

Ecological and evolutionary variation in community nitrogen use traits during tropical dry forest secondary succession

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Abstract. We assessed the role of ecological and evolutionary processes in driving variation in leaf and litter traits related to nitrogen (N) use among tropical dry forest trees in old-growth and secondary stands in western Mexico. Our expectation was that legumes (Fabaceae), a dominant component of the regional flora, would have consistently high leaf N and therefore structure phylogenetic variation in N-related traits. We also expected ecological selection during succession for differences in nitrogen use strategies, and corresponding shifts in legume abundance. We used phylogenetic analyses to test for trait conservatism in foliar and litter N, C:N, and N resorption. We also evaluated differences in N-related traits between old-growth and secondary forests. We found a weak phylogenetic signal for all traits, partly explained by wide variation within legumes. Across taxa we observed a positive relationship between leaf and litter N, but no shift in resorption strategies along the successional gradient. Despite species turnover, N-resorption, and N-related traits showed little change across succession, suggesting that, at least for these traits, secondary forests rapidly recover ecosystem function. Collectively, our results also suggest that legumes should not be considered a single functional group from a biogeochemical perspective.

Key words: leaf nitrogen; legumes; litter; neotropical dry forest; nitrogen use strategies; phylogenetic signal; resorption; tropical N cycling.

INTRODUCTION

Both evolutionary and ecological processes shape the distribution of functional traits in plant communities (Cavender-Bares et al. 2009), and thereby influence ecosystem properties (Lavorel and Garnier 2002). In areas such as the Neotropics, that are experiencing unprecedented land transformation (Foley et al. 2005), these processes determine the functional composition of human-modified forests that now make up large areas of the tropical landscape (Chazdon et al. 2009). As secondary forests regenerate, their ecological assembly from a regional species pool that includes introduced species from human interventions may change through the course of succession (Marin-Spiotta et al. 2007, Chazdon 2008, Bhaskar et al. 2014). The extent to which these forests resemble undisturbed forests in functional composition will have consequences for ecosystem functioning, including carbon and nutrient cycling (Lavorel and Garnier 2002). Thus it is critical to understand how both ecological and evolutionary processes structure functional traits in secondary forests.

Within the Neotropics, seasonally dry tropical forests (SDTFs) have experienced particularly widespread

conversion to pastures for cattle grazing (Sánchez-Azofeifa and Portillo-Quintero 2011). These pastures are often left abandoned for economic reasons after years of use, after which forests regrow (Burgos and Maass 2004). Despite their extent and vulnerability, SDTFs are a relatively understudied biome in the Neotropics (Sánchez-Azofeifa and Portillo-Quintero 2011), composed of species assemblages distinct from tropical rain forests and savannas (Pennington et al. 2009). The most species-rich plant family in these forests is the legume family, Fabaceae (Pennington et al. 2009); this dominant family comprises a large component of the canopy (Gentry 1995, Crews 1999). The abundance of legumes has important biogeochemical implications related to ecosystem nitrogen (N) input, resulting from their role in symbiotic N fixation (Houlton et al. 2008). The majority of legumes that have been evaluated have the potential to form root nodules, allowing for a symbiotic relationship with N-fixing rhizobia bacteria (Sprent 2007, Doyle 2011). Species that form N fixation symbioses have been observed to exhibit higher foliar nitrogen concentrations (Cornelissen et al. 1997). Their abundance may help explain why tropical forests may tend to cycle N in excess of biological demand, though the amount of N actually fixed by legumes remains an area of active research (Hedin et al. 2009, Menge et al. 2009, Vitousek et al. 2013).

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Legumes may have a potentially unique role in tropical N cycling, however, few studies have assessed the extent to which they contribute to evolutionary and ecological patterns of N-related trait variation in tropical communities (but see Townsend et al. 2007). This is true even for relatively commonly measured traits such as foliar and litter N, and N resorption, which provide insight into nutrient use strategies (Vitousek 1984, Kobe et al. 2005), and multiple other aspects of nutrient cycling (Wardle et al. 2006, Cornwell et al. 2008, Hättenschwiler et al. 2008). While foliar N is strongly linked to photosynthetic capacity (Reich et al. 1997), the difference between litter and green leaf N reflects how well a plant retains N through resorption (Kobe et al. 2005). High resorption efficiencies may be part of a strategy of nutrient conservation, and is expected to be an adaptation to low-nutrient environments (Chapin 1980, Vitousek 1982). Thus foliar resorption may help assess ecosystem nutrient status (Reed et al. 2012, but see Aerts 1996). Previous studies have evaluated the evolutionary pattern underlying species variation in green leaf nutrients, and have found mixed evidence for phylogenetic signal in foliar N concentrations (e.g., Fyllas et al. 2009, Kraft and Ackerly 2010, Stock and Verboom 2012, Swenson et al. 2012); substantially less is known about the evolutionary patterns of litter chemistry or nitrogen resorption. Yet for belowground processes, it is arguably litter, rather than foliar, N that is of greater importance, emphasizing the need to evaluate phylogenetic patterns in these traits.

Following land-use change, ecological assembly processes play a large role in structuring the functional composition of plant communities (Lebrija-Trejos et al. 2011, Bhaskar et al. 2014). Environmental factors, including soil fertility and air temperature, that influence foliar nutrient concentration (McGroddy et al. 2004, Townsend et al. 2007, Fyllas et al. 2009, Zhang et al. 2012) can vary along secondary-succession post-management (Guariguata and Ostertag 2001, Lebrija-Trejos et al. 2011). Secondary forests of different ages may therefore differ systematically in their resource use strategies along succession. Species in young successional dry forests have been found to display more acquisitive strategies related to carbon and growth rate, representing a fast return on investment, while those in mature stages tend to exhibit more conservative strategies; the opposite trend has been observed in traits related to water (Lohbeck et al. 2013). If nutrient limitation changes as forests develop post-management there may be ecological selection for different nutrient use strategies, through a combination of phenotypic plasticity and species turnover, including changes in the relative abundance of legumes. Among successional stages this may manifest as changes in plant traits such as leaf nitrogen concentration (Garnier et al. 2004), litter chemistry (Cortez et al. 2007), or nutrient resorption (Reed et al. 2012).

Plant traits, including foliar and litter chemistry, play a key role in C and N cycling (Lavorel and Garnier 2002, Quéfier et al. 2007, Fortunel et al. 2009), thus

changes in the community-aggregate values directly impact ecosystem properties and processes. For example, acquisitive ecological strategies may be linked with rapid litter mass loss at the site-level, as was observed initially following vineyard abandonment; communities in later successional stages displayed more conservative internal cycling of resources, and correspondingly slower ecosystem-level rates of processing (Garnier et al. 2004). In the wet and dry tropics successional changes in the identity and abundance of N-fixing legumes (Gehring et al. 2005, De Souza et al. 2012) may influence N cycling along succession (Batterman et al. 2013). Litter production can also shift substantially following land-use change, with increases in annual production and more pronounced seasonality of litterfall as forests age (Lawrence 2005). These changes to litterfall patterns have the potential to influence nutrient dynamics, which will be further magnified if they are coupled with changes in litter quality (Chapin 2003).

Along the Pacific coast of Mexico, secondary forests cover an ever-increasing portion of the landscape, and thus the region provides an ideal context in which to assess changes in community assembly and nutrient strategy patterns as secondary forests age. Here we evaluate the role of both evolutionary and ecological processes in determining the distribution of N-related plant traits in seasonally dry tropical forests, in and around the Chamela-Cuixmala Biosphere Reserve, on the Pacific coast of Mexico. Our objectives were to assess: (1) phylogenetic patterns in N-related traits for both green leaf and litter, and how these traits vary between legumes and non-legumes, (2) whether the relationship between leaf and litter nitrogen concentration shifts with secondary forest age, and (3) how community-aggregate values vary among different successional stages, to infer the potential consequences for ecosystem-level processes.

MATERIALS AND METHODS

Site location

This study was conducted within and around the Chamela-Cuixmala Biosphere Reserve, on the Pacific coast of Mexico. The area experiences a tropical dry climate, with strong seasonality of rainfall. Mean annual temperature is 24.9°C, and precipitation is 748 mm, with over 80% falling between July and November (Bullock 1986). The 13,142 ha reserve is dominated by tropical deciduous forest, with semi-deciduous species found along streams (Lott et al. 1987). The period of maximum leaf senescence occurs immediately following the rainy season, and most litterfall occurs in the dry season (Martinez-Yrizar and Sarukhan 1990). The reserve is strictly protected, and there is little evidence of forest clearing (Bullock 2000). In contrast, the landscape surrounding the reserve has been modified to varying degrees

by human activity, particularly since the 1970s (Castillo et al. 2009). Patches of the forest have been transformed via slash-and-burn clearing into pasture for cattle grazing; sometime thereafter pastures are often abandoned. As a result, the region hosts a patchwork of intact forest, actively managed pastures, and secondary forests of varying ages.

We worked across a chronosequence of secondary forests in abandoned agricultural land, as well as undisturbed mature forest. The sites were established in 2002 by scientists from the Centro de Investigaciones en Ecosistemas, UNAM Campus-Morelia (Martínez-Ramos et al. 2012). Four stages were represented: old-growth undisturbed forest (OGF), and successional forests regrowing from pasture representing three age categories (ages in years at time of establishment, 2002): early (1–2 yr; SS1), intermediate (3–5 yr; SS2), and advanced (8–12 yr; SS3). Each stage had three replicate 20 × 50 m plots of similar elevation, slope, aspect, and previous land-use intensity distributed across the landscape. Though parent material and soil type were not controlled for, no systematic bias is found with respect to the location of stages across the landscape (M. Gavito, *personal communication*). Parent material for the reserve is Cretaceous rhyolite (Campo et al. 2001); this has not been characterized in the successional stages. Soils in the region are variable and classified only at very large scales, but the most common soil type is poorly developed sandy-loam (Sandoval-Perez et al. 2009). The successional stages cover the age range where the most dynamic changes in basal area, plant density, and species richness in this system occur, given this particular land-use history (Mora et al. 2015); by the most advanced stage they largely mirror old growth forest (Martínez-Ramos et al. 2012, Bhaskar et al. 2014).

Species selection

Within all plots, species identity, diameter, and height was recorded for all living woody stems using a size-stratified design, with diameter at breast height (DBH) greater than 1 cm for half the plot (10 × 50 m), and greater than 2.5 cm for the other half (see Mora et al. [2015] for details). The identity of all individuals has been verified by a specialist of the region (Emily Lott) and voucher specimens have been stored in the Balvanera laboratory (UNAM, Campus Morelia). Using the field census as well as the litter collection data (see *Litter analysis*) we selected dominant species in each plot, defined as those species that collectively accounted for between 85% and 95% of the total standing basal area, combined with those that contributed at least 80% of the total litter biomass, for a total across stages of 73 species. We expect the traits of those species to contribute most to ecosystem-level processes, as predicted by the biomass ratio hypothesis (Grime 1998, Fortunel et al. 2009). Therefore we focused on these species for determination of the litter characteristics described below.

Litter analysis

We placed 12 0.26-m² circular litter traps 0.5 m above the ground at random locations within each plot. From June 2007 to May 2008, litter was collected monthly, dried at <70°C, weighed, and sorted to species after removing all non-organic and woody material. We combined litter from species that occurred in multiple traps in a given month within a plot. Decomposition is relatively slow in dry tropical forests (Lugo and Murphy 1986), and collections at a monthly interval are typical for dry forest systems (Read and Lawrence 2003), and not expected to result in substantial changes in litter chemistry.

In total, leaf litter mass was recorded monthly for a total of 163 species across the 12 plots, and litter from selected dominant taxa was sent to Brown University where it was ground to a fine powder (size 60 mesh on a Wiley mill (Thomas Scientific, Philadelphia, Pennsylvania, USA), oven dried at 60°C, and analyzed for total C and N using a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA).

Nutrient proficiency, the lowest nutrient concentration found in leaf litter, has been proposed as a measure to standardize resorption across species (Killingbeck 1996). However, in this seasonal system, the lowest litter N values are found in senesced leaves at the end of a species' litter production (data not shown), similar to what has been observed in temperate systems (Niinemets and Tamm 2005). Using this scarce litter as a metric of proficiency would reflect only a small percentage of litter produced by an individual over a season. Thus, we report a seasonally weighted proficiency measurement of litter N, calculated as the product of litter N concentration and litter biomass for each month, averaged across the 12 months of litter collection. Almost all species in this study are drought-deciduous, and exhibit a fairly similar seasonal pattern of leaf phenology. Species flush out at the start of the rainy season (June), and subsequently drop the entire canopy of leaves during the dry season, thus leaf lifespan is less than 12 months (Bullock and Solis-Magallanes 1990). As a result, our green leaf and litter N concentrations largely come from the same cohort of leaves. In months where litter was scarce for a particular species, this approach meant an incomplete data set for that species. Species were included in the litter data analysis only if N concentration measurements could be made for at least 65% of the total litterfall within a given plot summed over a full phenological year (June 2007–May 2008).

Green leaf analysis

Green leaf N was measured as described in Bhaskar et al. (2014) for 63 species. This reflects the number of species that met the basal area criteria for dominance and had at least three similar individuals to provide replication within a plot. Measurements were repeated across all stages of the chronosequence in which that species occurred to incorporate intraspecific trait variation. For most species green

leaves were collected late in the wet season in November 2007. In order to collect young, fully developed leaves and stems we collected leaves from reverse deciduous species in the dry season (April 2007) and from early deciduous species earlier in the wet season (August 2008). For each individual, the youngest fully expanded leaf from a shoot was collected in the field and brought back to the lab in plastic press-seal bags. After leaves were dried for a minimum of 72 h in drying ovens at 60°C, samples were ground to a fine powder in a Wig-L Bug grinding mill (Dentsply International, Surrey, UK), 4-mg subsample weighed and encapsulated in tin, and combusted for analysis in an elemental analyzer (PDZ Europa Scientific 20/20, Cheshire, UK) for analysis of leaf N content. The green leaf nitrogen analysis was conducted at the Center for Stable Isotope Biogeochemistry at UC Berkeley.

Community phylogeny

In order to test for phylogenetic signal in traits, we first built a community phylogeny. We reconstructed phylogenetic relationships among our dominant species using almost entirely newly generated sequences, with additional molecular sequences available from National Center for Biotechnology Information (NCBI) as needed (GenBank [NCBI] accession numbers of all samples used in phylogenetic analyses are indicated in Appendix S3: Table S2.) We focused on two chloroplast gene regions, *matK* and *rbcL*, which have been proposed as part of the DNA Barcoding guidelines (CBOL Plant Working Group et al. 2009), and have been used to construct community phylogenies in diverse tropical systems (e.g., Kress et al. 2009, 2010). Tissue was either field collected from the sites in Chamela and preserved with silica gel desiccant, or collected from the Chamela herbarium voucher specimens. DNA extraction was conducted using DNEasy and QIAGEN isolation kits (QIAGEN, Hilden, Germany). Details of the PCR cycling conditions and primer pairs used for each gene region are available in Appendix S3: Table S1. PCR products were sent to the University of Rhode Island Genomics and Sequencing Center for Sanger sequencing. Consensus sequences were assembled using CodonCode, and all sequences were BLAST searched in GenBank. The *matK* and *rbcL* sequences were separately aligned with each other using MEGA version 5.1, and then concatenated with PHYUTILITY (Smith and Dunn 2008) for a two-gene alignment for 73 taxa. Sequences could not be obtained for one species, *Cordia eleagnoides*, thus phylogenetic analyses excluded this species. Three other *Cordia* species found in this site were successfully sequenced and included, thus the exclusion of this one congener should not significantly influence the phylogenetic patterns we observed. A maximum-likelihood consensus tree was inferred using RAxML version 7.0.4 (Stamatakis et al. 2004). The program BEAST version 1.7.5 (Drummond et al. 2012) was used to construct ultra-metric phylogenetic trees estimated from the set of sequences using

Bayesian inference, and the maximum clade credibility consensus tree was used in all subsequent analyses. Two independent runs were conducted using the GTR+ Γ DNA substitution model, with chains of 10 000 000 and 15 000 000 generations, and burn in of 10% (1000 trees). Effective sample size of >300 was achieved for all parameters.

Statistical analyses

We calculated species means for each trait, for each stage in which a species occurs. A minimum of three, and on average four, individuals were used as replicates for calculating green leaf traits for each species, pooling across plots in each stage depending on their distribution. The majority of species occurred in more than one stage, so separate species means were calculated for each stage to incorporate intra-specific variation as it relates to successional status.

To assess phylogenetic signal, we first calculated species mean values averaged across all the chronosequence stages. We estimated the phylogenetic dependence of leaf and litter traits using both Blomberg's *K* (Blomberg et al. 2003) and Pagel's λ (Pagel 1999), using phylo-signal in the R package picante (Kembel et al. 2010) and phylo-sig in phytools (Revell 2012), respectively. These metrics measure the fit of the phenotypic data to the phylogeny, under a Brownian motion model of evolutionary change. For both metrics, values close to zero indicate that species traits are distributed independent of the phylogenetic relationships, while values of one indicate trait variation among species reflects their evolutionary history as expected under Brownian motion (e.g., higher trait similarity among closely related species). For significance testing, 1000 reps were used in phylo-signal, to detect whether observed values were significantly different from randomized permutations, with traits reshuffled randomly along the tips.

Regression analyses

To assess the relationship between green leaf nitrogen concentration, resorption efficiency, and litter nitrogen, we followed the analysis method detailed in Kobe et al. 2005 and fit the data to a linear model:

$$\log_{10}([\text{N}]_{\text{litter}}) = B \times \log_{10}([\text{N}]_{\text{gr}}) + A. \quad (1)$$

Briefly, both *A* and *B* are parameters estimated from the data, describing nutrient efficiency. The slope, *B*, of the relationship between leaf N ($[\text{N}]_{\text{gr}}$) and litter N ($[\text{N}]_{\text{litter}}$) is an estimate of how resorption efficiency is a function of nutrient availability; *B* = 1 indicates resorption does not vary as a function of nutrient concentration in the leaf, and values greater than 1 indicate nutrient status does have an effect on resorption efficiency, with decreasing efficiency as green leaf concentration increases. We were interested in how the relationship between leaf and litter N might change across the successional stages,

TABLE 1. Phylogenetic signal analysis using Blomberg's K metrics and Pagel's λ .

Trait	$K(P)$	λ	P if $\lambda = 0$	P if $\lambda = 1$
log(leaf N)	0.11 (0.008)	0.779	0.007	<0.001
log(leaf C:N)	0.1 (0.02)	0.83	0.003	<0.001
log(Nres)	0.14 (0.08)	0.12	0.24	<0.001
log(litter C:N)	0.06 (0.37)	0.013	0.9	<0.001

Notes: Boldface type indicates significance at $P < 0.05$. Nres, N resorption.

so we tested across stages for differences in slope, or shifts along the same slope. Both green leaf N and litter N are expressed on a mass basis (see *Results* for methodological considerations of area- vs. mass-based analyses). Because both traits are estimated with error, the appropriate regression analysis is model II, as the more typically used ordinary-least-squares regression underestimates slopes under these conditions (Legendre 1998). Specifically we used model II linear major-axis regression to derive estimates of the slope, B , and intercept, A , because both traits are measured in the same units (Legendre 1998). Analyses were conducted using the "ma" function of the smatr package (Warton et al. 2012) in R (R Core Development 2011). Stage-level regressions were conducted by pooling the dominant species from the replicate plots, and using trait values from only those species with both leaf N and weighted litter N values. The total number of species that met this criteria ($N = 25, 26, 19$, and 13 in OGF, SS3, SS2, and SS1, respectively; Table 2) represented a large percent of the total standing basal area of each stage (72%, 70%, 65%, and 75% respectively). Separate analyses were conducted using the subset of taxa that were legumes, and non-legumes, to estimate the Eq. 1 parameters and trait correlations.

Stage-level means

We scaled up species-level trait values to the stage level through calculation of community-weighted mean (CWM) values for each plot, for leaf and litter traits. To the extent that ecosystem-level processes are determined by traits of species with greater dominance, as estimated by their biomass contribution in the community ("biomass hypothesis" of Grime [1998]), this metric provides a quantitative measure of how changes in vegetation influence ecosystem functions. We calculated plot-level CWM values as the sum of each species trait value weighted by either basal-area in the case of green leaf traits, or litter biomass for litter traits. We also calculated the plot-mean legume basal area, reported as a percentage relative to the total basal area of dominant taxa (percentages relative to all taxa were similar, data not shown). We analyzed CWM values from the three replicate plots using a one-way ANOVA, and tested for

statistical differences between stages using Tukey's hsd post hoc tests.

RESULTS

Phylogenetic signal

The Bayesian consensus phylogeny (Fig. 1) was generally congruent with the latest accepted topology of the Angiosperm Phylogeny Group, with the exception of the placement of Myrtales (Apg 2009). In our sampling, this lineage was represented by a single taxon, and fell within the asterids instead of the rosids.

Both Blomberg's K and Pagel's λ indicated leaf traits (leaf C:N, leaf N) were more convergent than would be predicted by a Brownian motion (BM) model, with values of both K and λ less than 1 (Table 2). However, at least some degree of correspondence was detected between phylogeny and trait distribution, since K values significantly differed from randomized permutations, and λ was significantly greater than 0. For litter C:N and N resorption, the association between traits and phylogeny could not be distinguished from random, with low K values that could not be distinguished from randomized permutations, and λ values that could not be distinguished from zero (Table 2).

Among species in the family Fabaceae, we observed wide variation in both green leaf N concentration and N resorption (Fig. 1), largely overlapping the range of foliar N in non-legumes in this study and spanning a relatively large range of the global spectrum (Appendix S1: Fig. S1). Mean trait values for legumes vs. non-legumes assessed using a one-tailed t test differed significantly for leaf N (3.27 ± 0.1 for legumes vs. 2.96 ± 0.1 for non-legumes, $P = 0.02$), litter N (2.16 ± 0.09 vs. 1.78 ± 0.06 , $P < 0.001$), and litter C:N (22.3 ± 0.8 vs. 25.7 ± 1.0 , $P = 0.01$).

Variation in resorption during succession

We found high interspecific variation in N resorption (Fig. 1), comparable to that observed in other studies of a subset of the species included in the present analysis (Rentería et al. 2005, Rentería and Jaramillo 2011). Across stages, we found a positive significant correlation between green leaf and litter N concentrations, weakest in the SS3 stage, though with the same tendency as in all the other stages (Fig. 2, Table 1). Testing for a common slope, we found that stages did not differ in the form of this relationship ($P = 0.48$); an analysis including just the legumes similarly found no difference among stages in N resorption ($P = 0.70$; Table 1). The common slope estimate for all taxa ($B = 1.59$) was significantly greater than 1, ($P = 0.012$, Table 1), suggesting disproportionately more nitrogen remained in leaf litter as green leaf nitrogen concentration increased. Analyses conducted with a subset of the data that included all legumes, and all non-legumes similarly found slope estimates greater than 1, though not significantly so (Table 1).

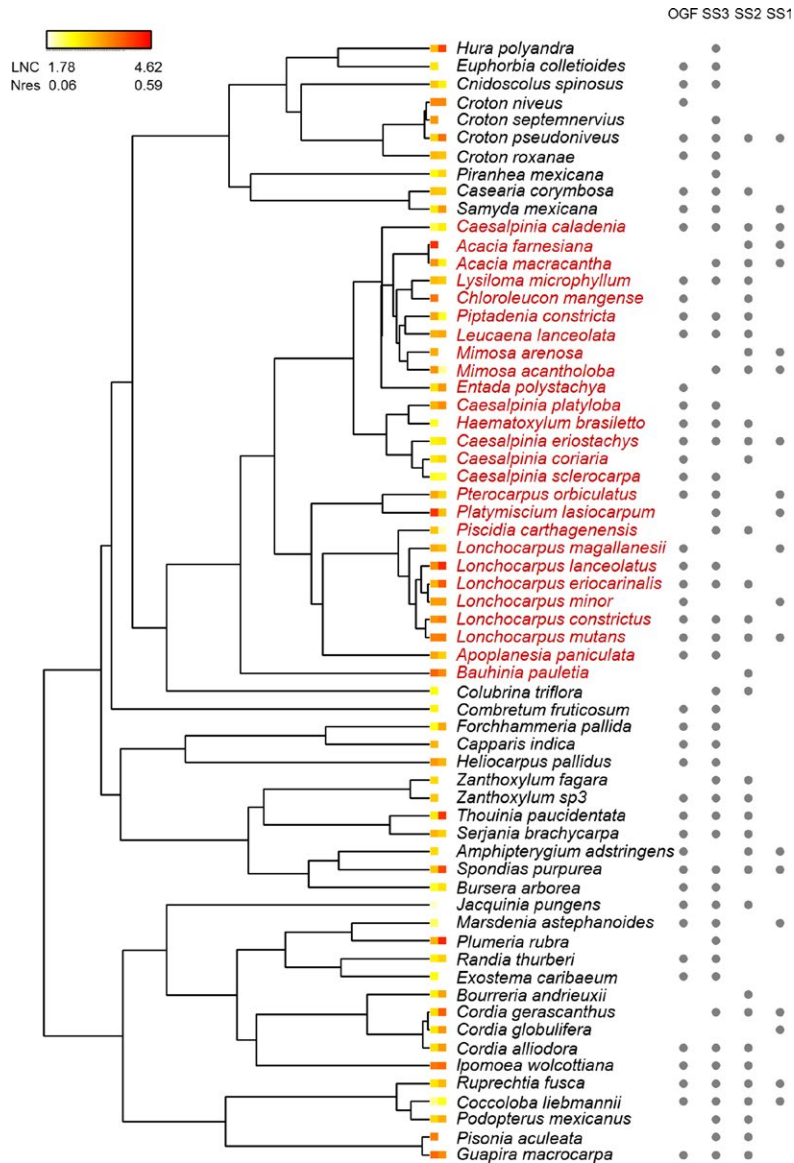


FIG. 1. Values of green leaf N concentration (LNC, first box at branch tip) and N resorption (Nres, second box at branch tip) mapped across a Bayesian consensus phylogeny, with species occurrence indicated (gray points) when present in old-growth (OGF), advanced (SS3), intermediate (SS2), or young (SS1) secondary forest. Species names in red are in the Fabaceae.

We did not detect any shift across stages along the leaf N–litter N axis ($P = 0.7$), despite the changes in communities across succession. Species richness has been observed to be distinct among stages, increasing rapidly by the advanced stages to levels comparable to old-growth forest (Bhaskar et al. 2014). Similarly, composition varied among stages (Fig. 1); a nonmetric multidimensional scaling ordination (NMDS) analysis revealed SS1 and SS2 were distinct while SS3 overlapped with old-growth plots (see Appendix S2: Fig. S1). Despite turnover in composition during succession, communities exhibited a similar range of leaf and litter N values.

Community-weighted mean shifts among successional stages

We observed community-level changes for fine litter biomass among stages ($F_{3,8} = 6.32$, $P = 0.02$; Fig. 3A). Values were lowest in the youngest secondary forests, and increased in later stages. The oldest secondary stands did not differ from old-growth forest. Legumes as a group varied in the percent of total basal area across stages, with significantly lower relative abundance in the advanced successional stage ($F_{3,8} = 7.91$, $P = 0.009$; Fig. 3B). We did not observe community-level changes

TABLE 2. Model II major axis regression estimate of slope parameter (B), and intercept (A) with 95% confidence limits and Pearson's correlation coefficient (r) testing the correlation between leaf N and litter N in each stage.

Stage	N	r	P	B	A
All taxa					
OGF	25	0.58	0.002	1.59 (1.11, 2.59)†	-1.11 (-2.07, -0.57)
SS3	26	0.31	0.08		
SS2	19	0.56	0.013		
SS1	13	0.68	0.011		
Legumes					
OGF	12	0.45	0.1	1.67 (0.88, 4.3)‡	-1.16 (-3.87, -0.27)
SS3	10	0.05	0.8		
SS2	11	0.45	0.1		
SS1	9	0.63	0.07		
Non Legumes					
OGF	13	0.63	0.02		
SS3	16	0.44	0.1	1.20 (0.66, 2.35)	-0.72 (-1.97, -0.14)
SS2	8	0.02	0.9		
SS1	4	0.83	0.2		

Notes: Boldface type indicates significance at $P < 0.05$. Separate analyses for all taxa, all legume taxa, and all non-legume taxa are reported, with data pooled across stages for the slope and intercept estimates. The number of species included in analysis is shown in column N ; all values \log_e -transformed. Stage codes: old-growth (OGF), advanced (SS3), intermediate (SS2), and young (SS1) secondary forest. Values in parentheses are 95% CL.

†Analysis of all taxa: no difference among stages in slope ($P = 0.48$), nor any shift along a common slope ($P = 0.70$).

‡Analysis of legumes only: no difference among stages in slope ($P = 0.70$), nor a shift along a common slope ($P = 0.36$).

in nitrogen traits associated with successional stage. Litter N values were highest in the earliest secondary stage, while the remaining secondary stages were not distinct from old-growth forest ($F_{3,8} = 23.4$, $P = 0.00026$; Fig. 3C). Community-weighted leaf N and litter C:N did not differ among any of the stages ($F_{3,8} = 0.84$, $P = 0.51$, $F_{3,8} = 1.75$, $P = 0.23$, respectively; Fig. 3D, E).

DISCUSSION

Legumes are numerous in this regional dry forest flora, which we expected to influence how N-related traits mapped onto the community phylogeny. Specifically, we expected to find a strong phylogenetic clustering of high N in leaf and litter in legumes, and in turn, that this pattern would drive variation at the stage-level between forests of differing ages due to taxonomic turnover and differences in the relative abundance of legumes. Surprisingly, neither of these expectations were met; on the contrary, we observed a very low phylogenetic signal for all traits, and despite changes in legume relative abundance we also demonstrated remarkably little community-level variation in N-related function. As we discuss below, these latter results are encouraging: basic ecosystem properties of these dry forest systems may be restored fairly quickly during the development of secondary forests. However, the results also suggest that our knowledge of the phylogenetic distribution of key plant functional traits remains poor: even an "obvious" case, such as that of legumes and nitrogen, quickly breaks down upon closer inspection.

N resorption along succession

Successional theory suggests that following a disturbance, resource-use strategies shift from acquisitive in early stages to conservative in later stages (Grime 1998). Changes along this acquisitive-conservative spectrum have been observed in carbon and water use traits during secondary succession in both wet and dry forests (Lohbeck et al. 2013). Nutrient use strategies, however, have been found to exhibit inconsistent directional changes. Based on changes in community-weighted mean leaf nitrogen, as well as direct measures of ecosystem processes related to nutrient cycling, young sites in old-field succession appear to display acquisitive strategies and fast resource processing, and later have more conservative strategies and slower cycling (Garnier et al. 2004). However, a recent meta-analysis suggests that the highest nutrient resorption values, which might be expected to be part of a more conservative resource use strategy, were observed in earliest stages of succession (Reed et al. 2012).

In contrast to both of these results, we found no consistent directionality in resorption during succession (Fig. 2, Table 1). While our data support the hypothesis that resorption efficiency decreases as a function of green leaf N (Vitousek 1984), each successional stage exhibited the same slope between green leaf and litter N. Even with a shared slope, if species in one stage occupied a lower range of values they would have higher nutrient resorption efficiency. However we found no significant evidence of a shift along the slope, thus stages were not separated along the common fit, and collectively the results suggest

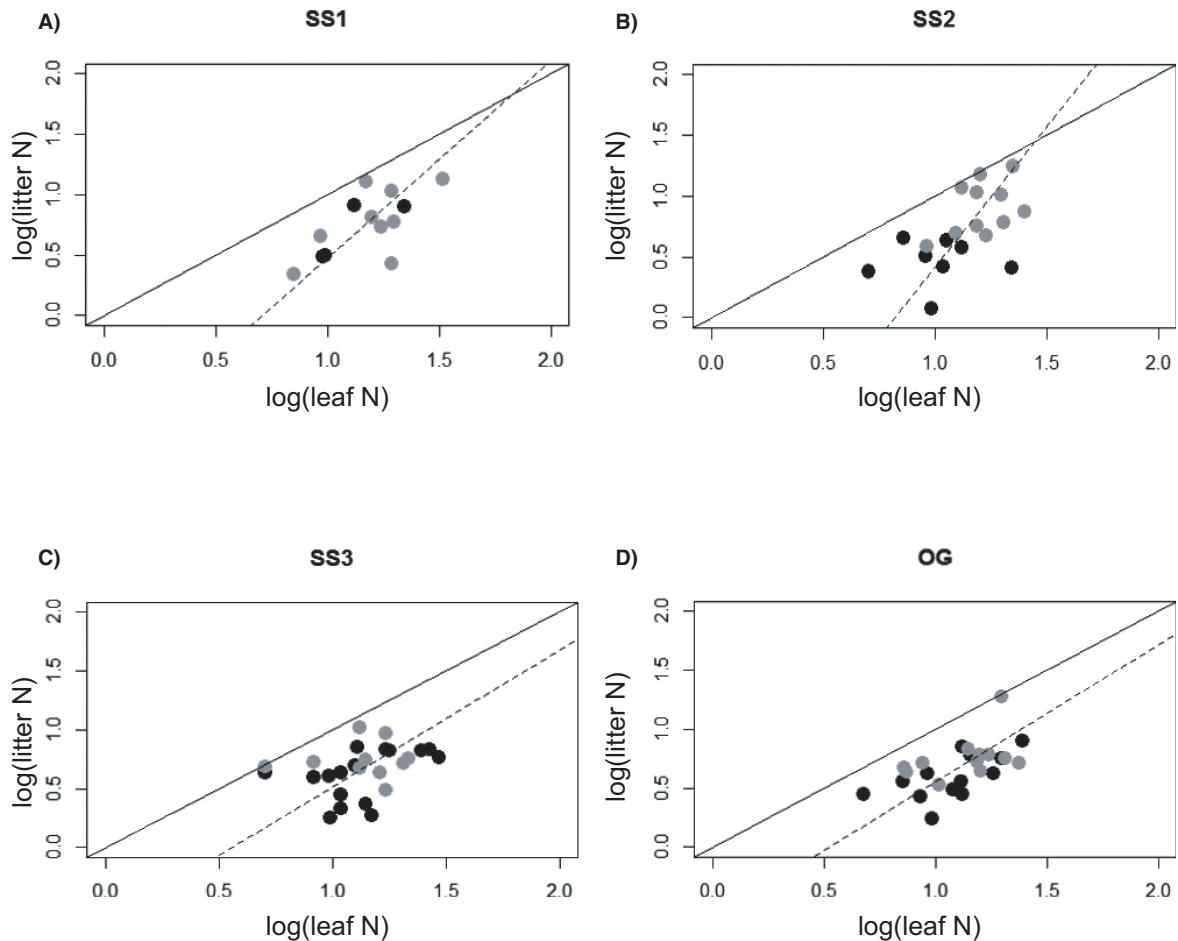


FIG. 2. Fit of scaling equation, Eq. 1, using major axis regression for log-transformed nitrogen concentration in litter vs. green leaves (mass-based) for (A) young (SS1), (B) intermediate (SS2), and (C) advanced (SS3) secondary stages, and (D) old-growth forest. 1:1 line shown in solid, major axis slope as dashed line. Gray points indicate species in the Fabaceae.

no community-level changes in resorption with successional stage.

Within all stages we observed wide variation among co-occurring species in N use traits, which, in addition to biological differences, may also reflect methodological choice. During senescence, changes in leaf mass as a result of soluble carbon translocation are not taken into account in the mass-based resorption estimate we report, which can lead to an under-estimation of actual nitrogen resorption (Van Heerwaarden et al. 2003). A comparison of mass- vs. area-based estimates for a subset of species from this study found resorption was under-estimated, however the larger differences between species and sites did not change after a correction was applied (Rentería et al. 2005). We therefore feel that the larger trends we observe across species and stages are valid.

Substantial variation in foliar chemistry has been found among co-occurring species in other tropical communities at small scales (Hättenschwiler et al. 2008), and interspecific differences may be a larger source of

variation than soil fertility (Townsend et al. 2007, Hättenschwiler et al. 2008). A multi-year study of old-growth species from the same region as this study found little evidence that green leaf and litter N vary as a function of annual rainfall (Rentería and Jaramillo 2011), though high interannual variability in N resorption was observed within species (Rentería et al. 2005). Thus, it is not clear what environmental controls drive nutrient resorption strategies.

Changes among forest stages and consequences for nutrient cycling

We observed substantial shifts in litter production following land-use change; annual production increased throughout secondary succession as observed in other modified systems (Lawrence 2005). The change in total litter biomass is not surprising, reflecting the structural changes during succession. Early successional stages contain fewer species with overall lower abundance and biomass (Arreola-Villa 2012, Mora et al. 2015) and thus

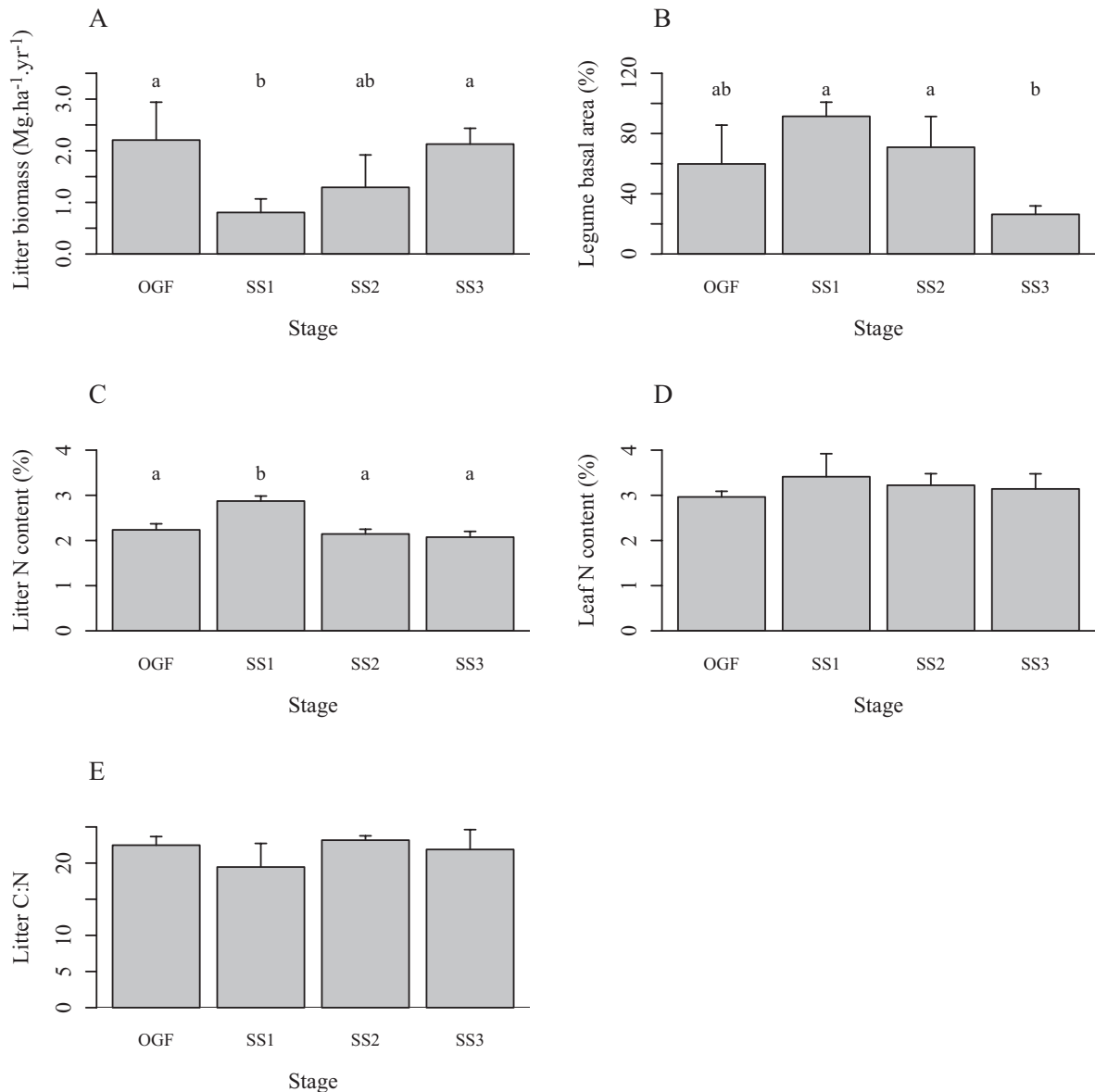


FIG. 3. Stage mean values of (A) litter biomass, (B) legume basal area, (C) litter N, (D) leaf N, and (E) litter C:N (mean \pm SD, $n = 3$ replicates for each stage). Stage codes as in previous figures, leaf traits weighted by aboveground basal area, litter traits weighted by litter biomass, and legume basal area reported as a percentage of the total basal area of dominant taxa. Means with different letters are significantly different (Tukey's hsd post hoc, $P < 0.05$).

less litter production. After the earliest stages, there is a rapid increase in litter biomass, such that advanced secondary stage cannot be distinguished from old-growth in production. This mirrors the recovery in this system of composition (Appendix S2), species richness and functional diversity (Bhaskar et al. 2014), and structure (Mora et al. 2015).

Changes in litter production were not coupled with changes in litter chemistry across successional stages (Fig. 3). Community mean litter C:N was relatively constant, and litter N was similar among all stages except the earliest stage, where high N values were observed.

Though species in the early stage spanned a similar range of leaf and litter N, this stage-level finding may reflect the abundance of particular species. Following pasture abandonment, secondary stages are dominated by *Acacia* and *Mimosa* species (Fig. 3B), potential N fixers, which, in this study, were observed to exhibit some of the highest leaf N values (Fig. 1). N resorption decreases as a function of green leaf status (reflected by B greater than 1, Table 1). Correspondingly, the litter of these species have disproportionately high N concentrations (2.8–3.1%). The higher biomass-weighted N value of early secondary plots is likely a result of the contribution of these species.

Despite changes in vegetation composition we found little change in community-mean trait values across successional stages. These functional traits have been found to link to ecosystem-level processes; higher leaf N, for example, relates to higher productivity and litter decomposition (Quétier et al. 2007, Cornwell et al. 2008), while litter N and C:N provide a measure of litter quality, which can impact decomposition rates (Pérez-Harguindeguy et al. 2000). Collectively, our results may imply stable litter decomposition across secondary succession, to the extent that plant traits determine decomposition rates (Cornwell et al. 2008). Similar rapid recovery of litter C cycling potential has been observed in modified vs. undisturbed wet forests (Ostertag et al. 2008); secondary forests may thus hold great potential to recover ecosystem processes related to litter nutrient dynamics comparable with undisturbed forests.

The community-mean value of plant functional traits has been found to be coupled to soil properties during succession (Garnier et al. 2004), therefore we might expect the recovery of soil nutrient characteristics to mirror that of leaf and litter traits. Though studies of soil in successional sites are limited in this region, one analysis of total and available nutrient content (N, P, C) and enzymatic activity found that pasture sites are degraded in soil quality, while secondary forest plots left fallow a number of years resembled old-growth in many facets (Sandoval-Perez et al. 2009). However, the secondary forests in that study did not span a range of successional stages, instead consisting of sites that had remained fallow for 26 yr, a time frame that extends beyond the oldest age considered in this study. Preliminary analyses of soil chemistry in the same successional sites as this study reveal substantial variation in soil nutrients and enzyme availability among plots within the same successional category (M. Gavito, *personal communication*), suggesting that spatial variability is another factor that needs further study. Future studies that include soil characteristics as covariates will improve our understanding of how spatial heterogeneity and successional stage may both influence the variation observed in leaf and litter traits across the landscape.

The complex role of legumes in forest nutrient cycling

Legumes are a notable clade from many perspectives. They are a remarkably speciose lineage and form a significant component of tropical biomes, in terms of both species richness and abundance (Pennington et al. 2009, De la Estrella et al. 2012, Hughes et al. 2013). From a functional perspective, many legumes are symbiotic N₂ fixers, thus can be an important source of N input to ecosystems (e.g., Roggy et al. 1999). In some studies, legumes, when averaged as a group, exhibit significantly higher foliar N concentrations when compared to other taxa in tropical forests (Davidson et al. 2007), and they did in our study here as well, though the difference was small.

However, focusing on a mean value of a family, or any other taxonomic rank, can obscure the wide variation

among taxa within those groups (Fig. 1 and Appendix S1: Fig. S1; Hättenschwiler et al. 2008, Davidson et al. 2007, Townsend et al. 2007), and can mislead about the true location of the actually relevant variation. For example, within the legumes in our study, two potential “high N” clades within legumes appear to exhibit much higher leaf nitrogen concentrations (Fig. 1), and it is this subset of legume taxa and a corresponding “trickle-down effect” to the family node that are responsible for the elevated family-level mean. This effect might explain the elevated family mean for leaf nitrogen observed in a dry forest community in Costa Rica (Powers and Tiffin 2010); the majority of legumes are from two subfamilies where nodulation is common (Sprent 2007). Incorporating the complexity within legumes could provide further insight into the interplay between the phylogenetic composition of a community and nitrogen dynamics.

We found little evidence for phylogenetic conservatism in the suite of traits relating to nitrogen use, partly a result of wide variation within legumes. Recent studies have, in contrast, reported evidence of phylogenetic signal in leaf N (e.g., Fyllas et al. 2009, Asner et al. 2014), but we suspect that these results reflect the methods used. These studies partition trait variance among family/genus/species rankings, an approach that treats taxonomic ranks as comparable evolutionary units, in spite of the wide variation in the evolutionary ages, and therefore, amount of shared history of different families or genera. Exploring trait variation using taxonomic ranks or other preselected phylogenetic groupings may mislead in precisely the same way as non-clade-based groupings (e.g., tree, shrub, herb; Edwards et al. 2007), and is plagued by the trickle-down effects mentioned previously. It was these sorts of considerations that motivated the development of “phylogenetic community ecology” over a decade ago (Webb 2000, Webb et al. 2002). The wealth of data collected in recent studies presents an exciting opportunity to apply a phylogenetic rather than taxonomic approach that can further our understanding of evolutionary patterns in functional traits.

Our analyses add to recent findings highlighting that generalizations about legumes, particularly in the context of their role in nitrogen-related processes, should be evaluated with caution. In our study, legumes occurred across the range of conditions found along the successional chronosequence. Collectively, the range of leaf, litter, and N resorption values for legumes and non-legumes largely overlapped. Although the group as a whole shows ecological and functional breadth, the identity and abundance of different species within Fabaceae varied across successional stages in this study, as has been found along other secondary successional gradients in the tropics (Gehring et al. 2005, De Souza et al. 2012, Batterman et al. 2013), potentially reflecting ecological sorting across environments within this group. Looking more closely within legumes illustrates a further complexity, namely the interaction between ecological and evolutionary factors that shape N distribution. Though only ~20% of the family has been investigated for N-fixing ability (De Faria et al.

2010), differential nodulation tendencies seem to correspond with different clades within legumes (Sprent 2007). However, even taxa with the potential to nodulate may be facultative fixers, not always actively engaged in biological N fixation in the field (Menge et al. 2009). Relevant to this study, active N fixation in some taxa has been found to differ at different stages of secondary succession (De Souza et al. 2012, Batterman et al. 2013). A more complete understanding of N fixation across this large, complex clade would provide broader insight into how N-related strategies vary along succession.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1890/15-1162.1/supinfo>