were clipped, and all material was removed from the six HR plots. Thereafter, HR plots were weeded/clipped two times annually, in spring (early May) to remove early season, ephemeral species, and again mid-summer (August) to remove peak biomass. For the OMA treatment, herbaceous material from nearby mesic, cove forests with similar herb composition was collected. Herbs were clipped, shredded, and spread evenly over each of the OMA plots as an organic matter amendment of 105 g m^{-2} fresh weight (≈42 g m^{-2} dry weight, estimated from initial 36–1.0 m^2 clipped plots) to double the amount of organic matter input expected from the NR plots. OMA was applied once annually in August; all treatments were maintained in subsequent years for a total of 14 years (1998–2011). Due to the timing of the OMA treatment, we likely missed weeding many of the spring ephemerals; however, they typically contribute less than 5.0% to the total HFG biomass (Elliott, unpublished data). Based on visual observation, forest floor disturbance due to plant removal was minimal during the first 2 years of treatment when understory biomass was greatest. By year 5, herbaceous and deciduous shrub biomass was substantially reduced (10.6 ± 0.3 g m^{-2} in 2003) and weeding requirements were minimal thereafter (for example, 0.5 ± 0.1 g m^{-2} in 2011). Hence, we would expect that any abiotic disturbances associated with the weeding process were minor and had no impacts on observed responses. To assess the effects of the herbaceous layer removal and organic matter addition on ecosystem function, we measured several key ecosystem level parameters: forest floor mass, carbon (C) and nitrogen (N); soil carbon dioxide (CO_2) efflux; soil temperature and moisture; soil C, N, and N-mineralization rates; litterfall mass, C, and N; and tree growth and regeneration. All sample collections were randomly placed, with the restriction that placement be at least 2-m from the plot boundary to avoid potential edge effects.

Forest Floor Mass, Carbon, and Nitrogen

Forest floor was sampled in December–January of 4 years (2002, 2004, 2007, and 2011). We collected four 0.09-m^2 samples using a 0.3 × 0.3 m wooden frame within each treatment plot (NR, HR, and OMA). Material was separated into Oi and a combined Oe + Oa layers. Forest floor materials were placed in a paper bag and transported to the laboratory where they were dried at 60°C to a constant weight, and weighed to the nearest 0.1 g. All samples were ground to less than 1 mm and mixed thoroughly. Total C and N concentrations were determined by combustion using an elemental analyzer (Perkin-Elmer 2400 CHN Elemental Analyzer, Norwalk, Connecticut, USA). Total forest floor C and N content were estimated by multiplying C or N concentration by dry mass for each forest floor layer. Ash-free dry weight of the Oe + Oa layer was determined by loss-on-ignition by incinerating a 5 g sample for 12 h in a muffle furnace at 450°C and then calculating by weight difference between the organic and mineral fractions of the sample to allow weight correction of the Oe + Oa layer for mineral material.

Soil C, N, and N-Mineralization

We measured soil N-mineralization rates using a modified 28-day closed core in situ incubation technique (Knoepp and others 2011). Measurements were conducted in the growing season, periodically from 1999 to 2011. We randomly selected sample locations on four transects dissecting each 20- × 20-m plot and three transects on each 10- × 20-m plot. At each location two PVC cores (4.3 cm internal diameter) were driven 10 cm into the mineral soil, one core was removed to determine NO_3-N and NH_4-N concentrations at the time of collection (t = 0) and one core was left in place for a 28-day incubation period (t = 1). Within 1 h

of collection, soils were mixed thoroughly and a subsample (approximately 10 g) of soil was added to pre-weighed 125 ml polyethylene bottles containing 50 ml 2 M KCl. The bottles plus soil were kept cool until returning to the laboratory, where they were weighed to determine the actual weight of soil extracted. The bottles plus soil were shaken and allowed to settle overnight (refrigerated at 4°C); 15 ml of the clear KCl was pipetted into a sample tube and analyzed for NH_4-N and NO_3-N on an autoanalyzer (Alpkem model 3590, Alpkem Corporation, College Station, Texas, USA) using alkaline phenol (USEPA 1983a) and cadmium reduction (USEPA 1983b) techniques, respectively. A second subsample (~20 g) was dried at 105°C for more than 12 h to obtain oven-dry weight. The remaining $t = 0$ soil samples were air-dried, composited by plot, sieved (<2 mm), and analyzed for pH, total C, and total N. Soil pH was determined in a 1:1 air-dried soil to 0.01 M $CaCl_2$ slurry with an Orion 9165BN combination pH electrode and Thermoscientific Orion 3 star pH meter (Thermo Fisher Scientific, Waltham, Washington, USA). A subsample of each soil was powdered by mortar and pestle and analyzed for total C and total N concentrations by combustion as described above. Total C and total N content of the surface soil (0–10 cm) were calculated by multiplying C and N concentration by soil bulk density of each plot. Average plot bulk density values were obtained by weighing all soil sampled in $t = 0$ soil cores in 2002, 2006, and 2011. Bulk density (g cm^{-3}) values represented weight of material less than 5.6 mm in a 145.2 cm^3 core.

Total Soil CO_2 Efflux

We measured total soil CO_2 efflux (soil plus forest floor; hereafter referred to as soil CO_2 efflux; μmol $m^{-2} s^{-1}$) in each NR, HR, and OMA plot using a gas exchange analyzer with a soil respiration chamber attachment (LiCor 6400, LiCor Inc., Lincoln, Nebraska, USA). We installed five PVC collars (10 cm diameter × 5 cm deep) randomly located in each treatment plot (3 treatments × 6 replicate plots × 5 collars = 90 total). Collars were imbedded 2 cm into the mineral soil and left in place throughout the measurement period for each sample year. Measurements began 1 week after collar installation to minimize the effects of disturbance. Concurrent with soil CO_2 efflux, we measured soil temperature (LI-6400 Soil Temperature Probe, LiCor Inc., Lincoln, Nebraska, USA) and soil moisture content using time domain reflectometry (CS620, Campbell Scientific Inc., Logan, Utah, USA) at 10 cm soil depth adjacent to each collar. We conducted these measurements seasonally (summer and winter) from 1999 to 2004 and monthly during the growing season in 2007 and 2011.

Litterfall

In August of 1999, we installed four, 0.11 m^2 plastic litter baskets within each plot (3 treatments × 6 replicate plots × 4 baskets = 72 baskets). Litterfall (leaves, seeds, and small twigs) was collected about every 3 months, from September 1999 to March 2010. After collection, samples dried at 60°C to a constant weight, and weighed. Litter was separated into components of deciduous leaves, *T. canadensis* needles, and other (other leaves, seeds, and small twigs). *T. canadensis* needles were a minor component of overall litterfall mass (2.3 ± 0.4% over the study period across all plots); however, we excluded *T. canadensis* needles from all litterfall analyses to avoid any potential biases associated with HWA. All component samples were ground to less than 1 mm, mixed thoroughly, and analyzed for total C and total N as described above.

Tree Growth

In winter 1999, dendrometer bands (Cattelino and others 1986) were installed to measure diameter at breast height (dbh; 1.37-m from ground level) on all trees at least 10.0 cm dbh in each plot. Tree stems at least 2.0 cm dbh and less than 10.0 cm dbh were measured with a diameter tape to the nearest 0.1-cm at dbh. All stems were number tagged and measured annually from 1999 to 2004, and again in 2008, 2009, 2011, and 2012. During the surveys, if a stem 2.0 cm or greater dbh had not been tagged and measured in the previous year, it was measured and considered recruitment from the understory layer. We calculated basal area increment (BAI), beginning in 2000, from the difference of successive annual diameter growth measured at the end of each year for the first 5 years of the study, and periodically thereafter. We estimated aboveground biomass using measured dbh and allometric equations developed for woody species in the southern Appalachians (Martin and others 1998). Aboveground net primary productivity (ANPP) was calculated from the difference of successive annual wood biomass increment plus annual litterfall.

Tree Seedling Recruitment

We counted tree seedlings by species in a 1.0-m × 15.0-m belt transect across each NR and HR

treatment plot. Seedlings were counted in July–August of 9 years (1998, 1999, 2000, 2002, 2004, 2005, 2006, 2007, and 2011). We did not survey tree seedlings in OMA treatment plots over time. Seedlings were counted in OMA and NR treatment plots in August 2013.

Statistical Analyses

We used a mixed linear model with repeated measures (PROC MIXED, SAS 2002–2013) to evaluate the main effects of year and treatment, and year * treatment interactions on litterfall, tree growth, and regeneration (BAI, ANPP, and tree seedlings), forest floor, and soil properties (CO_2 efflux, N-mineralization, total C, total N, and pH). In the repeated statement, the experimental unit ('subject') was the plot within treatment. We used either the unstructured covariance or the autoregressive order one option in the repeated statement depending on which covariance structure produced the smallest value for the Akaike's Information Criterion (AIC) and Schwarz' Bayesian Criterion (SBC) (Littell and others 1996) for each parameter model. If overall F tests were significant ($P \leq 0.05$) then least squares means (LS-means) tests were used to evaluate significance among year and treatment (NR, HR, and OMA) interactions. We used the Satterthwaite option in the model statement to obtain the correct degrees of freedom (Littell and others 1996). We examined the correlation between treatment differences (that is, OMA *minus* HR, HR *minus* NR) and climatic variables (annual and seasonal precipitation and air temperature) when the analyses indicated a significant year * treatment interaction.

Results

Inter-annual Variation

Main effects of year were significant for all parameters measured in our study (Table 1), indicating that our sampling methods were sensitive enough to detect year-to-year variation in ecosystem processes. Some of this inter-annual variation may have been related to climatic variation, which was substantial over the 14-year treatment period (Figure 1). For example, annual precipitation ranged from 1,250 to 2,400 mm y^{-1}, a near twofold difference (Figure 1A); growing season precipitation ranged from 350 to 1,300 mm y^{-1}. Mean annual air temperature ranged from 12.5 to 14°C, growing season temperature ranged from 18.5 to 20°C (Figure 1B). When analyzing the influence of precipitation and air temperature on ecosystem process responses, we expected (and observed) the strongest relationships where we doubled (OMA) or removed (HR) the entire high quality HFG biomass. Hence, we focused primarily on OMA *minus* HR responses when evaluating the influence of inter-annual variation in precipitation and air temperature (below). Additional temporal variation may reflect parameter responses that are systematically changing over time (either decreasing or increasing) in response to the treatments.

Forest Floor and Soil Responses

Despite increasing (OMA) or reducing (HR) herbaceous organic matter inputs to the forest floor for 14 years, no significant treatment effects were found for forest floor (Oi and Oe + Oa layers) mass and C concentration (Table 1). The year * treatment interaction was significant for the Oe + Oa layer C:N ratio. In 2004, NR had lower C:N ratio than HR ($F_{1,15} = -3.42$, $P = 0.004$) and OMA ($F_{1,15} = -2.16$, $P = 0.048$). C:N ratio was not different among treatments in 2002, 2007, or 2011. Across time and treatment, mean forest floor (Oi + Oe + Oa) mass, C, and N were 1172 ± 74 g m^{-2}, 506 ± 31 g C m^{-2}, and 14.9 ± 1.1 g N m^{-2}, respectively (Appendix 3 in Online publication). Forest floor C and N pool sizes were greater than the herbaceous material added or removed annually (18 g C m^{-2} y^{-1}; 0.7 g N m^{-2} y^{-1} dry weight).

Main effects of year and treatment, and year * treatment interaction were significant for soil N-mineralization rate (Table 1). OMA had greater N-mineralization than NR ($F_{1,15} = 2.39$, $P = 0.030$) and HR ($F_{1,15} = 2.48$, $P = 0.026$); but, there was no difference between NR and HR ($F_{1,15} = 0.08$, $P = 0.9342$) (Figure 2). Soil N-mineralization was greater on the OMA treatment in 2006 (NR, $F_{1,15} = 2.58$, $P = 0.021$; HR, $F_{1,15} = 2.85$, $P = 0.012$) and 2011 (NR, $F_{1,15} = 2.72$, $P = 0.016$; HR, $F_{1,15} = 3.07$, $P = 0.008$) (Figure 2). By 2011, N-mineralization was 0.734 g N m^{-2} 28 day^{-1} for OMA and 0.310 g N m^{-2} 28 day^{-1} for HR. If we assume that this rate is representative of the summer season (that is, June, July, August, and September) (Knoepp and others 2008), total summer N-mineralization was 3.193 g N m^{-2} 4-$months^{-1}$ and 1.348 g N m^{-2} 4-$months^{-1}$ for OMA and HR, respectively, a difference of 1.845 g N m^{-2} 4-$months^{-1}$. Thus, OMA *minus* HR N-mineralization was greater than the 0.7 g N m^{-2} y^{-1} added in the OMA treatment. The significant year * treatment term (Table 1) indicated precipitation and/or air temperature had an effect on the N-mineralization response. The difference between OMA and HR in

Table 1. Mixed Model Repeated Measures Analysis with *F* and *P* Values for Forest Floor Parameters (Oi and Oe + Oa Mass, C Concentration (%), N Concentration (%), and C:N Ratio); Soil CO_2 Efflux, Temperature, and Moisture (Growing Season and Dormant Season); and Soil Parameters (pH, N-mineralization, Total C, Total N, and C:N Ratio); Litterfall Parameters (Mass, C Concentration (%), Total C Input, N Concentration (%), Total N Input, and C:N Ratio); Tree Growth (BAI, Basal Area Increment; and ANPP, Aboveground Net Primary Production); and *Acer rubrum* Seedlings and Other Tree Seedlings

Forest floor:	Oi mass		Oi C %		Oi N %	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**25.06**	**<0.0001**	**7.28**	**0.0031**	**5.22**	**0.0115**
Treatment	0.75	0.4912	0.92	0.4213	0.18	0.8391
Year * treatment	0.63	0.7079	0.93	0.5003	0.54	0.7711
Forest floor:	**Oi C:N ratio**		**Oe + Oa mass**		**Oe + Oa C %**	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**3.40**	**0.0455**	**7.53**	**0.0027**	**27.76**	**<0.0001**
Treatment	0.06	0.9462	1.09	0.3619	1.55	0.2444
Year * treatment	0.42	0.8570	1.15	0.3826	2.10	0.1150
Forest floor:	**Oe + Oa N %**				**Oe + Oa C:N ratio**	
Parameter:	*F*	*P*			*F*	*P*
Year	**6.82**	**0.0041**			**6.20**	**0.0060**
Treatment	0.73	0.4970			0.21	0.8164
Year * treatment	2.70	0.0554			**3.53**	**0.0222**
Soil:	**Soil pH**		**N-mineralization**		**Soil C content**	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**73.91**	**<0.0001**	**5.83**	**0.0049**	**16.59**	**<0.0001**
Treatment	0.01	0.9923	**3.96**	**0.0417**	3.08	0.0756
Year * treatment	0.71	0.6825	**2.74**	**0.0443**	2.46	0.0735
Soil:	**Soil N content**				**Soil C:N ratio**	
Parameter:	*F*	*P*			*F*	*P*
Year	**17.46**	**<0.0001**			**21.74**	**<0.0001**
Treatment	3.18	0.0708			0.15	0.8612
Year * treatment	2.22	0.0990			0.67	0.6735
Soil:	**Soil CO_2 efflux**		**Soil temperature**		**Soil moisture content**	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Growing season						
Year	**21.69**	**<0.0001**	**160.52**	**<0.0001**	**61.88**	**<0.0001**
Treatment	0.25	0.7818	0.16	0.8550	0.30	0.7422
Year * treatment	1.14	0.3167	0.43	0.9878	0.25	0.9996
Dormant season						
Year	**36.55**	**<0.0001**	**133.09**	**<0.0001**	**44.46**	**<0.0001**
Treatment	0.38	0.6870	0.01	0.9994	0.14	0.8675
Year * treatment	0.43	0.9921	0.04	1.0000	0.29	0.9995

Table 1. Continued

Litterfall:	Mass		C %		Total C input	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**115.46**	**<0.0001**	**27.55**	**<0.0001**	**13.98**	**<0.0001**
Treatment	2.76	0.0953	0.07	0.9311	**5.62**	**0.0151**
Year * treatment	**3.13**	**0.0153**	0.56	0.9229	0.38	0.9891

Litterfall:	N %		Total N input		C:N ratio	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**38.86**	**<0.0001**	**146.73**	**<0.0001**	**105.84**	**<0.0001**
Treatment	0.39	0.6814	3.25	0.0670	0.02	0.9734
Year * treatment	0.47	0.9662	**2.31**	**0.0532**	1.56	0.1958

Tree:	BAI		BAI (trees ≤ 10 cm dbh)		ANPP	
Parameter:	*F*	*P*	*F*	*P*	*F*	*P*
Year	**40.01**	**<0.0001**	**18.55**	**<0.0001**	**56.13**	**<0.0001**
Treatment	0.83	0.4546	1.67	0.2215	0.88	0.4370
Year * treatment	1.64	0.1692	0.52	0.9342	1.08	0.3885

Tree seedlings:	*Acer rubrum*		Other seedlings	
Parameter:	*F*	*P*	*F*	*P*
Year	**200.5**	**<0.0001**	**14.64**	**0.0001**
Treatment	**8.83**	**0.0140**	4.31	0.0647
Year * treatment	**8.09**	**0.0014**	**43.25**	**<0.0001**

Parameters that were significant at the α ≤ 0.05 level are shown in bold.

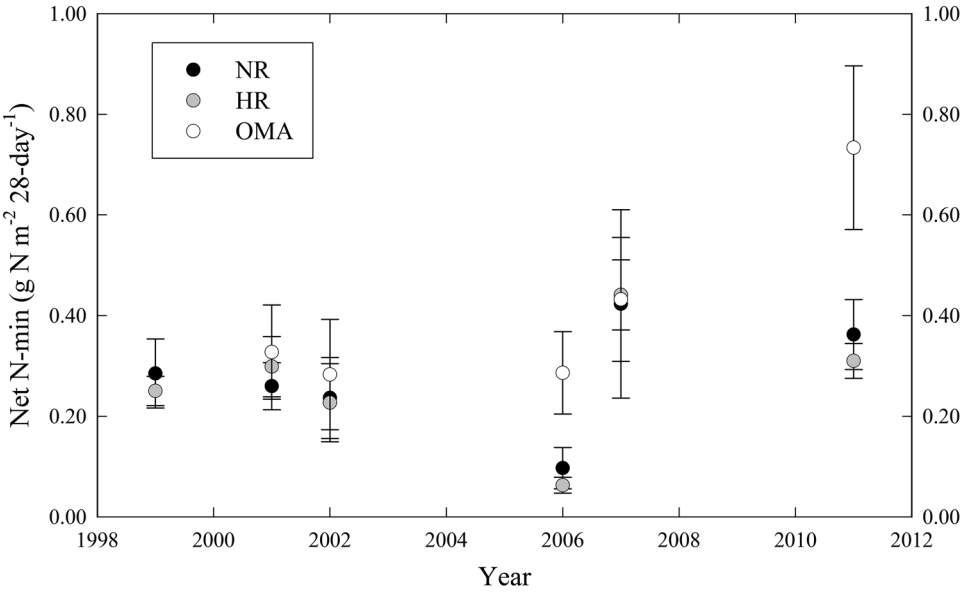

Figure 2. Soil net nitrogen (N)-mineralization for the no removal (NR), herbaceous removal (HR), and organic matter addition (OMA) treatments over time (1999–2011): Soil N-mineralization was measured in June–July of the sample years 1999, 2001, 2002, 2006, 2007, and 2011. Measurements began the first year after herbaceous removal and organic matter addition treatments.

N-mineralization increased with dormant season precipitation (r^2 = 0.670, P = 0.090), in contrast, no relationship was found for OMA *minus* HR N-mineralization and air temperature (r^2 = 0.048, P = 0.723). Year also had a significant effect for other soil parameters (pH, N, C, and C:N ratio)

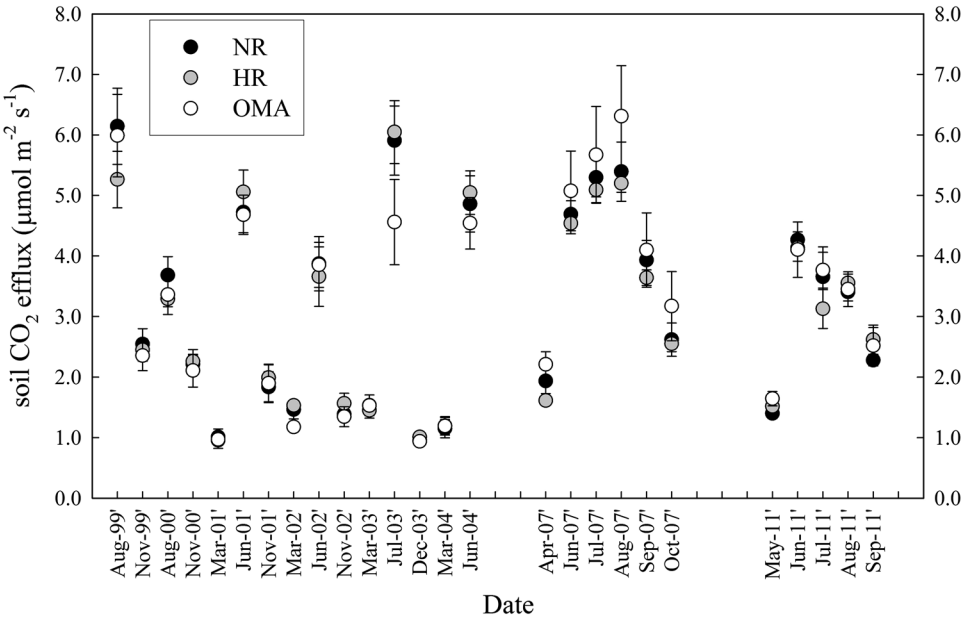

Figure 3. Soil CO_2 efflux for the no removal (NR), herbaceous removal (HR), and organic matter addition (OMA) treatments over time (1999–2011). Measurements were taken in spring, summer, and fall/winter months.

(Table 1); whereas, treatment had only a marginal effect on soil C content ($P = 0.076$) and soil N content ($P = 0.071$). In 2011, soils in OMA had 6649 ± 792 g C m^{-2} and 400 ± 22 g N m^{-2}; HR had 7445 ± 322 g C m^{-2} and 442 ± 23 g N m^{-2}; and NR had 6553 ± 418 g C m^{-2} and 397 ± 34 g N m^{-2} (Appendix 4 in Online publication). Thus, soil C and N pools in OMA were less than HR soil pools (OMA *minus* HR; −796 g C m^{-2}, −42 g N m^{-2}), even after 14 years of HFG addition.

Soil CO_2 efflux was higher in the growing season than dormant season months (Figure 3); averaging 4.8 ± 0.3 μmol m^{-2} s^{-1} in the growing season (Apr–Sep) and 1.8 ± 0.1 μmol m^{-2} s^{-1} in the dormant season (Oct–Mar). Treatment had no effect on soil CO_2 efflux (Figure 3); however, the main effect of year was significant in the repeated measures models for both the growing season and dormant season months (Table 1).

Soil temperature and soil moisture content varied across years (Appendix 4 in Online publication), but did not differ among treatments in any sample year or season (Table 1). Mean growing season soil moisture ranged from 16.2 to 40.2% and mean soil temperature ranged from 15.8 to 19.0°C. Instantaneous rates of soil CO_2 efflux ranged from 0.44 to 11.9 μmol m^{-2} s^{-1} depending on time of year. Across time and treatments, soil CO_2 efflux was strongly and exponentially related to soil temperature (soil CO_2 efflux = $\beta 0 * e^{\beta 1 * Temp}$, $r^2 = 0.572$, $P < 0.0001$), but not related to soil moisture content ($r^2 = 0.018$, $P = 0.265$). There was a significant relationship between growing season precipitation and OMA *minus* HR for soil CO_2 efflux, where response magnitude was greater at lower growing season precipitation (Figure 4). No significant response was found between OMA *minus* HR soil CO_2 efflux and air temperature ($r^2 = 0.313$, $P = 0.149$).

Aboveground Responses

We found significant treatment effects for both litterfall mass and total litterfall N (Table 1). The OMA treatment had greater litterfall mass ($F_{1,15} = 2.35$, $P = 0.033$) than HR, but there were no differences between OMA and NR ($F_{1,15} = 1.13$, $P = 0.277$) or between NR and HR ($F_{1,15} = 1.22$, $P = 0.240$) (Figure 5). Litterfall C and N concentrations were not different among treatments (Table 1) averaging 419.9 ± 19.8 mg C g^{-1} and 8.3 ± 0.3 mg N g^{-1} across years and treatments. Total litterfall N ranged from 2.2 to 5.5 g N m^{-2} y^{-1} and had a significant year * treatment interaction effect that was largely due to the greater contribution of litterfall mass (Figure 5A, B). Total litterfall N was greater for OMA than HR ($F_{1,15} = 2.55$, $P = 0.022$) in most years; but, there was no difference between OMA and NR ($F_{1,15} = 1.26$, $P = 0.226$) or NR and HR ($F_{1,15} = 1.29$, $P = 0.217$). Averaged across all years, litterfall N was 3.42 ± 0.16 g N m^{-2} y^{-1} for NR, 3.07 ± 0.13 g N m^{-2} y^{-1} for HR, and 3.76 ± 0.26 g N m^{-2} y^{-1} for OMA; and the mean difference between OMA and HR was 0.69 g N m^{-2} y^{-1}. The enhancement of both litterfall mass and litterfall N in OMA compared with HR increased with growing season precipitation (Figure 6A, B), but

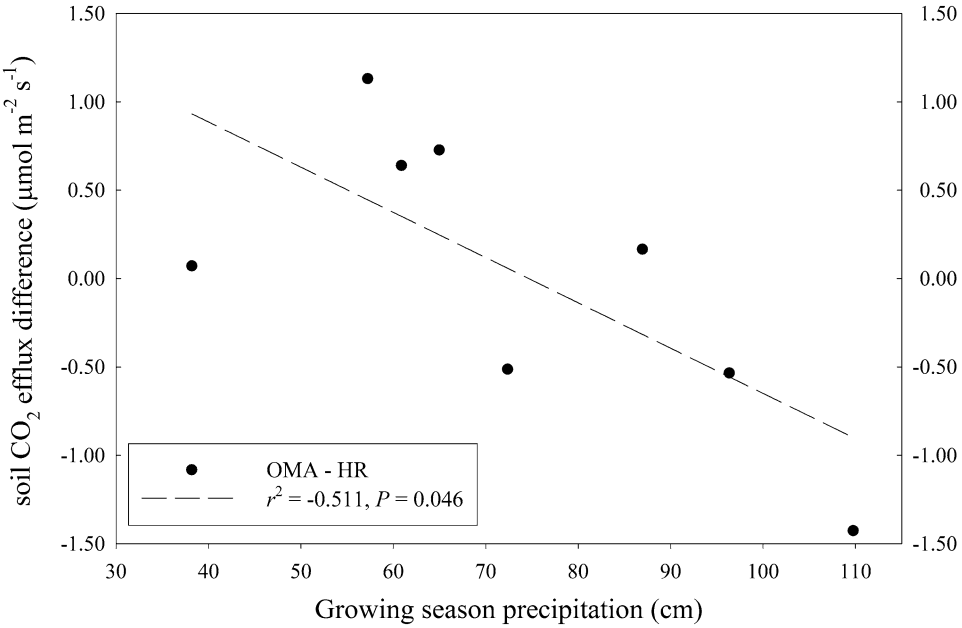

Figure 4. Regression between OMA *minus* HR soil CO_2 efflux and growing season precipitation.

not growing season air temperature (litterfall mass, $r^2 = 0.003$, $P = 0.997$; litterfall N, $r^2 = 0.001$, $P = 0.949$).

Nitrogen concentration in the herbaceous material was higher than canopy litterfall N (1.61 ± 0.03% N vs 0.83 ± 0.01% N, averaged across years and treatments), and C:N ratio was much lower (26.9 ± 0.6 vs C:N = 49.9 ± 0.8) verifying that higher quality material was added or removed as HR. At the initiation of the study (1998), average herbaceous mass was ca. 42 g m^{-2} resulting in addition or removal of approximately 0.7 g N m^{-2} annually for 14 years; representing from 13 to 30% of the annual litterfall N input depending on year and treatment (Figure 5B).

Tree BAI generally increased over time (Appendix 6 in Online publication) and the main effect of year was significant in the repeated measures model (Table 1); however, the main effect of treatment and year * treatment interaction were not significant. In addition, BAI of only small trees (≤ 10 cm dbh) did not respond to treatment (Table 1). For ANPP, we found a significant effect of year, however, neither treatment nor year * treatment effects were significant (Table 1). Litterfall mass contributed 15–42% of the ANPP depending on year.

Year and year * treatment interactions were significant for seedling recruitment, there was also a significant treatment effect in the recruitment of *Acer rubrum* seedlings (Table 1). Starting in 2002, seedling recruitment of *Acer rubrum* was higher in the HR plots (Table 1; Figure 7); seedlings of other tree species were less abundant overall compared to *A. rubrum*. There were significantly greater numbers of *A. rubrum* seedlings in HR than NR in 2004, 2005, 2006, 2007, and 2011 (Figure 7). Although we did not measure seedling recruitment changes on the OMA treatment over time, a 2013 survey in the OMA and NR plots indicated that seedling densities were comparable between OMA and NR treatments (for example, 4.9 ± 1.5 *Acer rubrum* and 0.5 ± 0.2 other tree seedlings m^{-2} for OMA vs 4.8 ± 0.7 *A. rubrum* and 0.6 ± 0.1 other tree seedlings m^{-2} for NR).

Discussion

Our first hypothesis was that the herbaceous functional group (HFG) plays an important role in regulating ecosystem C and N cycling pools and processes by supplying high quality litter to the forest floor. To maximize the likelihood of observing a treatment response, we doubled (OMA) or removed (HR) the entire high quality HFG biomass over a 14-year period. The OMA plots had greater soil N availability as demonstrated through greater rates of soil N-mineralization, which is consistent with our hypothesis. Numerous other factors regulate soil N-mineralization including soil moisture and temperature, pH, availability of other nutrients, and soil flora and fauna (Rothe and Binkley 2001; Knoepp and Swank 2002; Lovett and others 2004; Laughlin 2011; Norris and others 2013). We found that these factors did not respond to treatment suggesting that altering inputs of litter mass and quality was the primary regulating factor of N mineralization. At the initiation of our experiment, N-mineralization rates across treatments were

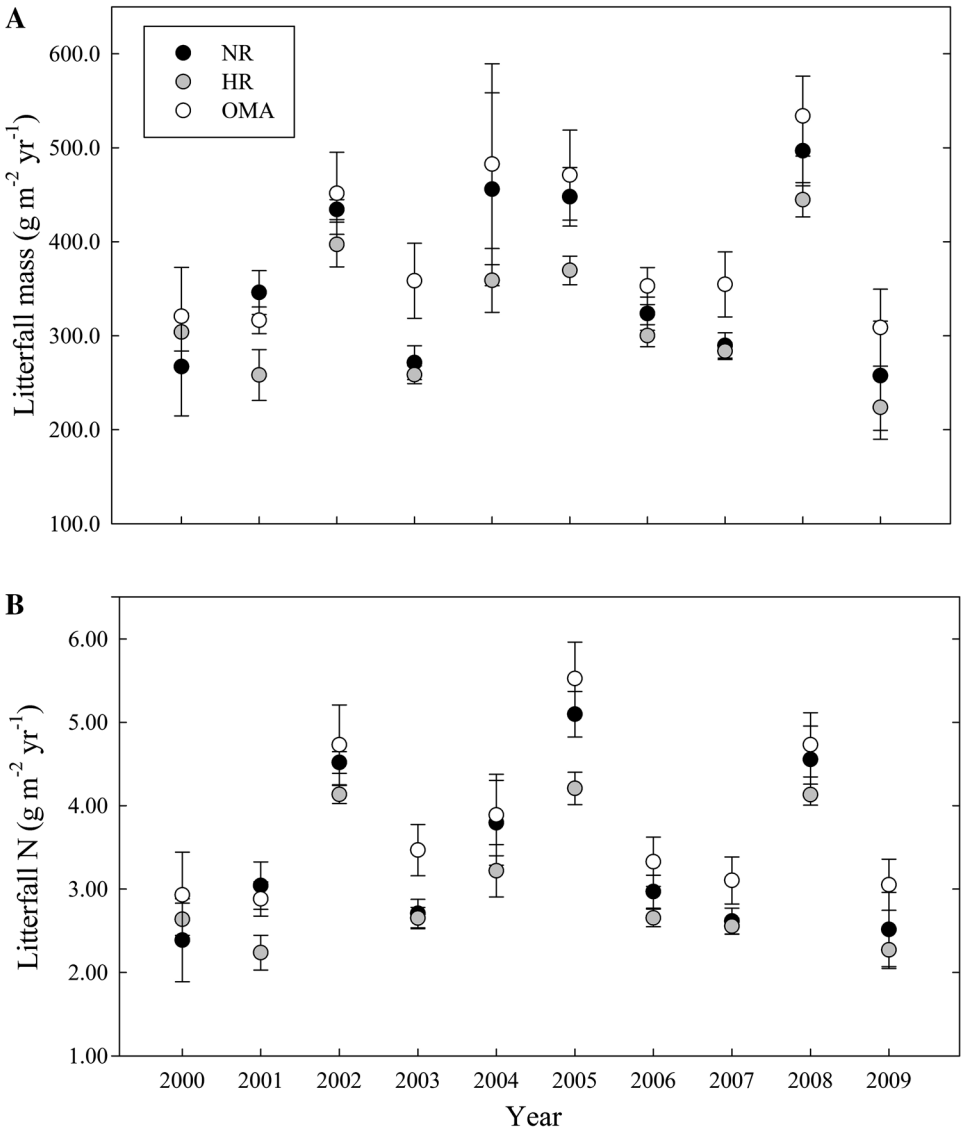

Figure 5. Litterfall input for the no removal (NR), herbaceous removal (HR), and organic matter addition (OMA) treatments over time (2000–2011): **A** total litterfall mass; **B** total litterfall nitrogen (N) content. Measurements began 2 years after the initial removal and organic matter addition treatments. Total litterfall includes leaf tissue of deciduous species, seeds and small twigs, and a small component of rhododendron leaves.

comparable to the values found in other mesophytic coves within the Coweeta basin (Knoepp and Vose 2007; Knoepp and others 2008). However, by 2011, N-mineralization rates in the OMA treatment were more than two times greater than the NR and HR treatments, and OMA *minus* HR N-mineralization was much greater than the amount of N added suggesting a priming effect (Kuzyakov 2010; Vos and others 2013) through the addition of high quality organic material. There was also an interaction between N-response and precipitation. For example, OMA *minus* HR N-mineralization was positively correlated with dormant season precipitation, suggesting that greater soil moisture storage from late dormant season (months March and April) precipitation may have stimulated N-mineralization in the OMA treatment. Interestingly, removing herbaceous organic material was not mirrored by an inhibitory effect with reduced organic material inputs, over the 14-year study period. Although soil N-mineralization was stimulated in OMA we measured little or no effect on forest floor mass, indicating that decomposition of the added organic material was rapid. Other research studies have shown that the priming effect stimulates microbial activity and accelerates decomposition rates (Cornelissen and others 2003; Keiser and others 2013). This supports our measurement of higher C:N ratio in the Oe + Oa forest floor layer and the trend towards reduced soil C and N in the OMA plots. A review of the priming effect in both agricultural and natural soils by Fontaine and others (2003) suggests that this complex response varies not only with the type of material added to soils but also the presence of microbial populations able to decompose fresh versus recalcitrant organic

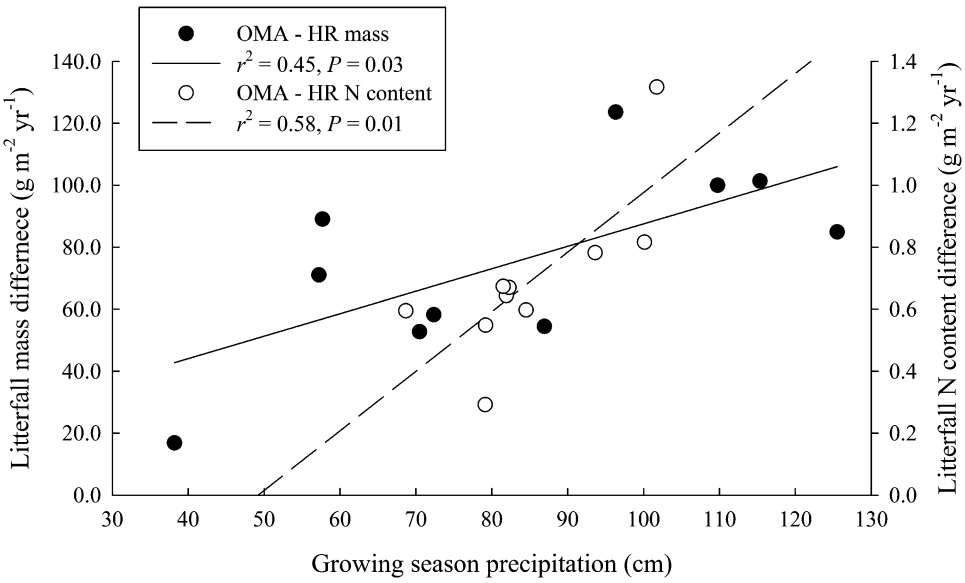

Figure 6. Regressions between OMA *minus* HR litterfall mass and growing season precipitation, and OMA *minus* HR litterfall nitrogen (N) content and growing season precipitation.

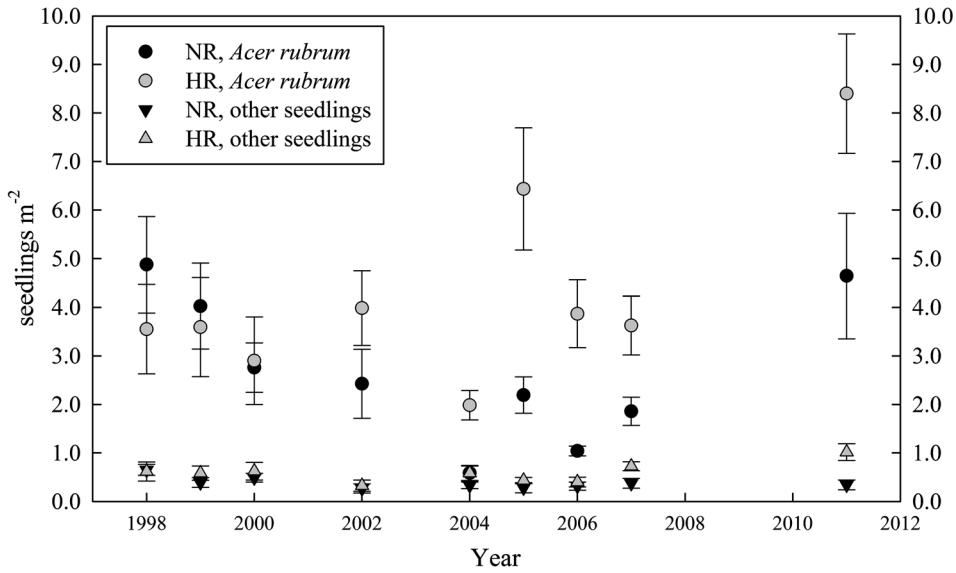

Figure 7. Number of *Acer rubrum* and other tree seedlings in the no removal (NR) and herbaceous removal (HR) treatments over time (1998–2011). The 'other' tree species category included: *Acer pensylvanicum, Amelanchier arborea, Betula lenta, Carya* spp., *Fraxinus americana, Hamamelis virginiana, Liriodendron tulipifera, Magnolia fraseri, Nyssa sylvatica, Quercus velutina*, and *Robinia pseudoacacia*. Nomenclature follows Kirkman and others (2007).

matter. For example, Holub and others (2005) found that organic matter manipulations had little to no effect on net N cycling rates for a conifer site in western Oregon and a dry oak forest (620 mm mean annual precipitation) in Hungary. They explained that microbes were probably N limited at the C-rich conifer site in Oregon, and that microbes were C limited at the Hungary site. In the Holub and others (2005) manipulations, the oak and conifer litter was much more recalcitrant than the HFG organic material added or removed in our study.

We found that removal of organic material (HR) had no effect on soil C or N; other researchers have found variable effects. In *Pseudotsuga menziesii* (Mirb.) Franco forests in the Oregon Coast Range, Yildiz and others (2011) found a decrease in total soil C and no change in total soil N following the removal of all shrubs (average biomass = 914.2 g m^{-2}) and herbs (average biomass = 96.9 g m^{-2}) compared to no re-

moval or shrub only removal after 5 years of annual treatment application. In their study, removing the additional small herbaceous component (~10% of total biomass removed) was sufficient to alter total soil C compared to shrub only removal. Abiotic factors likely interacted with microbial activity and decomposition of organic material (Rothe and Binkley 2001; Norris and others 2013), such that differences among treatments were found in some years but not others. Other studies have also shown differences in soil inorganic nitrogen in patches with or without HR (Gilliam and Dick 2010; Andreasson and others 2012). These studies often reflect the *circulus vitiosus (*sensu Jenny and others 1969), that is, the dilemma in distinguishing effects of soil on plants versus effects of plants on soil, such that one does not know if the plants are regulating soil nutrients by uptake and turnover (plant-soil feedback) or if the plants are colonizing sites with a certain level of soil nutrient availability (Laliberté and others 2013).

Our data showed no differences in soil CO_2 efflux among treatments over time despite the addition and removal of high quality herbaceous material. The soil CO_2 efflux rates we measured (growing season, 4.8 μmol m^{-2} s^{-1}) compared well to findings from other studies in the southern Appalachians (Vose and Bolstad 2007; Nuckolls and others 2009). This suggests that neither the abiotic (temperature and moisture) nor biotic (quality and quantity of litterfall and herbaceous material and root activity) factors were sufficiently altered by treatments to significantly affect soil CO_2 efflux. The lack of response may be due, at least in part, to the relatively small amount of fresh litter material added or excluded in our study. Other studies that have measured significant changes in soil CO_2 efflux or soil organic matter content, added or removed much more litter in their experiments (Nadelhoffer and others 2004; Prévost-Bouré and others 2010; Lajtha and others 2014; Fekete et al. 2014). For example, Prévost-Bouré and others (2010) were able to detect a change in soil CO_2 flux after doubling the amount of total fresh litter input (from ≈500 to ≈1,000 g m^{-2}). In our study, we were adding or subtracting approximately 42 g m^{-2} (dry weight) to the forest floor, a relatively small amount compared to the mean annual litterfall (320 ± 16 g m^{-2} y^{-1}, dry weight) and forest floor pool (1078 ± 41 g m^{-2}, dry weight, averaged across years) for NR. In mid-successional southern Appalachian forests, the forest floor contribution to total soil CO_2 efflux averages about 16% (Vose and Bolstad 2007), so large changes in forest floor decomposition rates would be required to directly impact total soil CO_2 efflux. The difference in soil CO_2 efflux between OMA and HR was marginally increasing beginning in April 2007 (Figure 3); however, the data were too variable to detect statistical significance. Despite no observed difference in CO_2 efflux between treatments, the difference between efflux in OMA and HR decreased with growing season precipitation (Figure 4). In dryer years, efflux in OMA exceeded HR, whereas this trend reversed in wetter years.

Our second hypothesis was that greater nutrient availability associated with HFG will have a positive influence on overstory tree growth. The higher overstory litter mass and litter N indicates that overstory trees were benefiting from the additional N availability assessed by enhanced soil N-mineralization in the OMA treatment. An increase in leaf biomass (or LAI) is a common response to fertilization (Albaugh and others 2012) or natural variation in soil N availability (Norris and others 2013); however, the increase in litter mass and N in our study, has not yet translated into greater tree BAI or a significant increase in ANPP. Lovett and others (2013) studied northern hardwood forest sites fertilized with 5 g N m^{-2} y^{-1}; they also saw no increase in ANPP or BAI over their 6-year study. N additions in our study and that of Lovett and others (2013) are below fertilizer applications intended to enhance ANPP, which are typically 10–40 g N m^{-2} (LeBauer and Treseder 2008; Campoe and others 2013). The total N provided by OMA is roughly equivalent to inputs from atmospheric N deposition in southern Appalachian forests (0.9–1.2 g N m^{-2} y^{-1}, Knoepp and others 2008); suggesting, the HFG contributes significantly to nutrient availability in these mesophytic coves. Appalachian forests rarely receive commercial fertilizer; their only external N sources are biological N_2-fixation and atmospheric deposition, making the internal cycling of nutrients via litterfall and HFG, and their subsequent decomposition essential for adequate nutrient availability (Alban 1982). In our study, litterfall N flux ranged between 2.2 and 5.5 g N m^{-2} y^{-1} depending on year and treatment. The difference between OMA and HR litterfall N flux averaged 0.7 ± 0.1 g N m^{-2} y^{-1} across all years (Figure 5), however, increased N availability during the growing season was greater (OMA *minus* HR N-mineralization was 1.845 g N m^{-2} 4-$months^{-1}$). This suggests that some of the internal N cycling contributed by OMA was allocated to belowground fine root and microbial production (Wurzburger and Hendrick 2009). This tradeoff in growth allocation between fine roots and wood production has been observed in numerous studies (see Valentine and Mäkelä 2012).

Our third hypothesis was that the HFG plays a significant role in inhibiting tree regeneration by competing for limited resources. The HR treatment increased regeneration of all tree species, however, the response was disproportionally greater for *A. rubrum*. *A. rubrum* typically has lower fecundity (seed production) than other mesophytic cove hardwoods, such as *Liriodendron tulipifera, Betula lenta, Nyssa sylvatica*, and *Amelanchier arborea* (Clark and others 2012). This response pattern (despite lower relative amounts of seed) reflects the ability of *A. rubrum* to germinate and become established with relatively small changes in resource availability. Because we observed no changes in soil nitrogen availability, soil moisture, or temperature following HR, we postulate that light was the primary limiting resource.

Others studies have documented reduced tree seedling density in forests with a well-developed herbaceous layer beneath an overstory canopy (George and Bazzaz 2014) due to further reductions in light reaching the forest floor. For example, in an Allegheny forest with a dense fern cover, Horsley (1993) found that light quantity and quality were reduced beyond that of the overstory canopy; however, no differences in soil moisture and nutrients were detected between fern and fern-removal treatments. In our study, the overstory canopy was dominated by *L. tulipifera* (Appendix 2 in Online publication), and this species has relatively high fecundity compared to other hardwoods (Clark and others 2012). Despite its high fecundity, *L. tulipifera* is a shade-intolerant species and the marginal increase in light with HFG removal was not sufficient to promote its recruitment.

Studies have documented increased *A. rubrum* density in forests across the eastern U.S. (Abrams 2005; Elliott and Swank 2008; Alexander and Arthur 2010), but none of these studies have implied an interaction between herbaceous vegetation and *A. rubrum* regeneration. Instead, a variety of mechanisms (fire exclusion, competitive ability, loss of *Castanea dentata*) have been advanced to explain this observed pattern (Alexander and Arthur 2010). The results of our study suggest that the understory flora may be another important controlling factor of tree seedling recruitment.

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