LEARNING FROM DYNAMIC TRAITS: SEASONAL SHIFTS YIELD INSIGHTS INTO ECOPHYSIOLOGICAL TRADE-OFFS ACROSS SCALES FROM MACROEVOLUTIONARY TO INTRAINDIVIDUAL

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Premise of research.   Phylogenetic comparative methods provide a powerful approach for exploring the macroevolution of plant functional traits. Such approaches can uncover trait-trait correlations through evolutionary time as well as provide evidence of the role of traits in adaptation across environmental gradients. For continuous traits, most phylogenetic comparative approaches to date employ a single trait value per species—often a mean of sampled individuals—or alternatively incorporate intraspecific variation as a distribution around such a mean. It has been known for quite some time that many of the most physiologically and ecologically important plant traits are actually highly plastic, changing dynamically across a growing season, with whole-plant development or in response to environmental conditions. Here we demonstrate one possible approach to assessing the evolution of such dynamic traits: the use of function-valued phylogenetic comparative methods.

Methodology.   Leaf traits were sampled across 25 taxa in the genus *Cornus* at six time points throughout the growing season in a common-garden context, followed by contrasting sets of alternative analyses to demonstrate the consequences of researcher decisions on study conclusions.

Pivotal results.   The vast majority of assessed traits exhibit substantial seasonal shifts. These shifts cause traditional macroevolutionary correlations assessed at different sampling dates to yield conflicting results. Function-valued approaches indicate that seasonal shifts in many traits are evolutionarily correlated, with implications for the origin of trait-trait trade-offs. Seasonal trait plasticity is also evolutionarily correlated with native habitat environmental gradients across *Cornus*.

Conclusions.   Because a very large number of plant functional traits are not fixed but vary dynamically over time or with environmental conditions, stronger insights into the evolution of plant functional traits can emerge when this dynamism is explicitly incorporated into phylogenetic comparative approaches. We encourage the adoption of such approaches, as well as the development of better tools for doing so.

Keywords: *Cornus*, function-valued traits, leaf economics, macroevolution, plant defense, plasticity, seasonality.

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Introduction

As highlighted in this journal over 15 years ago, the unification of plant ecophysiology with evolutionary biology requires understanding the role of plant functional traits in adaptation to diverse environments, including traits that are phenotypically plastic (Ackerly and Monson 2003). Over the past few decades, phylogenetic comparative methods have become one of the most effective approaches for understanding the macroevolution of functional traits, with substantial progress in the development and deployment of rigorous statistical methods for assessing the macroevolution of traits in relation to one another (e.g., suites and trade-offs sensu Reich et al. [2003]) and the abiotic and biotic environment (Felsenstein 1985; Martins and Hansen 1997; Garamszegi 2014). Such approaches allow for the explicit testing of hypotheses about the adaptive value of functional traits, with the repeated evolution of a focal trait in relation to other traits or environmental conditions interpreted as at least partial evidence of the response of that trait to selective pressures (Harvey and Pagel 1991; Weber and Agrawal 2012).

While the use of phylogenetic comparative methods has become widespread, until recently the most accessible and commonly used methods relied on the use of species mean values for traits (Martins and Hansen 1997) or else the representation

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of intraspecific trait variation as a distribution estimated around a mean value (Ives et al. 2007; Felsenstein 2008), with little way to explicitly incorporate the behavior of dynamic traits that change over time or in response to growing conditions. In our view, this has served as a roadblock for progress in understanding the evolution of plant functional traits, as many of the traits most recognized to play important roles in plant ecology are not fixed traits with unitary values for different species but instead highly dynamic traits that exhibit plastic responses to environmental conditions or large shifts with development (Stinchcombe et al. 2012; Donovan et al. 2014; Chitwood and Topp 2015; Leal et al. 2017). The timescales that are involved vary dramatically, from seconds to decades. At the very rapid end of the spectrum, consider the response of photosynthesis to changing light levels or the inducible upregulation of chemical defenses under pest or pathogen attack. At an intermediate timescale, consider the response of plant biomass allocation to changing soil moisture conditions or the response of phenological traits to differences in photoperiod or temperature. At the very slow end of the spectrum, consider the difference in the construction and makeup of plant organs in small seedlings versus adult trees. Furthermore, in many cases differences in these plastic responses among species are highly likely to play a role in adaptation. For instance, while photosynthesis can be summarized as a univariate trait (e.g., maximum assimilation rate), it has long been understood that differences in the response of photosynthesis to abiotic conditions (i.e., light response curves, A/C_i curves) represent adaptive differences among species—for example, sun-adapted versus shade-adapted plants (Boardman 1977; Givnish et al. 2004)—or the resilience of photosynthetic pathways to heat or drought (Wullschleger 1993; Huxman and Monson 2003). The adaptive importance of plasticity holds for many other classes of plant functional traits, including those governing water relations (e.g., stomatal responses, osmotic adjustment; Chaves et al. 2003; Bartlett et al. 2012), nutrient relations (e.g., transporter upregulation, root exudates, mineral resorption; Kudoyarova et al. 2015; Maillard et al. 2015; Bowsher et al. 2016), plant defense (e.g., induction under attack, differential investment given resource availability; Koricheva et al. 1998; Burghdardt 2016), and certainly many others.

The recent development of function-valued approaches now allows for the explicit incorporation of this trait plasticity into phylogenetic comparative methods, although there are at present a limited but growing selection of methods for handling such data (see Guo et al. 2007; Hadjipentalis et al. 2013; Goolsby 2015; Clavel et al. 2018). The function-valued perspective on plastic traits was developed for use largely in microevolutionary and genetic studies (e.g., Kingsolver et al. 2001; Kingsolver and Gomulkiewicz 2003; Izem and Kingsolver 2005; Stinchcombe et al. 2012) and has only recently begun to be adapted for use at the macroevolutionary scale. These emerging phylogenetic comparative approaches allow for the ancestral state reconstruction of polynomial functions or other curve-like representations of how traits change in response to an exogenous factor, such as time or an environmental variable. The consideration of trait shifts or responses to such exogenous factors has several advantages over univariate approaches, including higher statistical power, improved robustness to missing data and the ability to estimate missing trait values with higher confidence, and the explicit inclusion of the magnitude and directionality of trait re-

sponses (Kingsolver et al. 2001; Goolsby 2015). Despite these advantages, these methods have to date been primarily aimed at ancestral state reconstruction of curves, and there is not yet a clear set of best practices for incorporating function-valued traits when making statistical inferences about correlated trait evolution and the tempo and mode of trait evolution. Given the diverse nature of plastic traits, there is unlikely to be a one-size-fits-all solution for all applications and questions, but the family of function-valued methods represents an opportunity for more direct assessment of the evolution of trait plasticity. With innovations in high-throughput phenotyping and the emerging capacity to ask questions about the evolution of trait plasticity at scale (e.g., Stinziano et al. 2017; Silva-Perez et al. 2018), we are in desperate need of parallel innovation in analytical approaches.

The goal of this article is to introduce readers to function-valued approaches in the hope of stimulating both analytical development and the broader incorporation of these approaches into empirical studies. Here we demonstrate the utility of function-valued approaches to exploring the evolution of plasticity in functional traits. Specifically, we assess the macroevolution of seasonal shifts in leaf ecophysiological traits across the genus *Cornus* (commonly referred to as dogwoods, cornels, and osiers), exploring how incorporating within-individual plasticity in multiple traits allows for improved inference of the nature of trait-trait trade-offs at multiple scales (e.g., intraindividual and macroevolutionary), as well as improved understanding of the evolution of trait plasticity with native habitat environment.

Material and Methods

Study System and Sampling

The genus *Cornus* contains approximately 50 species (Xiang et al. 2006; The Plant List 2013) that vary widely in growth form. These include creeping herbaceous species; dwarf ground-covering shrubs; large, aggressively spreading rhizomatous shrubs; and trees ranging from small understory species to a few larger canopy trees (Xiang et al. 2006). This genus is quite old, dating to the late Cretaceous (Atkinson et al. 2016; Yu et al. 2017), and these species have diversified into habitats ranging from the subtropics to the Arctic (Xiang and Thomas 2008). In addition to wide variation in soil conditions and average climate, such habitats also strongly vary in seasonality, with growing-season lengths ranging from just a few months to year-round. To investigate leaf trait evolution in this group of diverse species, we employ a phylogenetic comparative approach coupled with common-garden phenotyping.

Twenty-five taxa within the genus *Cornus* were studied within the living collections of the Arnold Arboretum of Harvard University in Boston during the 2015 growing season. A total of 66 individual plants were included, varying between one and four individuals per taxa (an average of 2.6) based on availability within the living collections; most taxa were represented by three or four individuals, with three taxa represented by a single individual. Of note, there is taxonomic disagreement within *Cornus*, relating primarily to whether the genus should be subdivided into multiple genera (Eyde 1987) and secondarily to whether several taxa should be considered true species or more properly subtaxa within other species (Xiang et al. 2006; The

Plant List 2013). For the purposes of this study, these issues are unlikely to influence the questions and analyses being considered, and for clarity we use the taxonomy recognized by the Arnold Arboretum living collections (along with an established system of plant identity codes), which agrees well with the taxonomy used by most molecular phylogenies of the genus (Xiang et al. 2006, 2008; Xiang and Thomas 2008). In particular, we include in this study a named subspecies (*Cornus kousa* var. *chinensis*) as well as three named species of disputed species rank (*Cornus australis*, elsewhere considered *Cornus sanguinea* ssp. *australis*; *Cornus coreana*, elsewhere considered part of *Cornus walteri*; and *Cornus pumila*, elsewhere considered part of *Cornus alba*).

In mid-May, all focal plants were flagged on two branches, with one large branch on each tree or shrub reserved for repeated sampling and another large branch reserved for the assessment of relative rates of herbivory. All plants were sampled at six time points spaced 4 wk apart throughout the growing season, from late May (several weeks after initial leaf out) to just before the first frost in mid-October. At each time point, five fully expanded leaves were collected from the assigned branch on each plant and relative herbivory rate was assessed on the other branch (as the percent of leaves with damage present, along with an estimate of the average percent of leaf area removed on those damaged leaves). The *Cornus* species under study were observed to put out a single main flush of leaves at the beginning of the growing season and to produce few to no additional leaves during the growing season, so the collected leaves should primarily reflect changes within existing leaves across the growing season rather than changes due to differences in traits among cohorts of leaves over time (as might be the case in many other taxa). Only undamaged or lightly damaged leaves (when no undamaged leaves were available) were collected, and leaves were placed in zip-sealed plastic bags kept in an ice-cooled chest until being returned to the laboratory on the same day and kept in a refrigerator overnight, with next-day assessment of fresh traits. Only two individual plants became unsampleable halfway through the growing season due to defoliation (likely by browsing mammals). Growing degree days with a base of 10°C were calculated for each sampling date using temperature data from the Weld Hill Weather Station within the grounds of the Arnold Arboretum (http://labs.arboretum.harvard.edu/weather).

Leaf Trait Assessment

A large number of leaf traits were assessed on collected leaves at each time point and can be found in the supplement (data set S1; data sets S1–S7 are available online). First, fresh mass of the five sampled leaves was taken with an analytical balance, followed by imaging using a digital flatbed scanner at a resolution of 600 dpi. Next, chlorophyll content was assessed using a handheld meter (atLEAF CHL STD, FT Green), leaf lamina thickness was measured between veins with digital calipers, and leaf toughness was assessed as the force required to puncture the leaf lamina between veins using a digital penetrometer (FG-3006, Shimpo Instruments). For each of these three traits, one measurement was taken on each of the five leaves midway down the length of each leaf, and these measurements were averaged. Leaves were then dried in a forced-air drying oven at 60°C for 3 d until at constant weight and weighed to obtain dry mass. Leaf

water content was calculated as the mass of water present (leaf fresh mass minus dry mass) per gram of leaf dry mass. Leaf images were analyzed using ImageJ (Schneider et al. 2012) to obtain leaf area and leaf aspect ratio. Leaf mass per area (LMA) was calculated as the ratio of leaf dry mass to leaf area, and lamina density was calculated as the ratio of leaf dry mass to the volume of the leaf, estimated as the product of leaf area and lamina thickness. Chlorophyll meter values were converted to an area basis (mg/cm^2) based on manufacturer recommendations (and demonstrated linearity with extractable chlorophyll content; Zhu et al. 2012) and subsequently transformed to a mass basis using LMA.

A subset of dried leaves were ground into a fine homogenized powder, and methanolic extracts were prepared for use with four assays of secondary metabolite content. The colorimetric Folin-Ciocalteu assay was used to estimate total phenolics (Singleton et al. 1999), expressed in caffeic acid (CAS 331-39-5) equivalents per gram of dry leaf tissue using a standard curve under the Beer-Lambert law. The colorimetric aluminum complexation assay was used to estimate total flavonoids (Pękal and Pyrzynska 2014), expressed in quercetin (CAS 117-39-5) equivalents per gram of dry leaf tissue using a standard curve under the Beer-Lambert law. Total anthocyanin content was estimated using a colorimetric pH differential assay based on relative absorbance at 530 and 700 nm in buffers of 1.0 and 4.5 pH (Lee et al. 2005), expressed in cyanidin 3-glucoside equivalents using the molar absorptivity and molecular weight of that common anthocyanin. All colorimetric analyses used the same visible–near-infrared spectrometer (Vernier Software), which collected absorbance between 380 and 950 nm with 3-nm optical resolution and $\pm 5\%$ photometric accuracy. Last, the radial diffusion assay was used to estimate tannin activity (Hagerman 1987; Graça and Bärlocher 2005), whereby the visible precipitation of protein (bovine serum albumin; CAS 9048-46-8) by tannins present in leaf extract is measured in relation to a standard curve of varying concentrations of tannic acid standard (CAS 1401-55-4), allowing expression of tannin protein precipitation activity in tannic acid equivalents.

Remaining leaf tissue was sent for analysis by the Louisiana State University Soil Test and Plant Analysis Laboratory (Baton Rouge) via inductively coupled plasma mass spectroscopy (SPECTRO ARCOS; SPECTRO Analytical Instruments, Kleve, Germany) to obtain leaf concentrations for phosphorus, potassium, calcium, magnesium, sulfur, iron, boron, manganese, zinc, copper, sodium, and aluminum. Samples with sufficient tissue remaining were analyzed for nitrogen content using Dumas dry combustion (LECO 628 CHN Analyzer; LECO, St. Joseph, MI) at the same facility, yielding data for approximately half of all samples.

Environmental Data

To obtain data on habitat environmental characteristics, occurrence data for our focal species were compiled from the Global Biodiversity Information Facility (GBIF 2019) and Integrated Digitized Biocollections (iDigBio 2019) databases of digitized herbarium records (app. S1, available online). Records only from preserved specimens (i.e., herbarium records) and with geographic coordinates were included, and occurrence points were manually curated to remove duplicates, points outside of the native range (e.g., those in botanical gardens), and any other suspicious or

questionable records, as well as to align records using synonyms for the genus (e.g., *Swida*) with species names in *Cornus* (The Plant List 2013). Occurrences were not obtained for *C. australis*, *C. coreana*, *C. pumila*, or *C. kousa* var. *chinensis* given that these names are not accepted at species rank by The Plant List and the databases would not be expected to uniformly recognize these taxa; instead, many occurrences would likely be subsumed within other species.

We used the curated set of occurrence points to extract environmental variables from multiple sources, all presented in the supplement (data set S2). We used the WorldClim global climate model (Hijmans et al. 2005) to extract bioclimatic temperature and precipitation variables at a 30-arcsec resolution (~1 km²). We additionally extracted monthly minimum temperature data to calculate the number of frost-free months. Site annual average potential evapotranspiration and aridity index (the ratio of mean annual precipitation to potential evapotranspiration) were extracted from the CGIAR Global Aridity and Potential Evapotranspiration database (Zomer et al. 2008). Soil variables were extracted from the SoilGrids1km global interpolated soil model (Hengl et al. 2014), yielding soil texture and chemistry characteristics. Soil variables were obtained at six available depths between 0 and 200 cm and averaged across depths to provide an average description of the rooting zone. Last, we extracted smoothed weekly normalized difference vegetation index (NDVI) data at ~16-km² resolution from the National Oceanographic and Atmospheric Administration Center for Satellite Applications and Research's Global Vegetation Health Products (NOAA STAR 2016). These data were used to generate two metrics: first, the degree of vegetation seasonality was defined as the amplitude of NDVI across the year. A larger amplitude would occur in habitats that vary more strongly in greenness between summer and winter or a wet and dry season, indicating higher vegetation seasonality in response to environmental conditions. Second, the length of the growing season was defined as the number of weeks where NDVI was at least one-quarter of the annual amplitude above the minimum. This threshold is arbitrary but for these species yields a good relative measure of the length of time for which vegetation greenness is elevated above that of the dormant season. This generates a useful alternative metric to the frost-free period for defining the length of the growing season. These types of interpretations of seasonal NDVI patterns are common in remote-sensing studies and have been validated against ground-level data on multiple continents (Malingreau 1986; Reed et al. 1994).

Data Analysis

A traditional approach to analyzing sequentially sampled phenotypes is repeated-measures analysis (von Ende 2001). To examine general seasonal trends across the observed plants, a simple repeated-measures analysis of variance was performed on each leaf trait using a mixed model approach with month as a fixed effect, plant identity as a random effect, and an AR (1) covariance structure in JMP Pro version 13 (SAS Institute, Cary, NC), along with Tukey post hoc tests when appropriate.

A phylogeny of the genus *Cornus* was reconstructed from matK and ITS sequences originally published by Xiang et al. (2006). Sequences were concatenated, and a maximum likelihood phylogeny was reconstructed using a GTRCAT model in

RAxML version 8.2.10 (Stamatakis 2014) with the blue- and white-fruited clade set as the outgroup clade to provide well-established polarity for the genus (Xiang et al. 2006, 2008; Xiang and Thomas 2008). Based on taxonomic information, *C. australis* was manually added to the tree as sister to *C. sanguinea* and *C. coreana* was manually added as sister to *C. walteri* (The Plant List 2013).

Phylogenetic comparative analyses were performed (1) individually by month and (2) as seasonal curves. For analyses on individual months, trait data were treated as though the month of interest was the only time point sampled, yielding six parallel analyses. A phylogenetic generalized least squares model was fitted to estimate the correlation between all pairs of trait-trait and trait-environment correlations using restricted maximum likelihood in the Rphylopars package (Goolsby et al. 2017) in R version 3.5.1 (R Core Team 2018). *Cornus australis*, *C. coreana*, *C. kousa* var. *chinensis*, and *C. pumila* were omitted from analyses with environmental variables due to lack of data. To accomplish this, taxa were pruned from the phylogeny and trait data were removed from the data set. Because traits and environmental variables all exhibited varying levels of phylogenetic signal, Pagel's λ was simultaneously estimated for each pairwise correlation to minimize the occurrence of false positives (Pagel 1999; Revell 2010). When needed, maximum likelihood ancestral state reconstruction for traits and environmental variables was performed using the Rphylopars package (Goolsby et al. 2017).

For curve-based analyses, third-order polynomial regressions were fitted to the seasonal variation in each trait for each species, with growing degree days as the independent variable. Stepwise Akaike information criterion model selection was used to avoid overfitting (MASS package; Venables and Ripley 2002), allowing up to two terms to have coefficients of zero, as most appropriate for the data. Three metrics were derived from species' seasonal trait curves: (1) the baseline trait value at the start of the growing season in May, (2) seasonal breadth (the difference between the minimum and maximum values for each trait curve), and (3) net seasonal shiftiness, defined as the total integrated area under the curve when traits shift above the baseline trait value minus the total integrated area above the curve when traits shift below the baseline trait value (fig. 1). Seasonal breadth therefore gives the absolute range of trait plasticity over the growing season, while net seasonal shiftiness gives a directional magnitude of trait plasticity over the growing season. A positive net seasonal shiftiness value indicates that the trait shifts net higher than baseline over the growing season, while a negative value indicates that the trait shifts net lower than baseline over the growing season. A positive correlation between a univariate variable and net seasonal shiftiness would therefore indicate that as the univariate variable increases, the curve is observed to evolve to become more positive—either a stronger upward shift or less of a downward shift over the growing season. A negative correlation between a univariate variable and net seasonal shiftiness would indicate the opposite, where as the univariate variable increases, the curve is observed to evolve to become more negative—either less of an upward shift or a stronger downward shift over the growing season. Similarly, a positive correlation between net seasonal shiftiness metrics for two traits would indicate that seasonal curves are moving in the same direction (when one evolves to become more positive, the other does as well), whereas a negative
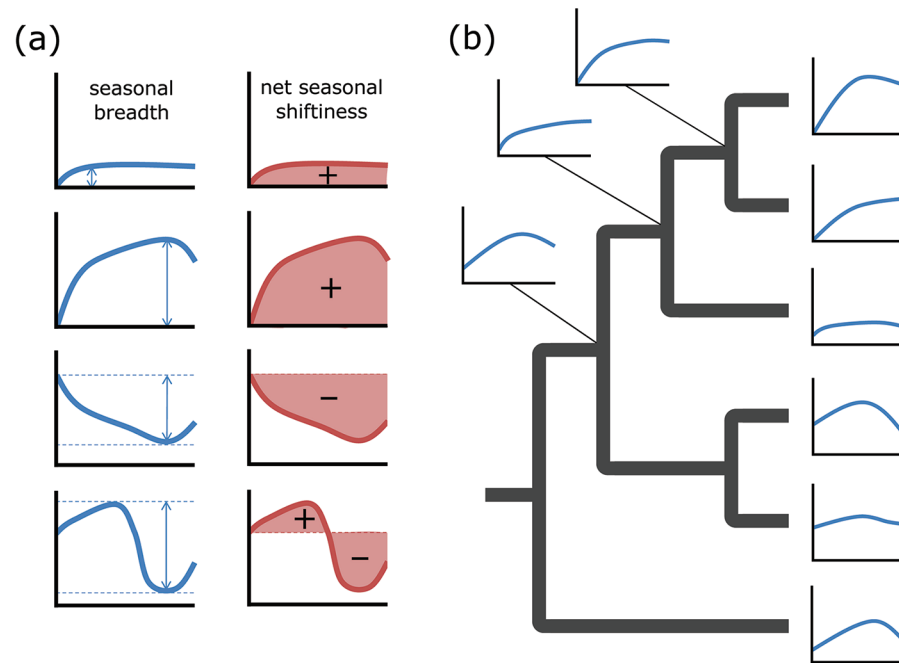
Fig. 1   Conceptual diagrams of the function-valued approach used in this study. *a*, Visual explanation of the three metrics of seasonal shifts calculated from the predicted curves fitted to the data: (1) the baseline trait value at the start of the growing season in May, represented by the *y*-intercept on each graph; (2) seasonal breadth (the difference between the minimum and maximum values for each trait curve); and (3) net seasonal shiftiness, defined as the total integrated area under the curve when traits shift above the baseline trait value minus the total integrated area above the curve when traits shift below the baseline trait value. Seasonal breadth therefore gives the absolute range of trait plasticity over the growing season, while net seasonal shiftiness gives a directional magnitude of trait plasticity over the growing season. A positive net seasonal shiftiness value indicates that the trait shifts net higher than baseline over the growing season, while a negative value indicates that the trait shifts net lower than baseline over the growing season. *b*, Conceptual explanation of the ancestral state reconstruction of function-valued traits (i.e., curves) for the assessment of the evolution of dynamic traits across phylogenies. This general approach, regardless of the specific metrics used, can provide valuable insights into the evolution of plastic traits in relation to fixed traits, environmental variables, and other dynamic traits during species diversification.

correlation between net seasonal shiftiness metrics for two traits would indicate that seasonal curves are moving in opposite directions (when one evolves to become more positive, the other becomes more negative).

Baseline trait value, seasonal breadth, and net seasonal shiftiness metrics were treated as univariate variables for each species trait curve, and phylogenetic comparative analyses were performed on all pairs of traits and between all trait-environment pairwise combinations using the same methods applied to analyses on individual months (see above). It is important to note that, although subtle, the individual by-month results for May are distinct from the curve-predicted trait values for May, as smooth curve-based predictions should be less sensitive to outliers (Kingsolver et al. 2001). For two species (*Cornus drummondii* and *C. walteri*), anthocyanin content was undetectable across all sampling dates and thus invariant, and so these two species were omitted from all curve-based analyses involving anthocyanins.

Results and Discussion

Repeated-Measures Analysis of Seasonal Trait Changes

Assessing seasonal trends in leaf traits across all sampled plants using repeated-measures analysis of variance, 23 out of 26 mea-sured leaf traits show significant changes across the growing season (fig. S1, available online). For most traits, these seasonal changes do not appear to be merely random walks driven by simple sampling variation around a static mean trait value but, rather, large directional shifts over time in leaf physiology. On average, leaves appear to continue to put on dry mass as well as area between May and June and during this period experience an increase in LMA. Across the growing season, leaves on average experience a decrease in water content, chlorophyll content, and concentration of key nutrients, such as nitrogen, phosphorus, potassium, iron, zinc, and copper, suggesting a general reduction in leaf productivity over time. Interestingly, concentrations of calcium and boron show steady increases over the growing season. Secondary metabolites differ in their behavior, with no significant changes in anthocyanins across the growing season but general declines in total flavonoids. Total phenolics and tannin activity both decrease on average between May and June, after which these chemical defenses experience general increases in investment, with the highest levels observed at the end of the growing season. During the last sampling interval between September and October, leaves clearly begin to experience the effects of nutrient resorption and the mobilization of other resources out of deciduous leaves, with sharp reductions in leaf mass, leaf area, chlorophyll, and nitrogen accompanied by decreases in lamina thickness and an increase in lamina density. During this period

there is also a large spike in leaf sodium, suggesting that this element is either being mobilized into leaves in advance of senescence or being disproportionately left behind while other leaf components are being removed from leaf tissues. This kind of repeated-measures analysis can be quite useful to gain a sense of general patterns of change over time but has the limitations of being univariate and blind to the phylogenetic nonindependence of species.

Considering a subset of these traits for several representative species in two-dimensional trait space, it is clear that many of these trait changes across the growing season are co-occurring shifts in leaf traits, often highly correlated (fig. 2). Despite the fact that species may start and end in different parts of trait space over the growing season, they typically experience similar directional trajectories over time. For example, as species experience increases in LMA and tannin activity over time, they experience reductions in water content, chlorophyll, and nitrogen, and those experiencing more of a shift in one trait seem to experience more of a shift in the other. This trait-space approach can yield useful insights, as it allows for the consideration of the interaction of multiple traits at once and the examination of differences among species, but of course it has the limitations of being largely descriptive, without the rigor of formal statistical tests.

Traditional Trait-Trait and Trait-Environment Analyses

Most studies of plant functional trait evolution employing a phylogenetic comparative approach collect trait data by sampling at one time point or plant developmental stage or else pool data collected from many different time points on the assumption that intraspecific variation is small relative to interspecific variation (often dramatically incorrect; Albert et al. 2010; Donovan et al. 2014; Shipley et al. 2016). Given that most comparative studies are not working with multiple time points, here we examine the effect of sampling time on the results obtained by traditional trait-trait and trait-environment phylogenetic generalized least squares regression, as if our study had chosen to collect data only at one point during the growing season and drawn inferences based on that data alone. The results of these six parallel analyses are contained in the supplement (in full in data sets S3 and S4 and summarized in tables S1 and S2, available online), with highlights presented here (tables 1, 2).

While some pairwise trait-trait macroevolutionary correlations hold no matter the time of sampling (e.g., calcium and magnesium, LMA and water content, chlorophyll and tannin activity), these are the exception rather than the rule. The majority of trait-trait correlations exhibit marked changes depending on the time of sampling, with major increases or decreases in strength and vacillations between statistical significance and nonsignificance (table 1). Perhaps most disturbing are the instances where correlations that would be considered strong and reliable relationships (e.g., $R^2 > 0.50$) when assessed in May completely disappear in some or all of the subsequent months—this is the case for relationships involving nitrogen, phosphorus, potassium, chlorophyll, LMA, and toughness. Given the importance placed on such traits when describing plant ecological resource-use strategies, this lack of consistency is troubling.

Trait-environment relationships yield a similar pattern, with wide swings in the strength of relationships (table 2). Looking across all six parallel analyses, very few trait-environment relationships hold across the majority of the months considered (table S2). Some relationships even change direction over the growing season—for example, those related to leaf nitrogen and descriptors of native habitat growing-season length and temperature seasonality and severity (table 2). These changes in direction actually make ecological sense if one knows that leaf nitrogen falls over the growing season in *Cornus* (which one would know only with time series sampling), as the evolution of higher early-season and lower late-season leaf nitrogen during adaptation to shorter, more seasonal environments would indicate the evolution of a resource-use strategy of rapidly producing resource-acquisitive high-productivity leaves to make the most of a shorter growing season and then resorbing that nitrogen investment relatively earlier in expectation of early frosts in such environments. However, sampling at a single midseason time point (e.g., June, July, or August) would miss almost all macroevolutionary associations between this classic resource-use trait and native habitat seasonality, despite this being among the strongest relationships in our data sets. That such a large difference in conclusions would exist based on a 4-wk difference in sampling time is unexpected given widespread sampling practices in the broader functional trait literature.

Value of Function-Valued Analyses

Function-valued analyses considering the entire growing season provide insights into how seasonal shifts in traits evolve, and full results for all traits are presented in the supplement (data sets S5, S6). The analyses performed here use two examples of simple metrics of plasticity derived from functions—in our case, a metric of the absolute range of trait plasticity (seasonal breadth) and a directional magnitude of trait plasticity (net seasonal shiftiness). For our purposes, the main goal is to understand how seasonal shifts in traits evolve with one another and with native habitat environmental characteristics, and these two metrics differ in their interpretation.

Seasonal breadth is agnostic to direction, such that a positive correlation between two traits indicates that as one trait evolves a larger seasonal shift, the other trait does as well, while a negative correlation indicates that as one trait evolves a larger seasonal shift, the other trait evolves a smaller shift. Examples of traits with strong correlations in seasonal breadth include total phenolics and zinc (positive; $R^2 = 0.55$), nitrogen and lamina thickness (negative; $R^2 = 0.46$), potassium and copper (positive; $R^2 = 0.41$), and lamina thickness and LMA (positive; $R^2 = 0.38$). Some of these, such as the relationship between lamina thickness and LMA or nitrogen, have an obvious mechanistic connection (Poorter et al. 2009; John et al. 2013, 2017; Onoda et al. 2017), while it is less clear why the evolution of seasonal plasticity would be correlated in other pairs of traits. Considering the evolution of seasonal breadth with native habitat environmental characteristics, examples of strong evolutionary correlations include water content and precipitation seasonality (positive; $R^2 = 0.43$), nitrogen and soil silt content (positive; $R^2 = 0.32$), and total phenolics and NDVI-derived growing-season length (negative; $R^2 = 0.37$). Possible interpretations of such correlations include that higher plasticity in water content evolves under more variable precipitation (perhaps reflecting a more substantial hardening of leaves; Nicotra and
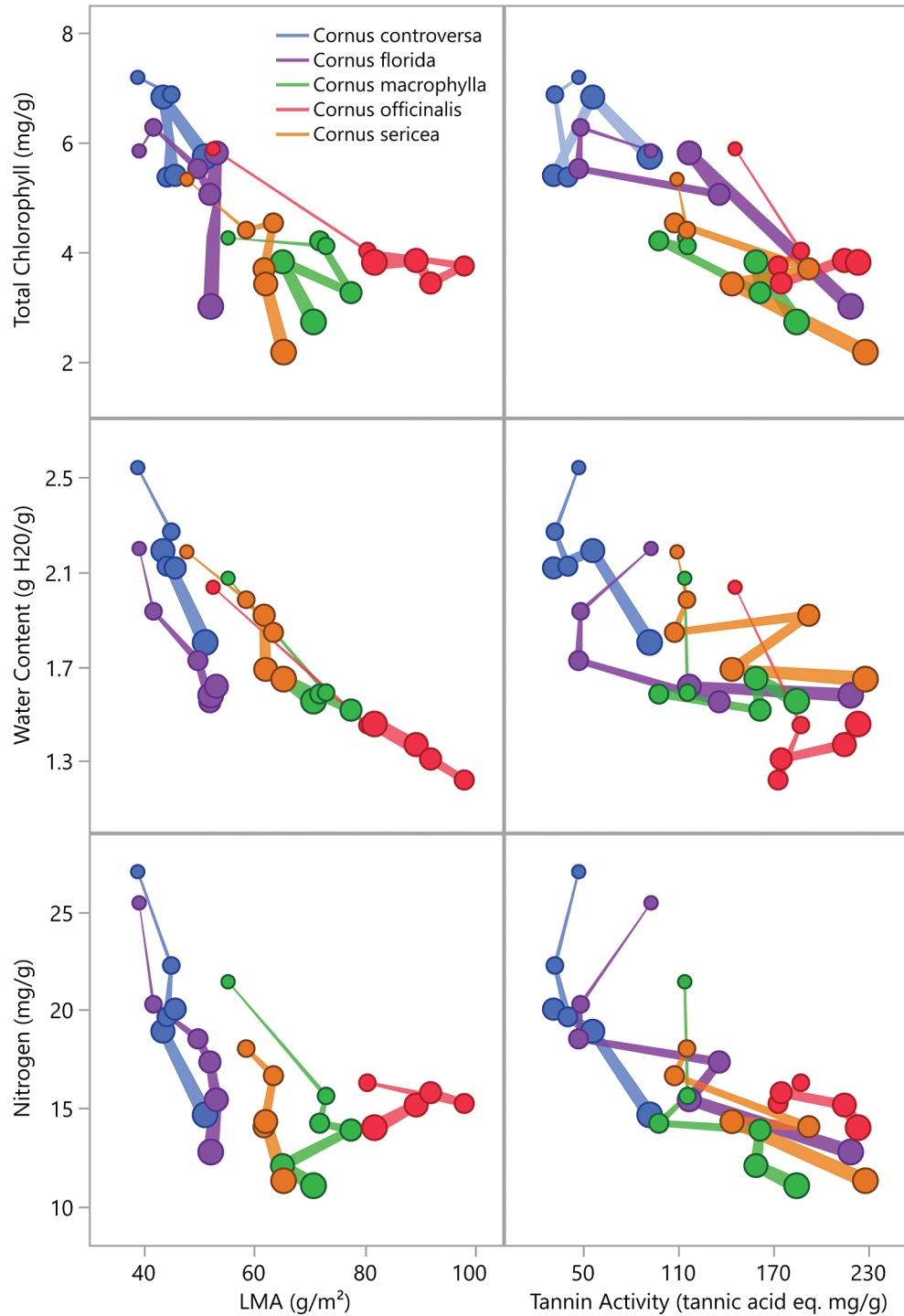
Fig. 2   Seasonal shifts in two-dimensional trait space for five *Cornus* species. Average trait values at the six sampled time points are shown for each species, with increasing size of markers and connections between markers by month (smallest size in May, largest in October). Note that trait data are missing for nitrogen for *Cornus sericea* and *Cornus officinalis* in May and so these points are omitted. LMA = leaf mass per area.

Davidson 2010), higher plasticity in nitrogen evolves on silt soils relative to sandy ones (perhaps suggesting a more resource-acquisitive early-season strategy and stronger end-of-season resorption; Reed et al. 2012; Yuan et al. 2015), and less variable

chemical defense in environments with longer growing seasons (perhaps reflecting more consistent defense investment under extended exposure to herbivore pressure; Hahn et al. 2019). While a metric such as seasonal breadth might be useful in some

Table 1

Demonstration of Variation across the Growing Season in the Strength of Pairwise Trait-Trait Macroevolutionary Correlations among *Cornus* Species, Attributable to Seasonal Shifts in Leaf Traits

Trait-trait relationship	Sign	May	June	July	August	September	October
Calcium + magnesium	(+)	.67	.62	.58	.53	.51	.48
Nitrogen + total chlorophyll	(+)	.63	.57	...	.44	.17	.45
Tannin activity + total phenolics	(+)	.41	.31	.28	.53	.53	.49
Potassium + water content	(+)	.28	.43	.29	.37	.56	.44
LMA + tannin activity	(+)	.35	.33	.51	.32	.23	...
Nitrogen + potassium	(+)	.53	.45	...	...	.23	.18
Nitrogen + phosphorus	(+)	.66	...	...	...	...	...
Total chlorophyll + water content	(+)	.34	.47	...	.37	.17	...
Nitrogen + total anthocyanins	(+)	.32	.19	...	...	.19	...
LMA + total phenolics	(+)	...	.20	.24	...	.17	.18
LMA + water content	(−)	.29	.54	.63	.67	.50	.30
LMA + total chlorophyll	(−)	.57	.88	...	.55	.24	...
LMA + nitrogen	(−)	.53	.56	.28	.22	...	...
LMA + potassium	(−)	.32	.55	.27	.25	.29	.21
Total chlorophyll + tannin activity	(−)	.65	.36	.20	.34	.24	.28
Nitrogen + toughness	(−)	.68	...	.30	...	...	...
Magnesium + total flavonoids	(−)	.33	.40	.40	.29	.19	.25
Nitrogen + tannin activity	(−)	.26	.41	.27	.21	.18	...
Tannin activity + water content	(−)	.17	.35	.37	.19	.20	...
Total phenolics + water content	(−)	.17	...	.18	.20	.17	.26
Total chlorophyll + total phenolics	(−)	.41	.14	.17	...	...	...
Calcium + total flavonoids	(−)	.36	.24	.23	...	...	...
Calcium + total phenolics	(−)	...	...	.22	.16	...	.31

Note. Each sampling month (May–October) was treated as a separate data set of leaf trait data on 25 species. Macroevolutionary correlations among traits were assessed using phylogenetic generalized least squares regression in the Rphylopars package. Coefficients of determination (R^2) are presented when statistically significant ($P < 0.05$). Note that while some trait relationships are largely invariant across sampling months, others vary dramatically. This suggests that studies making different decisions about the timing of sampling may arrive at different conclusions. A summary of variation in relationships across months is presented in the supplement (fig. S1, available online), along with full statistical results (data set S3, available online). LMA = leaf mass per area.

contexts, this kind of approach does not consider the baseline trait value or the directionality of the change in the two traits. Note that the interpretations made above rely on having a general sense of the directionality of trait change, here inferred using other approaches (e.g., fig. S1).

Net seasonal shiftiness is an example of a metric that incorporates both the baseline trait value (here the earliest sampling point) and the directionality of trait change alongside its magnitude. Under this metric, a positive correlation between two traits indicates that as one evolves a net upward (or downward) shift relative to baseline, the other trait moves in the same direction. Conversely, a negative correlation indicates that as one trait evolves a net upward shift, the other trait evolves a net downward shift (and vice versa). Examples of traits with strong correlations in net seasonal shiftiness include lamina density and tannin activity (positive; $R^2 = 0.48$), calcium and magnesium (positive; $R^2 = 0.46$), nitrogen and manganese (negative; $R^2 = 0.53$), and water content and LMA (negative; $R^2 = 0.50$). A finding that around half of variation in the evolution of a seasonal shift in one trait can be explained by the evolution of a seasonal shift in another trait indicates a strong underlying mechanistic interaction between these traits. Considering the evolution of net seasonal shiftiness with native habitat environmental characteristics, many of the associations found using seasonal breadth are recovered, such as water content and precipitation seasonal-

ity (negative; $R^2 = 0.36$) or nitrogen and soil silt content (negative; $R^2 = 0.31$), now with the sign of the relationship clear where before it could be inferred only from other approaches. Some relationships are no longer significant when accounting for the directionality of change from baseline, such as the relationship between total phenolics and NDVI-derived length of the growing season. Other strong evolutionary correlations that were previously missed emerge—for instance, relationships between certain micronutrients such as zinc and soil pH (negative, $R^2 = 0.44$) and between leaf area and precipitation seasonality (positive, $R^2 = 0.36$), as well as similar variables indicative of the intensity of dry periods (data set S6). These indicate the evolution of larger seasonal declines in leaf zinc (as well as copper and iron; data set S6) on more alkaline soils, where availability is poorer and perhaps resorption is higher (Lucas and Davis 1961; Hayes et al. 2014), as well as the evolution of larger early-season increases in leaf area in habitats with less consistent rainfall, perhaps indicating more prolonged leaf expansion in such species (as found in beech; Meier and Leuschner 2008).

Additional insights can be gained by explicitly considering the relationships between metrics of trait plasticity and baseline trait values at the start of the growing season. Here macroevolutionary correlations between May trait values and seasonal breadth or net seasonal shiftiness yield very strong relationships for many traits (table 3). Results for seasonal breadth are positive for most

Table 2

Demonstration of Variation across the Growing Season in the Strength of Pairwise Trait-Environment Macroevolutionary Correlations among *Cornus* Species, Attributable to Seasonal Shifts in Leaf Traits

Trait-environment relationship	Sign	May	June	July	August	September	October
Phosphorus + soil pH	(+)	.58	.21	.35	.37	.31	.21
Toughness + precipitation of wettest quarter	(+)	.21	.50	.41	.37	.32	.30
Copper + soil bulk density	(+)	.44	.43	.30	.25	.23	...
LMA + precipitation of warmest quarter	(+)	.23	.26	.27	.28	...	.23
Zinc + amplitude of seasonality (NDVI)	(+)	.29	...	.29	.31	.25	.33
Zinc + soil bulk density	(+)	...	.31	.25	.24	.30	.49
Nitrogen + amplitude of seasonality (NDVI)	(+)	.36	.23	.27	.19	...	...
Phosphorus + diurnal temperature range	(+)	...	.47	.29	.26	...	.25
Phosphorus + soil cation exchange capacity	(+)	.21	...	...	.23	.23	.23
Toughness + mean annual temperature	(+)	...	.44	.31	.26	.23	...
Toughness + mean annual precipitation	(+)	...	.38	.40	.23	...	...
Total flavonoids + precipitation of coldest quarter	(+)	.24	...	...	...	...	...
Nitrogen + temperature seasonality	Mixed	.53 (+)	...	...	...	...	.45 (−)
Nitrogen + growing-season length (NDVI)	Mixed	.52 (−)	...	...	...	...	.39 (+)
Nitrogen + minimum temperature of coldest month	Mixed	.61 (−)	...	...	...	...	.23 (+)
Nitrogen + mean temperature of driest quarter	Mixed	.73 (−)	...	...	...	...	.20 (+)
Zinc + precipitation of wettest month	(−)	.35	.29	.46	.53	.51	.48
Zinc + precipitation of warmest quarter	(−)	.42	.28	.33	.54	.64	.34
Toughness + amplitude of seasonality (NDVI)	(−)	.26	.49	.51	.37	.32	.22
Zinc + soil coarse fragment volume	(−)	...	.33	.41	.30	.38	.51
Zinc + precipitation seasonality	(−)	...	.30	.41	.24	.40	.55
LMA + precipitation of coldest quarter	(−)	.38	.34	.28	...	.38	.20
Manganese + precipitation of warmest quarter	(−)	.29	.21	.21	.20	.22	...
Phosphorus + frost-free period	(−)	.20	...	.26	.26	.28	.22
Phosphorus + mean annual precipitation	(−)	.35	.22	.37	.33	.23	...
Phosphorus + growing-season length (NDVI)	(−)	.39	...	.37	.34	.34	...
Nitrogen + precipitation of wettest month	(−)	.27	.20	.26	.22	...	...
Water content + mean temperature of warmest quarter	(−)	...	...	.21	.27	...	.51
Water content + mean temperature of wettest quarter	(−)	...	.32	.43	.35	...	...
Phosphorus + soil organic matter content	(−)	.29	.23	.22	...	...	...
Total anthocyanins + maximum temperature of warmest month	(−)	...	...	.28	...	.23	.36
Nitrogen + frost-free period	(−)	.61	...	...	...	...	...
Nitrogen + mean annual temperature	(−)	.54	...	...	...	...	...
Nitrogen + soil sand content	(−)	.56	...	...	...	...	...
Nitrogen + soil organic matter content	(−)	.42	...	...	...	...	...

Note.   Each sampling month (May–October) was treated as a separate data set of leaf trait data on 25 species. Macroevolutionary correlations among traits were assessed using phylogenetic generalized least squares regression in the Rphylopars package. Coefficients of determination (R^2) are presented when statistically significant ($P < 0.05$). Note that while some trait-environment relationships are largely invariant across sampling months, most vary dramatically, with some even changing sign. This suggests that studies making different decisions about the timing of sampling may arrive at different conclusions. A summary of variation in relationships across months is presented in the supplement (table S2, available online), alongside full statistical results (data set S4, available online). LMA = leaf mass per area; NDVI = normalized difference vegetation index.

traits, indicating that the evolution of higher May trait values is correlated with the evolution of higher seasonal trait plasticity. Results for net seasonal shiftiness are negative for most traits, indicating that for traits that typically shift downward over the growing season, the evolution of higher May trait values is correlated with the evolution of stronger declines over the growing season in those traits, while for traits that typically shift upward over the growing season, the evolution of lower May trait values is correlated with the evolution of stronger seasonal increases. In particular, the strong negative relationships for nitrogen ($R^2 = 0.76$), total phenolics ($R^2 = 0.72$), toughness ($R^2 = 0.50$), and tannin activity ($R^2 = 0.49$) indicate that there is a strong evolutionary coupling between the early-season resource-use or defense strategy employed by a species and the degree of plasticity it exhibits across the growing season (table 3). These relationships indicate that the evolution of differing degrees of seasonal trait plasticity is related to the evolution of species' early-season phenotypes for many classic plant functional traits.

Overall Insights into the Evolution of Leaf Trait Seasonality in Dogwoods

While each of the analyses described above gives useful pieces of information from different perspectives, considering the results

Table 3

Macroevolutionary Correlations between Function-Valued Metrics of Trait Seasonal Shifts (Seasonal Breadth, Net Seasonal Shiftiness) and Baseline Trait Values (May) among *Cornus* Species

Trait	Seasonal breadth	Net seasonal shiftiness
Leaf dry mass	…	…
Water content	.34 (+)	.18 (−)
Leaf area	…	.26 (−)
Aspect ratio	.19 (+)	…
LMA	.21 (+)	.17 (+)
Lamina thickness	…	…
Lamina density	.41 (−)	.62 (−)
Toughness	.21 (+)	.50 (−)
Total chlorophyll	.20 (+)	.24 (−)
Total anthocyanins	.62 (+)	.45 (−)
Total flavonoids	.45 (+)	.39 (−)
Total phenolics	.42 (+)	.72 (−)
Tannin activity	…	.49 (−)
Nitrogen	.77 (+)	.76 (−)
Phosphorus	.17 (+)	.22 (−)
Potassium	.17 (+)	…
Calcium	…	…
Magnesium	…	…
Sulfur	.68 (+)	.24 (+)
Iron	.74 (+)	.70 (−)
Boron	…	…
Manganese	.69 (+)	.36 (+)
Zinc	.31 (+)	.30 (−)
Copper	.40 (+)	.26 (−)
Sodium	…	.68 (−)
Aluminum	.28 (+)	…

Note. Correlations among traits were assessed using phylogenetic generalized least squares regression in the Rphylopars package. Coefficients of determination (R^2) are presented, followed by the sign when statistically significant ($P < 0.05$). Note that for many traits, the degree of seasonal shift (whether considered in terms of absolute plasticity or directional magnitude) is strongly evolutionarily correlated with the baseline trait value. Results for seasonal breadth are positive for most traits, indicating that species with higher baseline trait values at the start of the growing season also experience more seasonal plasticity. Results for net seasonal shiftiness are negative for most traits, indicating one of two situations—for traits that largely shift downward, species that have evolved higher baseline values tend to experience stronger downward shifts over the growing season; for traits that largely shift upward, species that have evolved lower baseline values tend to experience stronger upward shifts over the growing season. LMA = leaf mass per area.

together yields a more complete picture of functional trait evolution. Consider two contrasting scenarios of trait-trait and trait-environment interactions: first, the relationship between LMA and water content, and second, the relationships among nitrogen, toughness, and tannin activity, both in relation to an important native habitat environmental variable—precipitation of the wettest month (fig. 3). In the first scenario, net seasonal shiftiness for LMA and water content are strongly evolutionarily correlated (negative; $R^2 = 0.50$; data set S5), but May values are only weakly correlated with net seasonal shiftiness for these traits (table 3). Trait values for both traits considered individually by month show no evolutionary correlation with precipita-

tion regardless of month used (table S2), but net seasonal shiftiness is evolutionarily correlated with precipitation for both water content (negative; $R^2 = 0.42$) and LMA (positive; $R^2 = 0.26$; data set S6). In the second scenario, net seasonal shiftiness for leaf nitrogen, toughness, and tannin activity shows nonsignificant or only very weak pairwise evolutionary correlations (data set S5), but May values strongly predict net seasonal shiftiness for nitrogen (negative; $R^2 = 0.76$), toughness (negative; $R^2 = 0.50$), and tannin activity (negative; $R^2 = 0.49$). Trait values considered individually by month show significant evolutionary correlations with precipitation for toughness (all 6 mo) and nitrogen (two-thirds of months), though not tannin activity (table S2), but net seasonal shiftiness is not correlated with precipitation for any of these three traits (data set S6).

One interpretation of the differences between these two scenarios is that traits such as LMA and water content are tightly mechanistically linked at the scale of the leaf organ itself (John et al. 2013; Onoda et al. 2017), such that they shift in strong lockstep across the growing season within individuals, and because of this the degree of seasonal shift that these traits experience evolves together as species diversify. Conversely, for traits such as nitrogen, toughness, and tannins, the lack of correlated shifts across the growing season indicates that these traits are not strongly mechanistically linked at the leaf level but that correlations between these traits among species (e.g., nitrogen and tannins) might be considered more consistent with selection favoring certain combinations of trait values due to energy budgets and broader effects on fitness (Reich et al. 2014; Zust and Agrawal 2017) rather than direct trait-trait interactions generating constraints at the leaf level. Looking at the relationship between net seasonal shiftiness and May baseline values underscores this—for LMA and water content, there is only a weak relationship between baseline May values and the direction and magnitude of shifts observed, while for nitrogen, toughness, and tannins, the strong relationships between the baseline May value and the degree of seasonal shift show that the initial investment by the plant (in productivity, physical defense, or chemical defense) is strongly predictive of the degree to which that strategy changes over the growing season. When leaf nitrogen starts out at a higher concentration, it falls further over the growing season, reflecting a stronger shift from resource-acquisitive toward resource-conservative strategy in species that begin the growing season in a more resource-acquisitive part of trait space. When investment in tannins starts out at a low concentration, investment tends to increase fairly consistently over the growing season (adding chemical defense capacity over time to poorly defended leaves), whereas when tannins start out at a high concentration, they tend to fluctuate—often falling and then increasing again. Given that nitrogen and tannin activity considered by month correlate negatively across species in five out of 6 mo but their seasonal shifts are evolutionarily uncorrelated, these changes fit with expectations about trait trade-offs as part of larger plant resource-use and defense strategies (Reich 2014; Zust and Agrawal 2017), rather than fitting clear tissue- or organ-level hypotheses about structural or physiological interactions among traits (John et al. 2013; Onoda et al. 2017). When thinking about these two scenarios with respect to the environmental gradient of precipitation among species, for LMA and water content there is a strong relationship between net seasonal
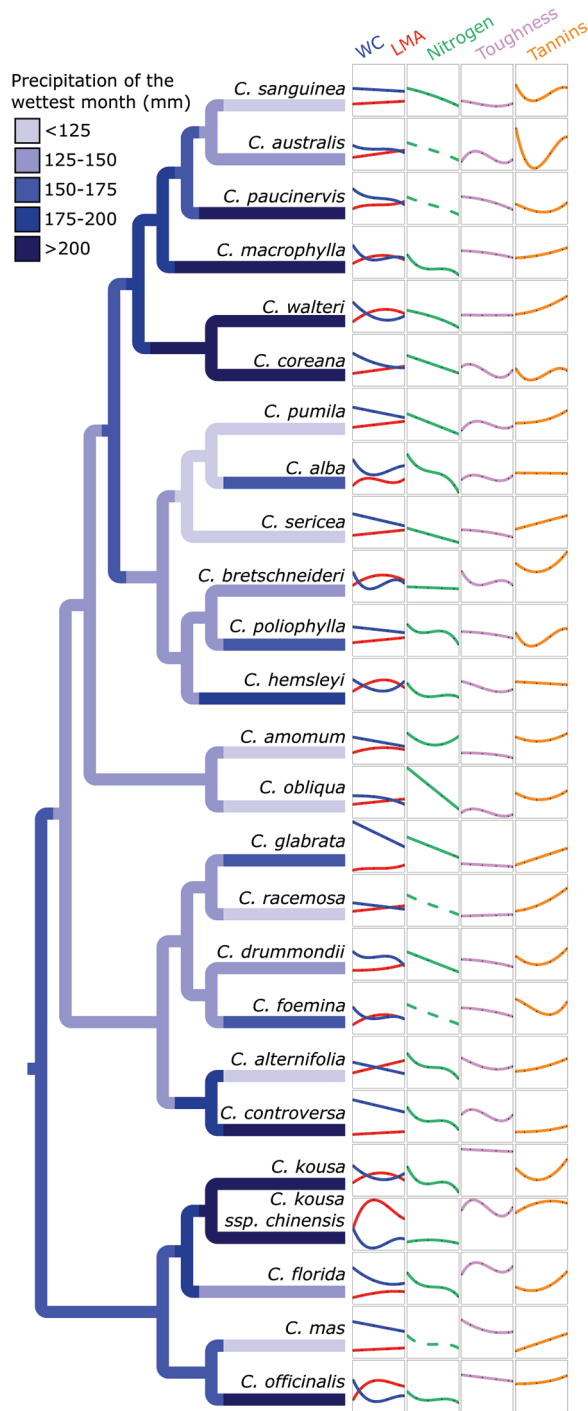
Fig. 3   Evolution of trait seasonality for leaf water content (WC), leaf mass per area (LMA), leaf nitrogen, leaf toughness, and tannin activity across 25 species of *Cornus*. The phylogeny reflects the ancestral state reconstruction of native habitat precipitation of the wettest month. Plots of curves all have the same *X*-axis (growing degree days across the growing season spanning May–October sampling dates) and have *Y*-axis values that are the same for each trait, scaled to the minimum and maximum trait values observed for that trait across species and months. Each trait curve can therefore be read based on its behavior across the growing season based on the relative trait space occupied by the *Cornus* species included in this study (i.e., showing large shifts or small ones, starting "high" or "low," etc.). This figure showcases two differing scenarios of trait-trait and trait-environment relationships, described in "Results and Discussion." Note that as nitrogen is the one variable in our data set with substantial missing data (~50%), we have used it to demonstrate curve fitting with partial data (shown by the absence of points for each sampling time in curves) and curve prediction for species that lack data entirely (represented by curves with dashed lines).

shiftiness and precipitation, while this does not exist for nitrogen, toughness, or tannin activity. This suggests that the capacity for seasonal plasticity in LMA and water content evolves in response to native habitat precipitation. Conversely, the actual trait values in any given month for LMA and water content are uncorrelated with precipitation, while the trait values for nitrogen (in four out of 6 mo) and especially toughness (in all months) are strongly correlated with precipitation. This suggests that while the capacity for seasonal plasticity in nitrogen or toughness may not evolve with this environmental variable, the relative resource strategies to which the absolute values of these traits contribute do evolve with native habitat precipitation.

Insights from the Evolution of Functional Trait Plasticity

In the *Cornus* system, a few core themes arise from examining the evolution of leaf trait plasticity alongside more standard comparative approaches. First, the large seasonal shifts observed in most functional traits alongside the variable trait-trait and trait-environment results that are observed when considering sampling dates separately highlight the risks of blindly sampling traits that exhibit strong plasticity. Second, trait shifts across the growing season typically move individuals from more resource-acquisitive to more resource-conservative trait combinations, and the degree of this shift appears often correlated with native habitat environment. Our findings of large seasonal shifts in leaf traits are certainly not unprecedented—many of these patterns have previously been identified in other temperate trees and shrubs (Riipi et al. 2002; Karolewski et al. 2013; Fajardo and Siefert 2016), including seasonal fluctuations in leaf dry mass, area, water content, LMA, toughness, nutrients, and secondary metabolites. The finding that phylogenetic correlations can change when plastic traits are assessed under different conditions or time points is also not a new observation (Goolsby 2015; Kariñho-Betancourt et al. 2015), though it is underappreciated and has substantial consequences for our ability to understand whether there are general principles that govern the evolution of plant functional traits. This is especially true because seasonal, ontogenetic, and environmental plasticity have often been ignored in favor of describing and synthesizing interspecific variation at large spatial and taxonomic scales (Wright et al. 2004; Reich et al. 2014; Díaz et al. 2016; Shipley et al. 2016; Pierce et al. 2017). While certainly a valuable endeavor for describing plant functional diversity and the boundaries of plant phenotypes, intraspecific trait data often challenge the applicability of the conclusions of broad interspecific syntheses with respect to "how plants work" (Niinemets 2015; Martin et al. 2017; Anderegg et al. 2018). Intraspecific trait variation is often treated as noise variation relative to species means (Kazakou et al. 2014; Siefert et al. 2015), despite the fact that much of this intraspecific variation is actually due to integrated plant changes over

time and space—that is, seasonal, ontogenetic, or environmentally driven variation, where traits will often change in nonindependent ways. Because this variation is "structured" (Martin et al. 2017), failing to account for it when collecting data across species may bias results in unclear ways—muddying the ability to understand the evolution of plant functional traits even when plasticity is not the focus.

Information on trait plasticity in key functional traits is not yet well incorporated into syntheses of the evolution of plant functional traits and resource-use strategies. At present, we lack a coherent understanding of how seasonal, ontogenetic, and environmentally driven variation in functional traits contributes to plant adaptation, though some excellent advances have been made in understanding how functional traits vary with these factors through meta-analysis (Poorter et al. 2009; Barton and Koricheva 2010; Endara and Coley 2011; Zvereva and Kozlov 2014; Barton et al. 2016; Cooke and Leishmann 2016; Gibert et al. 2016). To gain further insights into the evolution of plasticity in functional traits, we need sampling of functional traits across growing seasons, ontogeny, and environmental conditions and the examination of the correlated evolution of trait responses. Using reaction norms or function-valued approaches in a phylogenetic comparative framework is one way to accomplish this, but regardless of the specific approach used, the explicit consideration of how traits shift with one another at the intraindividual scale allows for insights into the nature of trait-trait relationships.

A particularly important distinction is whether apparent trade-offs arise from strong tissue- or organ-level structural or physiological interactions (John et al. 2013; Onoda et al. 2017) or whether they arise from a more general coordination of investment in traits that form ecological strategies (Reich 2014; Zust and Agrawal 2017). This is linked to the central distinction between "selection" and "constraint" (the former referring to selection against trait combinations with low fitness and the latter variously discussed as biophysical or ultimately genetic) that has been used to explain why parts of phenotypic space are empty—for instance, unobserved combinations of traits far off the main axis of the global leaf economic spectrum (Reich et al. 1999, 2014; Pigliucci et al. 2007; Donovan et al. 2011, 2014). When considering plasticity, if two leaf traits move in near lockstep at the intraindividual scale, this suggests that these traits either have a direct biophysical interaction at the leaf level itself (e.g., one structurally drives or physiologically requires the other) or are governed by shared gene expression (e.g., two secondary metabolites controlled by the same biosynthetic pathway). If two leaf traits are macroevolutionarily correlated but do not move in a coordinated fashion at the intraindividual scale under plastic responses, this suggests that these traits interact at a scale above the leaf level, perhaps arising from selection on plant ecological strategies that give rise to trade-offs or other strong trait relationships among species.

Of course, these are not two discrete categories of trait relationships but rather represent ends of a continuum of possible explanations for trait correlations. Especially in the middle of such a continuum, trait interactions are likely to be influenced by the relative location of species' phenotypic combinations in trait space (Reich et al. 1993; Donovan et al. 2014), where traits may be able to move more independently of one another when in the center of the global cloud of observed phenotypic combinations and may track more tightly when near the edges of such clouds at the margins of presumably structurally or physiologically incompatible combinations (Reich et al. 1999; John et al. 2013; Reich 2014; Onoda et al. 2017). There will likely also be effects of growth form, life history, and evolutionary lineage that limit the degree of plasticity possible and influence the relative tightness of pairwise trait-trait tracking at the intraindividual scale due to interactions from additional traits, similarly to differences in trait trade-offs observed at the macroevolutionary scale among lineages (Edwards et al. 2014; Mason and Donovan 2015; Muir et al. 2017). While relevant data sets to date are sparse, these principles provide a rough framework for how the explicit consideration of trait plasticity can assist with understanding the origins and nature of functional trait trade-offs.

The analyses performed here use simple metrics that are easy to understand to demonstrate the utility of function-valued approaches. There is a small but growing body of more nuanced function-valued phylogenetic comparative methods that can be used for particular purposes and data types (e.g., Guo et al. 2007; Hadjipentalis et al. 2013; Goolsby 2015; Clavel et al. 2018), but more important than the specific method employed is the recognition that a broad diversity of dynamic plant traits can be analyzed as function valued, allowing for the explicit assessment of the macroevolution of trait plasticity. Such traits include dose-response curves, reaction norms, allometric or stoichiometric relationships, and many other kinds of traits where phenotypes change with exogenous factors (e.g., time, resource availability, biotic pressures, abiotic stressors). We encourage others to explore (and develop!) approaches to incorporate dynamic traits into phylogenetic comparative studies, as addressing the evolution of plasticity in plant functional traits and its contribution to plant fitness, adaptation, and diversification has been recognized as one of the "loose foundation stones" of trait-based plant ecology (Shipley et al. 2016).

Acknowledgments

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