Evolution of nutrient resorption across the herbaceous genus *Helianthus*

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Abstract Foliar nutrient resorption is a key modulator of plant nutrient use. However, evolutionary patterns for nutrient resorption remain unclear, especially in herbs. We measured nitrogen and phosphorus resorption on preselected leaves across the *Helianthus* (sunflower) genus in a common garden in Athens, GA. We analyzed our data with published leaf traits and native habitat environmental data. Using phylogenetically controlled analyses, we tested if (1) nutrient resorption correlates with leaf economic, vasculature, and defense traits through evolutionary time, and (2) native habitat environment predicts nutrient resorption evolution. For *Helianthus*, nutrient resorption capacity is greater in resource-conservative species, as previously defined for *Helianthus* with a principle components analysis of leaf economic spectrum traits (photosynthetic rate, respiration rate, leaf lifespan, leaf mass per area, and green-leaf N and P concentrations). Nutrient resorption capacity also evolutionarily correlates with individual leaf economic traits, though not always as expected based on broad species

surveys. Greater nutrient resorption is also positively associated with leaf chemical defenses, but not leaf vasculature or senescence rate. Finally, nitrogen resorption evolution increases with native habitat precipitation, but native habitat soil fertility does not predict nitrogen or phosphorus resorption. Our results suggest nutrient resorption evolution is more closely tied to resource economic strategy than native habitat.

Keywords Nutrient resorption · Traits · Leaf economics spectrum · Tannins · Selection · Sunflowers

Introduction

Nutrient resorption is fundamental to plant nutrient economy. Resorption lowers net leaf nutrient construction costs, which somewhat frees plants from strict dependence on nutrient uptake capacity or availability (Aerts 1996; Chapin 1989; Eckstein et al. 1999; Zhang et al. 2015). Nutrient resorption may also contribute to an over-arching resource use strategy, which may result in predictive relationships between nutrient resorption and other traits related to how plants acquire, conserve, and defend nutrients in the varying climatic and soil environments plants contend with as they evolve.

One set of resource-use strategies is the leaf economics spectrum (LES). The LES is a pattern of

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leaf-level tradeoffs between traits conveying productivity and traits conveying persistence (Diaz et al. 2004; Reich 2014; Wright et al. 2004). High-productivity traits are found in “resource-acquisitive” species, and include high photosynthetic and respiration rates, high leaf nitrogen (N) and phosphorus (P) concentration, and low leaf mass per area (LMA) and leaf lifespan. Species toward the resource-acquisitive end of this spectrum rapidly repay initial carbon investment of leaf production at the expense of thinner, flimsier leaves that senesce sooner. Resource-conservative species generally have long-lived, durable leaves, but the structural carbon required for persistence results in lower productivity per unit time (Diaz et al. 2004; Reich 2014; Wright et al. 2004).

We hypothesize that resource-conservative species resorb more foliar nutrients than resource-acquisitive species. High nutrient resorption capacity may allow resource-conservative species to thrive despite their low nutritional status by increasing nutrient mean retention time (Wright and Westoby 2003; Zhang et al. 2015). Furthermore, resource-conservative species have longer-lived leaves, which may contribute to slower leaf senescence rates that allow more time for resorption. Studies examining nutrient resorption and the LES have mixed results, with some suggesting higher nutrient resorption is a resource-conservative trait (Wood et al. 2011; Wright and Westoby 2003; Zhang et al. 2015) and others concluding resorption is independent of resource use strategy (Freschet et al. 2010).

Leaf economic theory predicts nutrient-conservative, longer-lived leaves are also better protected (Diaz et al. 2004; Mason et al. 2016; Mason and Donovan 2015b). If higher resorption capacity is a resource-conservative trait, leaf defense compounds such as phenolics and tannins should increase along with resorption to protect hard-won nutrients from loss through herbivory. Mason et al. (2016) found tannin concentrations are higher in more resource-conservative *Helianthus* species. However, Aerts (1997) hypothesized phenolics precipitate leaf proteins during senescence and make them unavailable for resorption. If phenolics physiologically impede nutrient resorption, then evolution of high chemical defense via tannins may preclude evolution of high nutrient resorption and generate a negative relationship between these traits among taxa.

Nutrient resorption capacity may also have co-evolved with leaf vascular capacity due to mechanistic physiological dependencies. Leaf vein density, a common proxy for vascular capacity, plays an important role in nutrient metabolism and transport (Sack et al. 2012, 2013; Zhang et al. 2015). If evolution of high resorption capacity requires high vascular capacity for export from senescing leaves, vein density and nutrient resorption may have a positive relationship. Based on these interactions leaf chemical defenses, and vascular capacity as described by leaf vein density may all be evolutionarily correlated with resorption in *Helianthus*.

Whether the result of physiological dependencies or trade-offs, all trait–trait correlations evolve in the context of native habitat. Large-scale studies show nutrient resorption generally decreasing as habitat fertility or green-leaf nutrient concentration increases (Aerts 1996; Killingbeck 1996; Kobe et al. 2005; Wright and Westoby 2003; Yuan and Chen 2009). However, it is unclear if this trend is evolutionary or a plastic phenotypic response to soil fertility (Aerts 1996). Though many researchers have hypothesized increased nutrient resorption explains plant adaptation to infertile environments (Eckstein et al. 1999; Zhang et al. 2015; Distel et al. 2003), this has not always been supported by empirical evidence (Chapin 1980; Aerts 1996; Aerts and Chapin 2000; Brant and Chen 2015; Drenovsky and Richards 2006). If nutrient resorption enables plant survival in low-fertility conditions, species from lower-fertility habits should express higher capacity for nutrient resorption than species from higher-fertility habitats.

Nutrient resorption capacity is measured as either resorption efficiency or resorption proficiency. Resorption efficiency is the proportion of resorbed nutrients relative to green-leaf nutrients. Resorption-proficiency measures terminal resorption ability of the leaf and represents the biochemical limit of resorption; more proficient leaves have lower senesced-leaf nutrient levels (Killingbeck 1996). Although efficiency and proficiency are complementary traits, Killingbeck (1996) argues proficiency is under stronger selection and more useful in evolutionary studies. In contrast, efficiency is useful for comparing species’ relative capabilities of retaining acquired nutrients (Killingbeck 1996).

We used the *Helianthus* (sunflower) genus to explore the connection between nutrient resorption

evolution and other leaf traits and native habitat. *Helianthus* includes a diverse mix of annual and perennial species and spans a range of North American habitats with varying climate and soil characteristics (Heiser et al. 1969; Mason and Donovan 2015b; Sack et al. 2013). This is a good system for examining nutrient resorption because *Helianthus* species have been shown to vary in leaf economic traits and associated resource-use strategies (Mason and Donovan 2015b), defense traits (Mason et al. 2016; Mason and Donovan 2015a), vascular traits (Mason and Donovan 2015b), and secondary metabolites (Webber and Mason 2016), as well as stem and root traits (Bowsher et al. 2016; Pilote and Donovan 2016). Our study captured patterns of trait expression by minimizing environmentally induced plasticity using a common garden (de Vilmereuil et al. 2016; Donovan et al. 2014). We examined nutrient resorption variation through evolutionary time in the light of physiological trade-offs and resource availability in native habitats. We used novel nutrient resorption data and published leaf and habitat environment data for 28 sunflower species of known phylogenetic relationship (Stephens et al. 2015). Specifically, we asked (1) if nutrient resorption correlates through evolutionary time with LES, leaf vascular, and leaf defense traits, and (2) if native habitat environment predicts nutrient resorption evolution.

Methods

Plant growth

We present previously unpublished nutrient resorption data from a published common garden experiment assessing evolution of leaf economic and defense traits (Mason et al. 2016; Mason and Donovan 2015b; Webber and Mason 2016) (Table 1). Seeds from two

to four populations each of 27 *Helianthus* annual and perennial species and one *Phoebanthus* species were either wild collected or obtained from accessions maintained by the USDA National Genetic Resources Program. These diploid, nonhybrid species are evenly distributed across the most recent *Helianthus* phylogeny (Stephens et al. 2015). Populations are spread across each species range (83 populations total). For more information on population selection and seed sourcing, see (Mason and Donovan 2015b).

Eight replications of all populations were grown under high-resource conditions in identical pots and soil mixtures. The same experiment has additionally been used to assess floral trait evolution (Mason et al. 2017b), biomass allocation, and reproductive timing (Mason et al. 2017a). A detailed account of experimental conditions can be found in Mason and Donovan (2015b). See Table 1 for sources of all published data used in this study.

Leaf selection and trait measurement

To avoid confounding effects of leaf ontogeny on nutrient resorption and other leaf trait values (Mason et al. 2013; van Heerwaarden et al. 2003), leaves were predestinated for collection. The most recent fully expanded leaf (MRFEL) and its oppositely paired leaf were collected between the fourth leaf pair stage and the onset of reproduction and the newly expanding leaf pair immediately above was tagged. The tagged leaf pair was monitored for loss-of-greenness three times per week and harvested directly off the plant at full senescence (Mason and Donovan 2015b). We calculated leaf senescence rate as the number of days between 25% loss-of-greenness and 100% loss-of-greenness. Four undamaged senesced leaves from different replicate plants within each population were sampled for N and P concentrations for this study.

Table 1 Literature base on which the dataset was compiled for analysis with N- and P-resorption efficiencies and proficiencies

Trait(s)	Source
LES PC1	Mason and Donovan (2015b)
Leaf lifespan, LMA, photosynthetic rate, respiration rate	Mason and Donovan (2015b)
Green-leaf N, P, N:P, and C:N	Mason and Donovan (2015b)
Tannin activity	Mason et al. (2016)
Flavonoids and total phenolics	Webber and Mason (2016)
Native habitat environmental traits	Mason and Donovan (2015b)

All leaf economic traits, including green-leaf N and P concentrations, were measured on the MRFEL (Mason and Donovan 2015b). Green-leaf tannin activity (as determined by protein-precipitation capacity), total phenolics, flavonoids, and other chemical and structural leaf defenses were measured on the MRFEL (Mason et al. 2016; Webber and Mason 2016). Vein density was measured on the oppositely paired leaf (Mason and Donovan 2015b).

Leaf N and P concentrations were measured in leaves which were dried in a forced-air drying oven at 60 °C for 72 h before being finely ground with a ball mill. N concentration was determined via carbon–nitrogen analysis using Micro-Dumas Combustion (NA1500, Carlo Erba Strumentazione, Milan, Italy) at the University of Georgia Analytical Chemistry Laboratory. P concentration was determined via acid-molybdate spectrophotometry on ashed leaf powder (Varvel et al. 1976).

We calculated resorption efficiency sensu Killingbeck (1996) as:

$$\text{Resorption efficiency (\%)} = \frac{\text{nutrients}_{\text{GL}} - \text{nutrients}_{\text{SL}}}{\text{nutrients}_{\text{GL}}} \times 100$$

where $\text{nutrients}_{\text{GL}}$ is the population average green-leaf nutrient concentration and $\text{nutrients}_{\text{SL}}$ is the population average senesced-leaf nutrient concentration. We calculated resorption proficiency sensu Killingbeck (1996) as:

$$\text{Resorption proficiency} = \text{nutrients}_{\text{SL}}$$

Environmental data

Source habitat soils and climate data were obtained for all 83 populations as described in Mason and Donovan (2015b). Five soil cores were collected from each population source habitat, dried at 60 °C in a forced-air drying oven for 72 h, and homogenized. Each core was analyzed for pH, cation exchange capacity, organic matter content, and soil chemistry (phosphorus, potassium, magnesium, calcium) by A&L Eastern Laboratories (Richmond VA). N, carbon (C), and C:N ratio was determined using Micro-Dumas Combustion at the University of Georgia Analytical Chemistry Laboratory. Replicate cores were averaged; these

source habitat means were used for all subsequent analysis.

Temperature and precipitation data were obtained for each population from the WorldClim database (Hijmans et al. 2005). Aridity Index and potential evapotranspiration (PET) data were obtained from the CGIAR Global Aridity and PET database (Zomer et al. 2008).

Statistical analysis

Recent critiques in LES literature have addressed normalizations in trait calculation. Mathematical interconversion of mass- and area-based measurements using LMA causes spurious correlations because LMA varies more than traits like nutrient concentration (Lloyd et al. 2013; Osnas et al. 2013; Pearson 1897; Poorter et al. 2014; Tu 2016). In this study, we performed normalizations when we calculated area-based resorption proficiency by dividing senesced-leaf nutrient concentration by green-leaf LMA. However, critiques of nutrient resorption measurement techniques argue area-based normalizations are more reliable because mass-based senesced-leaf N and P may be biased by carbon resorption. Carbon resorption impacts the senesced-leaf's overall mass and therefore the concentration of the remaining nutrients in the senesced-leaf (Kandil et al. 2004; van Heerwaarden et al. 2003). To address these possibilities, we present and discuss our results using mass- and area-based normalizations (Poorter et al. 2014; Westoby et al. 2013).

In comparative analysis among species, phylogenetic signal can confound results because it can lead to trait correlation due to shared ancestry rather than adaptation or biophysical constraints (Felsenstein 1985). We tested for phylogenetic signal with Blomberg's K and Pagel's λ using the *phylosig* function in the phytools R package (Blomberg et al. 2003; Pagel 1999; Revell 2012). While no resorption traits displayed significant phylogenetic signal, many of the previously published leaf traits do, therefore we used phylogenetically explicit analyses.

Evolutionary trait–trait and trait–environment correlations were assessed on population-weighted species means with the *phylopars* function in the R package *Rphylopars* (Paradis et al. 2004; Goolsby 2016; Martins and Garland 1991). To assess correlations between nutrient resorption and the LES, we

Table 2 r^2 of evolutionary trait–trait correlations on population-weighted species means for (a) mass- and (b) area-normalized data

2 (a)	P _{eff}	P _{SL}	N _{eff}	N _{SL}	N _{eff} :P _{eff}	N _{SL} :P _{SL}	Flavonoid	Phenol	LSR
P _{eff}	1								
P _{SL}	(−) 0.57	1							
N _{eff}	(+) 0.35	(−) 0.38	1						
N _{SL}	(−) 0.37	(+) 0.48	(−) 0.82	1					
N _{eff} :P _{eff}	(−) 0.63	(+) 0.37	—	—	1				
N _{SL} :P _{SL}	—	—	—	—	—	1			
Flavonoid	—	—	—	—	—	—	1		
Phenol	—	(−) 0.21	(+) 0.21	(−) 0.27	—	—	(+) 0.99	1	
LSR	—	—	—	—	—	—	—	—	1
A _{mass}	—	—	—	—	—	—	—	—	—
R _{mass}	(−) 0.25	(+) 0.39	—	—	(+) 0.44	—	—	(−) 0.17	—
LMA	—	—	—	—	v	—	—	—	—
LL	—	—	—	(+) 0.15	—	—	—	—	(+) 0.19
N _{GL}	—	—	—	—	—	—	—	—	—
P _{GL}	—	—	—	—	—	—	—	—	—
N _{GL} :P _{GL}	—	—	—	—	—	—	—	—	—
C _{GL} :N _{GL}	—	—	—	v	v	—	—	—	—
Tannin	—	—	—	(−) 0.18	—	—	—	—	—
VD	—	—	—	—	—	—	—	—	—
LESPC1	—	—	—	—	—	—	—	—	—
2 (b)	P _{eff}	P _{SL}	N _{eff}	N _{SL}	N _{eff} :P _{eff}	N _{SL} :P _{SL}	Flavonoid	Phenol	LSR
P _{eff}	1								
P _{SL}	(−) 0.42	1							
N _{eff}	0.35	(−) 0.41	1						
N _{SL}	(−) 0.15	(+) 0.48	(−) 0.74	1					
N _{eff} :P _{eff}	(−) 0.63	(+) 0.22	—	—	1				
N _{SL} :P _{SL}	—	—	—	—	—	1			
Flavonoid	—	—	—	—	—	—	1		
Phenol	—	(−) 0.17	—	(−) 0.16	—	—	(+) 0.99	1	
LSR	—	—	—	—	—	—	—	—	1
A _{area}	—	—	—	(+) 0.27	—	—	—	—	—
R _{area}	(−) 0.17	(+) 0.45	—	—	(+) 0.23	—	—	—	—
LMA	—	—	—	(+) 0.22	—	—	—	—	—
LL	—	—	—	—	—	—	—	—	(+) 0.19
N _{GL}	—	—	—	(+) 0.22	—	—	—	—	—
P _{GL}	—	(+) 0.31	—	(+) 0.20	—	—	—	—	—
N _{GL} :P _{GL}	—	—	—	—	—	—	—	—	—
C _{GL} :N _{GL}	—	—	—	—	—	—	—	—	—
Tannin	—	—	—	—	—	—	—	—	—
VD	—	—	—	—	—	—	—	v	—

Table 2 continued

2 (b)	P_{eff}	P_{SL}	N_{eff}	N_{SL}	$N_{\text{eff}}:P_{\text{eff}}$	$N_{\text{SL}}:P_{\text{SL}}$	Flavonoid	Phenol	LSR
LESPC1	–	(+) 0.33	–	(+) 0.33	–	–	–	–	–

All values shown are significant at $p \leq 0.05$. **Bold** correlations are significant under FDR multiple comparisons. P phosphorus, N nitrogen, P_{eff} P-resorption efficiency, P_{SL} senesced-leaf P concentration, N_{eff} N-resorption efficiency, N_{SL} senesced-leaf N concentration, Flavonoid green-leaf flavonoid content, Phenol green-leaf phenolic content, LSR leaf senescence rate, A green-leaf photosynthetic rate, R green-leaf nighttime respiration rate, LMA green-leaf leaf mass per area, LL leaf lifespan, N_{GL} green-leaf N concentration, P_{GL} green-leaf L P concentration, Tannin green-leaf tannin activity, VD green-leaf vein density, *LESPC1* principle components 1 of leaf economics spectrum traits

used published principal components analysis of the six traits comprising the LES (photosynthetic rate, respiration rate, leaf lifespan, LMA, and green-leaf N and P concentrations) (Mason and Donovan 2015b). For correlations between nutrient resorption and leaf economic traits, we used these six traits individually. Missing values were handled using restricted maximum likelihood optimization of covariance parameters (Bruggeman et al. 2009; Goolsby 2016). Multiple comparisons correction was performed using False Discovery Rate (FDR) (Benjamini and Hochberg 1995). Ancestral state reconstructions were performed using restricted maximum likelihood on the most recent *Helianthus* phylogeny with the *phylopars* function (Paradis et al. 2004; Goolsby 2016; Stephens et al. 2015). To perform phylogenetic least squares ANOVA, we used the *gl*s function in the *nlme* package (Martins and Garland 1991; Pinheiro et al. 2014). We calculated p-values using the *aov* function. To detect differences between groups, we used the *TUKEY HSD* function in the *stats* package. Due to phylogenetically biased missing data for erect perennials from our flavonoid and total phenolics data, phylogenetic least squares ANOVA could not be performed for leaf phenolic or flavonoid content.

Results

Variation in nutrient resorption across Helianthus

The 28 species vary widely in N- and P-resorption efficiencies and proficiencies. Resorption efficiency varies almost twofold for P (57–93%) and less so for N (69.5–90%). N- and P-resorption proficiencies varies more than efficiency for both nutrients, with mass-based senesced-leaf N varying threefold (0.42–1.5%), mass-based senesced-leaf P varying sevenfold

(0.03–0.21%), area-based senesced-leaf N varying fourfold (18.3–70.7 g/m²), and area-based senesced-leaf P varying ninefold (1.06–9.16 g/m²). N- and P-resorption efficiencies positively correlate with each other, as do senesced-leaf N and P. Consequently, species with high N-resorption capacity have high P-resorption capacity. N-resorption efficiency is strongly negatively correlated with senesced-leaf N concentration, and P-resorption efficiency is strongly negatively correlated with senesced-leaf P concentration. Thus, species with high resorption efficiency capacity are also more proficient (Table 2).

N:P resorption efficiency ratio is only significantly correlated with senesced-leaf P concentration. Senesced-leaf N:P ratio does not correlate with any resorption metric (Table 2).

N-resorption efficiency displays significant differences by growth form, with basal rosette perennials recovering more N relative to their nutrient pool than either annuals or erect perennials ($p = 0.016$). We find no significant differences by growth form for senesced-leaf N concentration, although basal rosette perennials have substantially lower green-leaf N concentration (Mason and Donovan 2015b). Thus, basal rosettes resorb more N than erect perennials and annuals relative to their nutrient pool, but have equivalent resorption proficiency.

Correlations between nutrient resorption and leaf economic traits

We defined resource-conservative leaf economic strategy by lower values of first principal components axis of LES traits (photosynthetic rate, respiration rate, leaf lifespan, LMA, and green-leaf N and P concentrations) from Mason and Donovan (2015b). This axis explains 56.2% of variation in area-based LES traits and 51.5% of variation in mass-based LES traits and

Table 3 r^2 of evolutionary trait–environment correlations on population-weighted species means for **(a)** mass- and **(b)** area-normalized data

3 (a)	P_{eff}	P_{SL}	N_{eff}	N_{SL}	$N_{\text{eff}}:P_{\text{eff}}$	$N_{\text{SL}}:P_{\text{SL}}$	Flavonoid	Phenol	LSR
Latitude	–	–	–	(+) 0.17	–	–	–	–	–
Longitude	–	–	–	(–) 0.23	–	–	–	–	–
Altitude	–	–	–	(+) 0.16	–	–	–	–	–
MAT	–	–	–	(–) 0.14	–	–	–	–	–
MDR	–	–	–	–	–	–	–	–	–
ISO	–	–	–	–	–	–	–	–	–
TS	–	–	–	–	–	–	–	–	–
MTM _{warm}	–	–	–	–	–	–	–	–	–
MTM _{cold}	–	–	–	–	–	–	–	–	–
TAR	–	–	–	–	–	–	–	–	–
MTQ _{wet}	–	–	–	–	–	–	(–) 0.18	–	–
MTQ _{dry}	–	–	–	–	–	–	–	–	–
MTQ _{warm}	–	–	–	–	–	–	–	–	–
MTQ _{cold}	–	–	–	–	–	–	–	–	–
AP	–	–	–	(–) 0.17	–	–	–	–	–
PM _{wet}	–	–	–	(–) 0.15	–	–	–	–	–
PM _{dry}	–	–	–	(–) 0.19	–	–	–	–	–
PS	–	–	–	–	–	–	–	–	–
PQ _{wet}	–	–	–	(–) 0.16	–	–	–	–	–
PQ _{dry}	–	–	–	(–) 0.17	–	–	–	–	–
PQ _{warm}	–	–	–	–	–	–	–	–	–
PQ _{cold}	–	–	–	(–) 0.16	–	–	–	–	–
PET	–	–	–	–	–	–	–	–	–
ARI	–	–	–	–	–	–	–	–	–
$N_{\text{GL}}:P_{\text{GL}}$	–	–	–	–	–	–	–	–	–
$C_{\text{GL}}:N_{\text{GL}}$	–	–	–	–	–	–	–	–	–
Soil N	–	–	–	–	–	–	(+) 0.30	(+) 0.32	–
Soil C	–	–	–	–	–	–	–	(+) 0.15	–
Soil C:N	–	–	–	–	–	–	–	–	–
Soil N:P	–	–	–	–	–	–	–	–	–
Soil OM	–	–	–	–	–	–	(+) 0.34	(+) 0.42	–
Soil P	–	–	–	–	–	–	–	–	–
Soil K	–	–	–	–	–	–	–	–	–
Soil Mg	–	–	–	–	–	(–) 0.19	–	–	–
Soil Ca	–	–	–	–	–	–	–	–	–
Soil pH	–	(+) 0.14	–	–	–	–	–	–	–
Soil CEC	–	–	–	–	–	–	–	–	–
OM, N, C	–	–	–	–	–	–	(+) 0.25	(+) 0.31	–
P, K, Mg	–	–	–	–	–	–	–	–	–
Ca, pH, CEC	–	–	–	–	–	–	–	–	–
3 (b)	P_{eff}	P_{SL}	N_{eff}	N_{SL}	$N_{\text{eff}}:P_{\text{eff}}$	$N_{\text{SL}}:P_{\text{SL}}$	Flavonoid	Phenol	LSR
Latitude	–	–	–	–	–	–	–	–	–
Longitude	–	–	–	(–) 0.27	–	–	–	–	–

Table 3 continued

3 (b)	P _{eff}	P _{SL}	N _{eff}	N _{SL}	N _{eff} :P _{eff}	N _{SL} :P _{SL}	Flavonoid	Phenol	LSR
Altitude	–	–	–	–	–	–	–	–	–
MAT	–	–	–	–	–	–	–	–	–
MDR	–	–	–	–	–	–	–	–	–
ISO	–	–	–	–	–	–	–	–	–
TS	–	–	–	–	–	–	–	–	–
MTM _{warm}	–	–	–	–	–	–	–	–	–
MTM _{cold}	–	–	–	–	–	–	–	–	–
TAR	–	–	–	–	–	–	–	–	–
MTQ _{wet}	–	–	–	–	–	–	(–) 0.16	–	–
MTQ _{dry}	–	–	–	–	–	–	–	–	–
MTQ _{warm}	–	–	–	–	–	–	–	–	–
MTQ _{cold}	–	–	–	–	–	–	–	–	–
AP	–	–	–	(–) 0.14	–	–	–	–	–
PM _{wet}	–	–	–	–	–	–	–	–	–
PM _{dry}	–	–	–	(–) 0.15	–	–	–	–	–
PS	–	–	–	–	–	–	–	–	–
PQ _{wet}	–	–	–	–	–	–	–	–	–
PQ _{dry}	–	–	–	–	–	–	–	–	–
PQ _{warm}	–	–	–	–	–	–	–	–	–
PQ _{cold}	–	–	–	–	–	–	–	–	–
PET	–	–	–	–	–	–	–	–	–
ARI	–	–	–	(–) 0.16	–	–	–	–	–
N _{GL} :P _{GL}	–	–	–	–	–	–	–	–	–
C _{GL} :N _{GL}	–	–	–	–	–	–	–	–	–
Soil N	–	–	–	–	–	–	(+) 0.22	(+) 0.26	–
Soil C	–	–	–	–	–	–	–	–	–
Soil C:N	–	–	–	–	–	–	–	–	–
Soil N:P	–	–	–	–	–	–	–	–	–
Soil OM	–	–	–	–	–	–	(+) 0.25	(+) 0.32	–
Soil P	–	–	–	–	–	–	–	–	–
Soil K	–	–	–	–	–	–	–	–	–
Soil Mg	–	–	–	–	–	(–) 0.19	–	–	–
Soil Ca	–	–	–	–	–	–	–	–	–
Soil pH	–	–	–	–	–	–	–	–	–
Soil CEC	–	–	–	–	–	–	–	–	–
OM, N, C	–	–	–	–	–	–	(+) 0.17	(+) 0.24	–
P, K, Mg	–	–	–	–	–	–	–	–	–
Ca, pH, CEC	–	–	–	–	–	–	–	–	–

All values shown are significant at $p \leq 0.05$. **Bold** correlations are significant under FDR multiple comparisons. *MAT* mean annual temperature; *MDR* mean diurnal range, *ISO* isothermality (diurnal range/annual range of temperature), *TS* temperature seasonality, *MTM_{warm}* max temperature of warmest month, *MTM_{cold}* min temperature of coldest month, *TAR* temperature annual range, *MTQ_{wet}* mean temperature of wettest quarter, *MTQ_{dry}* mean temperature of driest quarter, *MTQ_{warm}* mean temperature of warmest quarter, *MTQ_{cold}* mean temp of coldest quarter, *AP* annual precipitation, *PM_{wet}* precipitation of wettest month, *PM_{dry}* precipitation of driest month, *PS* precipitation seasonality, *PQ_{wet}* precipitation of wettest quarter, *PQ_{dry}* precipitation of driest quarter, *PQ_{warm}* precipitation of warmest quarter, *PQ_{cold}* precipitation of coldest quarter, *ARI* aridity index, *OM* organic matter, *CEC* cation exchange capacity, soil PC1 of OM, N, and C; Soil PC1 of P, K, and Mg; Soil PC1 of Ca, pH, and CEC

(Mason and Donovan 2015b). Under area normalization, species with lower senesced-leaf nutrient concentration have a resource-conservative leaf economic strategy. Thus, resource-conservative species (i.e., species with lower photosynthetic and respiration rates and longer leaf lifespan) are more proficient. Senesced-leaf N (i.e., N proficiency) is evolutionarily correlated with every leaf economic trait except leaf lifespan and nighttime respiration rate (Table 2b). All significant correlations are in the direction predicted by the hypothesis that more complete nutrient resorption proficiency is a resource conservative trait except for the correlation with LMA. Senesced-leaf P (P proficiency) only correlates with two LES traits (respiration rate and green-leaf P concentration; Table 2b).

Mass normalization does not show an association between senesced-leaf nutrient concentration and the principal components based assessment of LES as a whole. However, senesced-leaf N correlates positively with leaf lifespan, and senesced-leaf P correlates positively with nighttime respiration (Table 2a). Leaf senescence rate correlates positively with leaf lifespan, but not with any measure of nutrient resorption (Table 2). N:P resorption-efficiency ratios correlate positively with nighttime respiration rate.

Correlated trait evolution of chemical leaf defenses, but not leaf vascular capacity, with resorption proficiency

Leaf phenolic content correlates negatively with senesced-leaf N and P concentrations regardless of normalization (Table 2). Tannin activity is negatively correlated with senesced-leaf N concentration under mass normalization (Table 2a). Thus, species with more leaf total phenolic compounds are more N and P proficient, and species with higher tannin content are more N proficient. Leaf minor vein density does not exhibit correlated evolution with nutrient resorption (Table 2).

Correlations between nutrient resorption and soil fertility and precipitation

In general, native habitat soil fertility does not correlate with nutrient resorption. N- and P-resorption efficiencies and area-based normalizations of N- and P-resorption proficiencies (senesced-leaf N and P) do

not correlate with any native habitat soil fertility measurement (Table 3). Mass-based senesced-leaf P concentration only correlates with soil pH, although this correlation loses significance under FDR correction; mass-based senesced-leaf N concentration does not correlate with any measure of fertility (Table 3).

In general, senesced-leaf nutrient concentration decreases in warmer habitats with more precipitation (Table 3). Under mass-based normalization, senesced-leaf N decreases as mean annual temperature increases (Table 3a). Multiple precipitation measures also correlate negatively with mass-based senesced-leaf N concentration, with species evolving more complete N proficiency in habitats with more precipitation. Senesced-leaf N concentration correlates negatively with annual precipitation and precipitation of the driest month under area normalization, though these correlations lose significance under FDR correction. Senesced-leaf P concentration and N- and P-resorption efficiency do not correlate with climate (Table 3).

Discussion

Evolution of nutrient resorption as a resource-conservative trait

Given the importance of foliar nutrient resorption to plant nutrient economy, determining whether nutrient resorption capacity corresponds with other trait syndromes is key to understanding plant adaptive strategy. Under area normalization, we find more complete resorption proficiency in resource-conservative *Helianthus* species, as defined by the first principal components axis of LES traits (photosynthetic rate, respiration rate, leaf lifespan, LMA, and green-leaf N and P concentrations) from Mason and Donovan (2015b). This is consistent with the hypothesis that resource-conservative species resorb more foliar nutrients than resource-acquisitive species (Wood et al. 2011; Wright and Westoby 2003; Zhang et al. 2015). However, under mass normalization, more complete resorption proficiency is not found in more resource-conservative *Helianthus* species, likely due to some of the unique aspects of bivariate LES trait relationships for *Helianthus*.

The bivariate relationships of resorption proficiency with individual leaf economic traits in

Helianthus are not always as might be expected based on broad cross-species patterns of LES (Diaz et al. 2004; Wright et al. 2004; but see Edwards et al. 2014 and Funk and Cornwell 2013). For example, higher-LMA leaves display less complete area-based resorption. This is probably because for this study system, LMA positively correlates with area-based photosynthetic rate (A_{area}) and not leaf lifespan (Mason and Donovan 2015b). At smaller phylogenetic scales such as within deciduous *Viburnum*, higher LMA occurs via thicker layers of photosynthetically active mesophyll cells (Edwards et al. 2014). More mesophyll cells increase LMA and A_{area} simultaneously because leaf area remains constant, while the photosynthetically active cells increase. Meanwhile, LMA remains negatively correlated with mass-based photosynthetic rate because increased mass from these cells dilutes mass-based photosynthetic gains (Edwards et al. 2014). It is not known if a similar association exists between mesophyll cell layers and LMA in *Helianthus* species; however, the positive relationship between LMA and A_{area} suggests it may. Since A_{area} and LMA may be mechanistically related, a similarly directional relationship between nutrient resorption and both these LES traits may be unavoidable.

Additionally, leaf lifespan does not correlate with senesced-leaf nutrient concentration in this herbaceous study system, where leaf lifespan is driven by growing season length and may not be influenced by selective pressure on mean retention time (Mason and Donovan 2015b; Mason et al. 2017a). We further find evolution of extended leaf senescence does not account for variation in nutrient resorption, nor the relationship between leaf economic strategy and nutrient resorption capacity, in *Helianthus*. Instead, leaf economic strategy and nutrient resorption appear to be governed by whole-plant processes ultimately driven by growing season and relative growth rate.

Thus, although N and P resorptions are strongly correlated with each other and some traits associated with resource-use strategy, they are still labile enough to evolve independent associations with other traits. These disparate relationships additionally support the emerging understanding that leaf economics strategy is a constellation of interacting traits rather than a tight linear spectrum (Mason and Donovan 2015b; Poorter et al. 2014).

Nutrient resorption correlates differently with other leaf traits than predicted by the literature

Researchers have hypothesized that leaf phenolics inhibit resorption by precipitating proteins (Aerts 1997; Chapin and Kedrowski 1983). In contrast, our data show that neither tannin activity (as measured by protein-precipitation capacity) nor total phenolics (as measured by Folin–Ciocalteu assay) constrain resorption. Indeed, higher phenolic content and tannin activity evolves across *Helianthus* as mass-based N-resorption proficiency becomes more complete. However, the absolute value of tannins in this herbaceous genus ranged from 0.005 to 2.516% tannic acid equivalents per leaf dry mass, which is much lower than levels reported in many woody species. This highlights the need to include herbaceous species in resorption studies because they often occupy different trait space than more heavily studied functional groups like trees. Studying herbaceous systems can provide demarcation for these hypotheses. For example, our data suggest that phenolics may not influence resorption at concentrations lower than 2.5%. Our data support the hypothesis that highly proficient species evolve higher secondary metabolite concentrations to defend the N and P fated to be recycled from senescing leaves. High molecular weight tannins increase with mass-based N proficiency in *Helianthus*. Tannins also increase as resource conservatism increases (Mason et al. 2016). These relationships generally explain less than 25% of regression variation, indicating direct physiological dependencies are not driving these correlations (Poorter et al. 2014). Overall, resorption, secondary metabolites, and resource economic strategy represent different axes of variation interacting based on more than just their shared carbon and nutrient economics.

Our data do not show any correlated evolution between leaf vein density and nutrient resorption. Rather, increased leaf vein density associates with a resource-acquisitive strategy in *Helianthus* (Mason and Donovan 2015b), suggesting high nutrient resorption is possible with relatively low leaf vein density. In their 2015 dipterocarp study, Zhang et al. found leaf vein density was strongly positively associated with nutrient resorption. While we cannot explain why our study system shows a different pattern, lack of evolutionary correlation between these two traits is not surprising since processes related to water supply

and photosynthate export mobilize orders of magnitude more material than processes involved in nutrient resorption.

Greater nutrient resorption capacity is beneficial in a variety of habitats

Our data show *Helianthus* species adapting to lower-fertility environments do not evolve higher resorption capacity. This finding differs from studies within global- and community-level datasets that show higher nutrient resorption capacity among species in lower-fertility environments (Aerts 1996; Killingbeck 1996; Kobe et al. 2005; Wright and Westoby 2003; Yuan and Chen 2009). These broad datasets are useful for determining nutrient-cycling dynamics within and across ecosystems, but cannot produce compelling evidence of evolutionary adaptation or underlying genetic and physiological linkages because they encompass many different variation sources. Our study prevents or accounts for environmental, phylogenetic, and ontogenetic variation sources. We acknowledge our single time-point soil sampling may not be a reliable proxy for whole-season fertility due to flushes and leaching that keep soil nutrients in constant flux. Still, given the wide variation in habitat occupancy across the genus (e.g., deserts to wetlands), the overall pattern obtained via our soil sampling is most likely sufficient for summarizing patterns of soil composition in habitats occupied by the diverse *Helianthus* genus.

Green-leaf N:P and C:N ratios, which are often used as proxies for nutrient limitation (Han et al. 2013), did not correspond to any resorption metric, including N:P resorption efficiency and proficiency ratios. This finding is not entirely unexpected, since N:P ratio is a poor proxy for nutrient limitation in other species (Drenovsky and Richards 2004), and poor nutrient resorption capacity actually reinforces plant-level nutrient limitation in desert shrubs (Drenovsky and Richards 2006). In contrast to soil fertility, climate predicts N-resorption proficiency. In general, N proficiency follows the same pattern as the LES, with resource-conservative, N-proficient species occupying warmer and/or wetter habitats than their resource-acquisitive, less proficient congeners.

In conclusion, our results show that nutrient resorption falls within the general framework of the LES in this phenotypically diverse herbaceous genus.

Fast-growing, resource-acquisitive, annual *Helianthus* species live in lower-precipitation habitats and exhibit lower resorption capacity. Meanwhile, slow-growing, resource-conservative basal rosette perennial species in warm and rainy habitats have evolved higher resorption capacity. Furthermore, the role of leaf phenolics in protecting green-leaf resources appears compatible with the role of resorption in conserving leaf resources during senescence. Our results suggest nutrient resorption evolution may be tied to resource economics strategy more than native environment, with resource-conservative, proficient species capable of thriving in a variety of habitats.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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