ECOPHYSIOLOGY AND EVOLUTION OF RESOURCE USE AND LOW-RESOURCE RESISTANCE IN *HELIANTHUS*

by

ASHLEY M. REA

(Under the Direction of Lisa A. Donovan)

ABSTRACT

A foundational goal of plant ecology is to identify co-varying traits across the climatic and soil environments plants contend with through ecological and evolutionary time. These patterns can be observed in ecological time through trait-trait relationships in favorable and stress environments, and through evolutionary time through trait-trait relationships among species. This dissertation focuses on trait-trait and trait-environment relationships in *Helianthus* at the ecological and evolutionary scale. Using controlled environmental and ontogenetic conditions, I explored variation in drought resistance and nutrient use efficiency in cultivated *H. annuus,* and variation in nutrient resorption across wild *Helianthus* species. In water limitation response studies, I found that resistant genotypes of cultivated *H. annuus* (as assessed by geometric mean productivity and absolute achene mass of the primary head) exhibit high early stem elongation rate under well-watered conditions. In nutrient limitation response studies, I found that nitrogen and phosphorus acquisition and utilization efficiencies often related differently to performance than predicted by single-nutrient limitation studies. I further find evidence suggesting that root nutrient use efficiency traits are independent are independent from shoot nutrient efficiency traits at lower nutrient levels, and that nitrogen and phosphorus efficiency traits are positively associated. At the evolutionary scale, I examine patterns of nutrient resorption variation across wild *Helianthus* species in light of the paired influences of constraints and selective pressure on foliar nutrient resorption evolution. Using phylogenetically-informed analyses, I found that nutrient resorption capacity is greater in slower-growing species exhibiting a relatively more resource-conservative leaf functional strategy. Furthermore, native habitat soil fertility does not predict nutrient resorption capacity in this genus. This suggests that nutrient resorption evolution is more closely tied to resource economic strategy than native habitat. In conclusion, this dissertation has expanded knowledge of how individual traits predict yield and productivity across resource supply in the globally important sunflower crop, and elucidated the evolutionary relationship between nutrient resorption, leaf functional traits, and native habitat. These insights may inform studies exploring the genetic or physiological basis of the trait relationships I here describe, which will be important for the development of sunflower as a valuable and sustainable oilseed crop.

INDEX WORDS: drought, stem elongation rate, ontogeny, nutrient use efficiency, nutrient acquisition efficiency, nutrient utilization efficiency, sunflower, nutrient resorption, leaf economics, adaptation

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DEDICATION

To my mother, Jeanne Pim, who has been an endless source of creativity, optimism, energy, and encouragement.

To my husband, Michael Rea, for believing in me, and for being so easy to love.

And to my daughter, Emma Rose, the delight of my life. You will forever be my greatest accomplishment.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v
LIST OF TABLES ix
LIST OF FIGURES xi
CHAPTER
1 INTRODUCTION AND LITERATURE REVIEW 1
References 7
2 ASSOCIATIONS BETWEEN EARLY VIGOR AND YIELD TRAITS UNDER WATER LIMITATION IN CULTIVATED SUNFLOWER 14
Abstract 15
Introduction 16
Methods 19
Results 24
Discussion 27
References 31
Figures 35
Tables 48
3 NITROGEN AND PHOSPHORUS USE EFFICIENCY IN CULTIVATED SUNFLOWER UNDER MULTI-NUTRIENT STRESS 53
Abstract 54

Introduction....56

Methods....61

Results....64

Discussion....67

References....73

Tables....82

4 EVOLUTION OF NUTRIENT RESORPTION ACROSS THE HERBACEOUS GENUS *HELIANTHUS*....92

Abstract....93

Introduction....94

Methods....98

Results....102

Discussion....106

References....111

Tables....119

5 CONCLUSIONS....124

References....128

LIST OF TABLES

Page

Table 2.1: ANOVA tables for yield metrics under R-stage water limitation (Fall 2014) and V-stage water limitation (Summer 2015). 48

Table 2.2: Spearman's ρ2 with sign for correlations between percent reduction and geometric mean productivity for each trait. 48

Table 2.3 Spearman's ρ2 with sign for traits for the 2014 R-stage experiment. 49

Table 2.4: Spearman's ρ2 with sign for traits measured in the 2015 V-stage experiment. 50

Supplementary Table 2.1: Percent reduction for each genotype 51

Supplementary Table 2.2: Geometric mean for each genotype..51

Supplementary Table 2.3: List of genotypes 52

Table 3.1: Full terms and calculations for each nutrient use efficiency trait…………82

Table 3.2: Correlation coefficients between nutrient acquisition efficiency, nutrient utilization efficiency, and nutrient use efficiency in crop species 82

Table 3.3A: Least-squares means of nitrogen nutrient use efficiency traits for roots and shoots 83

Table 3.3B: Least-squares means of phosphorus nutrient use efficiency traits for roots and shoots 84

Table 3.4: Spearman's ρ^2 with sign for correlations between least-squares means of nutrient acquisition efficiency or utilization efficiency and nutrient use efficiency...86

Table 3.5: Spearman's ρ^2 with sign for correlations between least-squares means of root nitrogen and phosphorus acquisition efficiency, utilization efficiency, and use efficiency and shoot nitrogen and phosphorus acquisition efficiency, utilization efficiency, and use efficiency 86

Table 3.6: Spearman's ρ^2 with sign for correlations between least-squares means of organ nitrogen acquisition efficiency and utilization efficiency to equivalent organ phosphorus acquisition efficiency and utilization efficiency................................87

Supplementary Table 3.1: Spearman's ρ^2 with sign for correlations between least-squares means of traits measured at budding and nitrogen acquisition efficiency 88

Supplementary Table 3.2: Spearman's ρ^2 with sign for correlations between least-squares means of traits measured at budding and phosphorus acquisition efficiency........89

Supplementary Table 3.3: Spearman's ρ^2 with sign for correlations between least-squares means of traits measured at budding and nitrogen utilization efficiency 90

Supplementary Table 3.4: Spearman's ρ^2 with sign for correlations between least-squares means of traits measured at budding and phosphorus utilization efficiency 91

Table 4.1: Literature base on which the dataset was compiled for analysis with N and P resorption efficiency and proficiency 119

Table 4.2: Evolutionary trait-trait correlations on population-weighted species means for mass- and area-normalized resorption, chemical defense, and leaf senescence rate data…..119

Table 4.3: Evolutionary trait-environment correlations on population-weighted species means for mass- and area-normalized resorption, chemical defense, and leaf senescence rate data 120

LIST OF FIGURES

Page

Figure 2.1: Genotype least-squares means for early stem elongation rate and days to bud (2014 R-stage)....................35

Figure 2.2: Genotype least-squares means for performance traits for 2014 (R-stage) data….....................36

Figure 2.3: Genotype least-squares means for early stem elongation rate, early stem expansion rate, and early number of leaf pairs (2015 V-stage)....................39

Figure 4: Genotype least-squares means for 2015 V-stage water limitation biomass traits....................41

Supplementary Figure 2.1: Scatterplot of R-stage least squares means of early stem elongation rate verse least squares means of performance traits....................42

Supplementary Figure 2.2: Scatterplot of R-stage least squares means of days to bud verse least squares means of performance traits.....................44

Supplementary Figure 2.3: Scatterplot of V-stage least squares means of early stem elongation rate verse least squares means of performance traits.....................46

Supplementary Figure 2.4: Scatterplot of V-stage least squares means of early stem expansion rate verse least squares means of performance traits....................47

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

A foundational goal of plant ecology is to identify co-varying traits in the climatic and soil environments plants contend with through ecological and evolutionary time. For this dissertation, I define “traits” as morphological and/or physiological attributes expressed by individual plants. Trait correlations can occur due to selective pressure, mechanistic dependencies, or genetic linkages (Donovan et al. 2011, Donovan et al. 2014). Traits governing plant acquisition and investment of resources such as nutrients and water strongly influence plant success in a given environment, which in turn influences ecological processes and adaptation (Chapin 1980, Wright et al. 2004, Diaz et al. 2004). Understanding relationships between plant traits, and the repeated evolution of specific trait combinations, informs explanations and predictions for plant success across environments.

On an ecological timescale, meaning plant responses to environmental changes within a generation, trait-trait correlations can be used in the context of crop species to predict agronomically important performance metrics such as yield or above-ground biomass (Upadhyaya et al. 2017, Rao et al. 2016, Pepo and Novak 2016). Where strong correlations between traits are present, research identifying them can set the stage for selection on secondary traits associated with yield. This strategy is particularly useful as a part of indirect selection breeding strategies (Richards 2000, Richards et al. 2010), which occurs when breeders select for traits related to crop performance and yield in non-stressed conditions.

Indirect selection relies on the phenomena by which some traits which strongly associate with production in non-stressed conditions also do so in stressed conditions, and requires knowledge of which traits these are (Atlin and Frey 1989, Ceccarelli et al. 1998, Bowsher et al. 2017, Richards et al. 2010). As the relationships between performance and concomitant traits often shifts with resource level, observing the shift in trait-trait relationship and what traits remain associated with performance across resource supply can help inform efforts to breed crops that produce acceptable yield across resource levels. Low-resource environments are a common challenge to global agricultural production (Dathe et al. 2013). This dissertation focuses on traits relating to productivity under different water and nutrient supply.

Water-limitation (i.e., drought) is one of the most limiting abiotic factors on crop production, and is expected to increase in frequency and severity in the United States as a result of climate change (Meyer et al. 2014, Zhao and Running 2010, Hatfield et al. 2013, Groisman and Knigth 2008). Desirable drought-resistant cultivars generally do so via a drought “avoidant” or a drought “escape” strategy (Richards, 2010, Blum 2011, Juenger 2013). Drought avoidant species maintain a more favorable internal plant-water status by maximizing water uptake and minimizing water loss, while drought escaping species have more rapid phenology where they flower and set seed before a drought occurs or intensifies (Juenger 2013; Ludlow 1989). Based on principles of indirect selection, it has been argued that early vigor under well-watered conditions is a key suite of traits for predicting and improving genotypic performance under moderate drought in cereals

(Richards et al. 2010, Araus 2008). If increased early vigor leads to increased drought resistance, it may do so either by supporting a suite of drought-avoidant or drought-escape traits.

Low-nutrient stress is found across agricultural regions and often occurs in combinations of concurrently low nutrient levels (Dathe et al. 2013). Crop performance under low nutrient stress is often characterized as “nutrient use efficiency,” which is calculated as productivity per unit applied nutrient. It is the product of nutrient acquisition efficiency (amount of nutrient acquired per unit applied) and internal nutrient utilization efficiency (productivity per unit acquired nutrient). Based on single-nutrient limitation studies, nutrient acquisition efficiency is generally expected to associate strongly with overall nutrient use efficiency regardless of nutrient supply (Earley et al., 2011; Presterl, Groh, et al., 2002; Manske et al., 2001; Mundim et al., 2013; Sandana 2016). Nutrient utilization efficiency either associates strongly with overal nutrient use efficiency regardless of nutrient supply (Presterl, Groh, et al., 2002; Mundim et al., 2013) or shows no relationship with nutrient use efficiency at low nutrient supply (Earley et al., 2011; Manske et al., 2001; Sandana 2016). However, under more complex soil conditions, such as soils with low availability of multiple nutrients, interactions between applied nutrients may impact nutrient use efficiency and the relationship between nutrient use, acquisition, and utilization efficiencies (Rietra et al. 2017). Thus, understanding whether the relative contributions of nutrient acquisition and utilization efficiency to nutrient use efficiency follow patterns established in single-nutrient stress studies could inform breeding strategies for complex soil nutrient conditions. Since efforts to breed low-input crops benefit from maximizing production under all conditions (Richards et al.

2010), studies investigating trait relationships across resource levels can inform breeding efforts to modify these traits with the end result of improving overall plant performance.

On an evolutionary time scale, identifying repeated instances of correlated trait-trait evolution in like environments can help explain the physiology influencing patterns of species range distribution (Grime et al. 1997, Reich et al. 1999, Ackerly 2004). However, evolutionary adaptations to environment are often influenced by physiological or genetic constraints. I examine patterns of foliar nutrient resorption evolution across *Helianthus* in light of these paired influences of constraints and selective pressure. Nutrient resorption is the process by which plants retransolocate nutrients from senescing leaves to other plant tissues. Resorption lowers net leaf nutrient construction costs, which somewhat alleviates strict dependence on nutrient availability (Aerts 1996; Chapin 1989; Eckstein et al. 1999; Zhang et al. 2015), but it may also contribute to an over-arching resource use strategy dictated by trait relationships at the leaf level. One such suite of trait relationships is the leaf economic spectrum, which describes a global pattern of leaf-level tradeoffs between traits conveying productivity and traits conveying persistence (Diaz et al. 2004; Reich 2014; Wright et al. 2004). Species towards 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). Research on the evolution of these trait-trait and trait-environment relationships in *Helianthus* will inform our understanding of the mechanistic basis of nutrient resorption in herbaceous species.

This dissertation examines the ecophysiology and evolution of resource use and low-resource resistance within the *Helianthus* genus. As a genus, *Helianthus* includes a diverse mix of annual and perennial species and spans a range of North American habitats, with considerable variation in suites of traits relating to plant leaf productivity, stem architecture, and root architecture (Heiser 1969, Mason and Donovan 2015b, Sack et al. 2013, Mason et al. 2016, Mason and Donovan 2015a, Webber and Mason 2016, Bowsher et al. 2016, Pilote and Donovan 2016). *Helianthus* contains the globally important oilseed crop, cultivated sunflower (*H. annuus* L.) (Foreign Agricultural Service, 2016, Sheoran et al., 2016, FAOSTAT, http://faostat3.fao.org). Due to its intensive root system and short-term drought-resistance relative to other crops, sunflower is often grown on fringe arable land (National Sunflower Association, *Limited Irrigation Farming*, 2001, Vear, 2016, FAOSTAT, http://faostat3.fao.org). As a result, sunflower potential as a low-input crop for fertilizers and water is being investigated (Škorić 2009). Additionally, breeding strategies for cultivated sunflower have changed from breeding strictly for maximum possible yield under intensive agriculture to breeding for yield with resistance to abiotic stresses (Vear 2016). Improved physiological understanding of sunflower should be useful to both traditional and genetic breeding efforts because powerful genetic resources have been developed in sunflower, such as a high-quality reference genome (Badouin et al. 2017) and associated mapping populations (Mandel et al. 2011).

For this dissertation, I employ a traits-based approach to answer the following questions: (1) Does early vigor predict cultivated sunflower performance and yield under water limitation; (2) What traits associate with nutrient acquisition and utilization efficiency under low-nutrient stress; and (3) How has a specific nutrient utilization trait (nutrient resorption) evolved across habitat type and functional strategy in *Helianthus*.

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CHAPTER 2

ASSOCIATIONS BETWEEN EARLY VIGOR AND YIELD TRAITS UNDER WATER LIMITATION IN CULTIVATED SUNFLOWER[1]

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[1] Rea AM, Donovan LA. To be submitted to *Helia.*

ABSTRACT

Drought is a major limiting factor on cultivated sunflower (*Helianthus annuus* L.) production. Sunflower improvement breeding is increasingly prioritizing cultivars which maximize production across favorable and water-limited conditions. We examine the relationship between early vigor traits and responses to water limitation imposed at two different ontogenetic stages (vegetative, V-stage; reproductive, R-stage) and using different response indices. The greenhouse experiments were conducted for six to ten inbred genotypes from an association mapping population, using individually potted plants and an automatic irrigation system that allowed maintenance of consistent well-watered and water-limited treatments. Analyses of variance indicate that both R-stage and V-stage water limitation reduced plant performance, and that there was variation among genotypes for seed yield traits in response to R-stage water limitation and for biomass in response to V-stage water limitation. Responses assessed as Geometric Mean Productivity (GMP) and percent reduction are positively associated for biomass traits, but not seed yield traits. Importantly, we found that early vigor assessed as stem elongation rate (SELR) predicts GMP and absolute yield traits under water limitation. The strong positive correlations between early SELR and seed mass suggest the possibility of accelerated, indirect selection programs breeding for early SELR in favorable conditions. Finally, we recommend the uniformly resistant genotype PI 597368 for additional research for future sunflower development programs.

INTRODUCTION

Drought is one of the most limiting abiotic factors on crop production (Meyer et al., 2014; Zhao and Running, 2010). Climate modeling predicts an increase in drought frequency, severity, and unpredictability in the continental United States (Hatfield et al., 2013; Groisman and Knight, 2008). Breeding programs are increasingly prioritizing the development of drought-resistant genotypes, with resistance to moderate drought being of particular importance (Vear 2016). We here define "drought resistance" as the ability to grow and produce economic yield under sub-optimal water supply (Farooq et al. 2009). Current and future efforts to develop more drought-resistant crops will require a better understanding of how plant traits associate with yield metrics.

Determining which traits predict yield under drought is important for informing indirect selection. Indirect selection is a breeding strategy by which crop performance under stress is improved by selecting for performance under non-stress conditions (Atlin and Frey 1989, Bänzinger et al. 1997, Ceccarelli et al. 1998). This strategy has proved successful in recent years for three reasons. Additionally, agronomically valuable cultivars are increasingly those which maximize performance under favorable conditions while maintaining acceptable yield under stress (Bowsher et al. 2017; Richards et al. 2010, Vear 2016). Finally, many traits confer an advantage under both favorable and water-limited conditions, and selection for these specific traits can improve performance under both conditions (Richards et al. 2010; Araus et al. 2008).

Based on these principles of indirect selection, Richards et al. (2010) have argued that early vigor under well-watered conditions is a key suite of traits for predicting and improving genotypic performance under drought in cereals. Early vigor traits include greater early leaf area, faster aboveground growth, and faster early root system growth. These traits can also contribute to a “drought-avoidant” strategy, whereby plants avoid unfavorable internal water status with extensive root systems that access deeper groundwater (Juenger 2013; Ludlow 1989). Greater early leaf area and aboveground growth can manipulate the environment into a more beneficial microclimate via soil shading (Richards et al. 2010, Araus et al. 2008). They also increase carbon capture to fuel root growth. While it is difficult to select for early root vigor due to the logistical constraints of root phenotyping, root vigor is thought to associate strongly with shoot vigor (Richards et al. 2010). However, under severe drought or conditions precluding ground water access, enhanced early vigor may deplete soil water too quickly, leading to reduced yield (Richards et al. 2010).

Early vigor can also be associated with rapid phenology, which has also been presented as an effective way to improve yield in drought-prone localities, especially when drought timing is predictable (Araus et al. 2008, Blum 2011). Rapid phenology, i.e. early onset of reproduction, can allow plants to “escape” drought by flowering before drought stress can impact yield (Juenger 2013, Ludlow 1989, Blum 2011). However, selection for drought escape via earlier flowering may lead to decreased yield in favorable conditions because a positive correlation has repeatedly been found between growth duration and yield under favorable conditions (Blum 2011, Araus et al. 2008). As a result, evaluation for early vigor among genotypes must also evaluate phenology.

The cumulative impact of drought on plant performance is dependent in part on the developmental stage of the plant during drought (Blum 1996). For example, water limitation at the early reproductive stage (hereafter: R-stage) can directly impact the development of reproductive organs, leading to lowered viability of ovaries and pollen (Blum 2011). Meanwhile, drought at vegetative stage (hereafter: V-stage) can impact leaf growth and photosynthesis (Andrianasolo 2016). As climate change is expected to lead to less predictable droughts, genotypes that can maintain acceptable yield under drought at multiple ontogenetic stages are preferable. However, traits conveying resistance at one ontogenetic stage may be less beneficial at another. Thus, the relationship between early vigor traits and crop performance under drought should be examined for water limitation at multiple ontogenetic stages before determining which genotypes merit further research.

When evaluating genotypes for maximized performance under favorable and water-limited conditions, geometric mean productivity (GMP) is often employed as an index to characterize the combined performance of genotypes across environments with a stronger penalty for poor performance. GMP is a popular stress index because it distinguishes between genotypes that perform well under both favorable and water-limited conditions from genotypes that perform well (relative to other genotypes) only under stress (Arnauld et al. 2016). However, GMP cannot always differentiate between genotypes that perform well under both favorable and water-limited conditions from genotypes that perform well only under favorable conditions (Arnauld et al. 2016). If consistently high performance across environments is desired (Blum 2011, Reitz 1974), evaluation of genotypes must take into account absolute performance under drought and percent reduction of yield as well as geometric mean of yield in favorable and water-limited environments. However, genotypes with low percent reduction in yield may be unable to simultaneously exhibit high yield GMP due to a possible trade-off between stress resistance and yield potential (Grime 1977).

Here, we use a genotypically diverse subset of cultivated sunflower (*Helianthus annuus* L.) to examine the relationship between early vigor traits and yield under water-limitation in light of developmental timing and different performance indices. Worldwide, cultivated sunflower is a very important oilseed crop (Foreign Agricultural Service, 2016; Sheoran et al., 2016, FAOSTAT; http://faostat3.fao.org.) Sunflower is commonly grown in rain-fed agriculture systems and fringe arable land and is therefore often subjected to drought conditions (National Sunflower Association; *Limited Irrigation Farming*, 2001; Vear, 2016; FAOSTAT, http://faostat3.fao.org, Andrianasolo 2016).

Our main objective was to determine if predictive relationships between early vigor and yield traits are present in cultivated sunflower across stress indices and ontogenetic stages. Specifically, we asked: (i) Is there variation among sunflower genotypes for response to moderate sustained water limitation at V-stage and R-stage? (ii) How well does water limitation response assessed as percent reduction agree with GMP estimates? (iii) Does early vigor (assessed as SELR) predict response to water limitation? (iv) Do genotypes respond similarly to V-stage and R-stage water limitation?

METHODS

Plant growth

In 2014, six diverse genotypes (NSL 202284, PI 386230, PI 597368, PI 599778, PI 599984, and PI 617099) were used to investigate sunflower resistance to R-stage water limitation. Between 13 and 17 November 2014, the blunt end of each achene (hereafter: seed) was scarified and placed on filter paper moistened with 0.2% Banrot anti-fungal solution before being transplanted into seedling trays containing standard potting soil and placed in a growth chamber until the emergence of one to two true leaf pairs. The growth chamber was set to 16h/8h light/dark cycle, with 25˚C days, 20˚ nights, and 55% humidity. Seedlings were transplanted to 2.92L pots containing a 1:1 sand to turface mixture (Turface, MVP ®, Buffalo Grove, IL) with 20g Osmocote Plus 15-9-12 9-month slow-release fertilizer with micronutrients (Scotts, Marysville, OH) and supplemental Calcium. Pots were arranged in a greenhouse in a randomized block design with two blocks each containing two replicates of each species and treatment (6 genotypes x 2 treatments x 2 blocks x 2 replicates per block = 48 plants total). Supplemental lighting of 16h/8h light/dark cycle was provided, and greenhouse temperature was set to 25˚C days and 20˚ nights. All plants were grown under well-watered conditions (35% volumetric water content, hereafter: soil moisture) until budding. When one half of all individuals in a genotype (i.e., four out of eight plants) reached budding, pots designated for water-limited treatments were lowered to 20% soil moisture. Treatments were maintained until harvest at reproductive maturity.

In 2015, four genotypes (NSL 208764, PI 476853, PI 597373, PI 603989) were used to investigate sunflower resistance to V-stage water limitation in addition to the six genotypes grown in 2014. For more information on the 10 total genotypes used, see Table S2.3. Beginning on 31 July 2015, plants were scarified, germinated, and transplanted into seedling trays in a growth chamber as in 2014. On 5 August 2015, seedlings were transplanted to 1.7L pots containing a 1:1 sand to turface mixture with 20g Osmocote Plus and supplemental calcium. Pots were arranged in a greenhouse in a randomized block design with two blocks containing three replicates of each species and treatment

(10 genotypes x 2 treatments x 2 blocks x 3 replicates per block = 120 plants total). Due to seasonal light, no supplemental lighting was provided. Greenhouse temperature was set to 25˚C days and 20˚ nights. All plants were grown under 35% soil moisture conditions until the 4-6 leaf pair stage, at which point, pots designated for water-limited treatments were incrementally lowered to 20% soil moisture over the duration of one week. Genotypes reached this early vegetative stage at approximately the same time; thus, treatment implementation occurred at the same time for all genotypes. After two weeks of sustained 35% (well-watered treatment) or 20% (water-limited treatment) soil moisture, plants were harvested.

Irrigation regimes

Well-watered treatment moisture levels of 35% represents field capacity for this substrate. The water-limited treatment moisture level of 20% was chosen based on preliminary studies that determined this to be a strong limiter of plant growth.

Irrigation regimes were maintained using an automatic irrigation system programmed to continuously measure soil moisture and irrigate when soil moisture dropped below the programmed threshold values of 35% or 20% (Nemali and van Iersel 2006). Soil moisture was measured with soil moisture probes (EC-5 probes, Decagon Devices, Pullman, WA), which were connected to a data logger (CR23X, Campbell Scientific, Logan, UT) via a multiplexer (AM416, Campbell Scientific, Logan, UT). The soil moisture for each genotype/treatment group was averaged (2014 R-stage: 4 pots total, 2015 V-stage: 4 of 6 pots measured and averaged), and this average was compared to the set-point (35% or 20%). When the average soil moisture for a group of pots dropped below the programmed threshold, a signal was sent via a relay driver (SDM-CD16/AC-16 Channel AC/DC Controller, Campbell Scientific, Logan, UT) to a solenoid valve to deliver drip irrigation to those 4 pots (for 2014 R-stage experiment) or 6 pots, two of which were not measured for the group average (2015 V-stage experiment).

Trait measurement and calculation of performance indices

In 2014, plant height was measured twice weekly beginning immediately after transplant. Early stem elongation rate (SELR) was calculated as height growth in cm per day for the first three weeks after transplant to permanent pots. Plants were checked for budding daily. Days to bud was calculated as number of days since germination before budding was first recorded. When 90% of disc florets on a head were flowering, the head was self-pollinated by rubbing a new paper towel on each flower head. For branching genotypes, all heads were selfed as they reached 90% flowering. Each selfed head was covered with a nylon mesh bag to prevent cross-fertilization. When all individuals in a genotype were fully matured for a given treatment, the genotype/treatment group was harvested. Tissue was dried and weighed for organ biomass. Seed yield was calculated as number of seeds per primary head and 25-seed weight.

In 2015, plant height, stem diameter, and number of true leaf pairs were measured thrice weekly beginning immediately after transplant. Early SELR was calculated as height growth in cm per day for the first two weeks after transplant to permanent pots. Early stem expansion rate (SEPR) was calculated as stem diameter expansion in mm per day for the first two weeks after transplant. Early number of true leaves was assessed as number of true leaves two weeks after transplant. Plant tissue was dried and weighed for organ biomass.

Statistical analysis

For 2014 R-stage data, we performed least-squares ANOVA using block, genotype, treatment, and genotype-by-treatment interaction for all traits (early SELR, days to bud, shoot biomass, plant biomass, and primary head seed count, seed mass, and 25-seed mass) using JMP PRO ver. 13 (SAS Institute, Cary, NC, USA). Shoot and plant biomass were natural log transformed before performing ANOVA. All traits were tested for normality of the residuals using the Shapiro-Wilk Goodness-of-fit test in JMP.

We generated Tukey-adjusted least-squares means for each genotype for shoot biomass, plant biomass, and primary head seed count, seed mass, and 25-seed mass using block, genotype, treatment, and genotype-by-treatment interaction as explanatory variables. We performed least-squares ANOVA using block and genotype to obtain least-squares means for stem elongation rate and days to bud after confirming that plants designated for future treatments did not differ prior to treatment initiation. All least-squares means were backtransformed when necessary before performing subsequent calculations and analyses. Least-squares means were used to calculated GMP and percent reduction, which we calculated as:

$$\text{GMP} = \surd(Control\ performance \times Stress\ performance)$$

$$\text{Percent reduction} = \frac{Control\ performance - Stress\ performance}{Control\ performance} \times 100\%$$

We used these least-squares means, as well as calculations of GMP and percent reduction for biomass and seed yield traits, in Spearman's rank correlation tests using the Hmisc package in R (Harrell, 2017). Using Spearman's rank, we tested for correlations between genotype rankings for GMP and percent reduction values for biomass and seed yield traits. We also tested for correlations between genotypic rankings for least-squares means of early vigor traits and well-watered and water-limited absolute biomass and seed yield traits. Finally, we tested for correlations between genotypic rankings for least-squares means of early vigor traits and GMP and percent reduction for biomass and seed yield traits.

For 2015 V-stage data, we followed the same procedure as in 2014. However, early vigor traits for this experiment were early SELR, early SEPR, and early number of leaf pairs. Only shoot and plant biomass were assessed as performance traits in 2015.

RESULTS

Variation in genotype response to water limitation at different ontogenetic stages

Under R-stage water limitation, all genotypes exhibited reduced biomass (Figure 2.2A and B). Total plant biomass ranged from 17.180 (NSL 202284, water-limited treatment) to 152.128 g (PI 386230, well-watered treatment). Shoot biomass ranged from 14.530 g (NSL 202284, water-limited treatment) to 111.848 g (PI 386230, well-watered treatment). The majority of genotypes also exhibited reduced seed count, seed mass, and 25-seed mass; however, PI 386230 exhibited increased values for all three seed traits (Figure 2.2C, D, and E). Primary head total seed count ranged from 10 (PI 386230, well-watered treatment) to 596 (NSL 202284, well-watered treatment); primary head total seed mass ranged from 0.263 g (PI 386230, well-watered treatment) to 32.454 g (NSL 202284, well-watered treatment); primary head 25-seed mass ranged from 0.296 g (NSL 202284, water-limited treatment) to 1.883 g (PI 386230, well-watered treatment).

Under V-stage water limitation, the majority of genotypes exhibited reduced biomass, with the exception of NSL 202284, which increased both whole-plant and shoot biomass, and PI 297368, which trended towards an increase in biomass under water limitation (Figure 2.4). Total plant biomass ranged from 3.35 g (PI 597373, water-limited treatment) to 66.89 (PI 476853, well-watered treatment). Shoot biomass ranged from 2.21 g (PI 597373, water-limited treatment) to 18.76 g (PI 599778, well-watered treatment).

Genotypic variation in response to R-stage water limitation is present in primary head seed count, primary head seed mass, and 25-seed mass, as evidenced by significant genotype and genotype-by-treatment effects for seed count and seed mass, and significant genotype effects for 25-seed mass (Table 2.1). No genotypic variation is present in total plant or shoot biomass in response to water limitation at R-stage. Genotypic variation in total plant and shoot biomass in response to V-stage water limitation is present, with significant genotype and genotype-by-treatment interactions (Table 2.1).

Correlations between percent reduction and geometric mean productivity

Under moderate R-stage water limitation, genotypes that rank highly for shoot and whole-plant biomass GMP rank low for percent reduction (Table 2.2). Thus, a high-performing genotype also maintains consistent biomass across favorable and water-limited conditions. Genotype rankings for percent reduction of seed count are not significantly associated with genotype rankings for GMP, and seed mass and 25-seed mass are not strongly associated with genotype rankings for GMP.

Under moderate V-stage water limitation, genotypic rankings for total plant biomass as assessed by percent reduction are strongly negatively correlated with GMP (Table 2.2). For shoot mass, genotypic rankings as assessed by percent reduction are strongly negatively correlated with GMP (Table 2.2).

Correlations between early vigor and water limitation response, and between developmental rate and water limitation response

Early SELR is strongly positively correlated to shoot mass, total seed mass of the primary head and 25-seed mass under moderate R-stage water limitation when assessed as absolute resistance (i.e., trait values under water limitation; Table 2.3A) but not total plant biomass (Table 2.3A). Early SELR is strongly positively correlated to plant mass, shoot mass, and 25-seed mass under moderate R-stage water limitation when assessed as GMP (Table 2.3B) but not seed count or total seed mass of the primary head (Table 2.3B). Early SELR does not strongly correlate to percent reduction and biomass or seed trait (Table 2.3B).

Number of days to budding under favorable conditions is negatively correlated to absolute plant and shoot biomass under water limitation, but does not strongly correlate with any seed traits (Table 2.3A). Number of days to budding under favorable conditions does not strongly associate with any biomass or seed traits assessed as GMP or percent reduction (Table 2.3B).

Early SELR under favorable conditions is not associated with GMP, percent reduction, or absolute shoot or total plant biomass or GMP of shoot and total plant biomass under V-stage water limitation (Table 2.4). Early SEPR and the number of early leaf pairs under favorable conditions are not associated with GMP, percent reduction, or absolute biomass or seed traits under V-stage water limitation.

DISCUSSION

Using an automatic irrigation system where volumetric soil moisture was maintained, we investigated the relationship between early vigor traits and yield in diverse cultivated sunflower genotypes. We used two separate experiments to examine trait-trait relationships at two ontogenetic stages: reproductive stage (early budding) and vegetative stage (4-6 leaf pairs). We found genotypic variation for seed yield traits in response to R-stage water limitation, and for biomass traits in response to V-stage water limitation. We also found that two indices, GMP and percent reduction, are moderately or strongly associated for biomass traits, but not seed yield traits. Importantly, we found that early stem elongation rate predicts absolute shoot biomass and seed mass traits and GMP of plant and shoot biomass and 25-seed mass under reproductive stage water limitation. The strong positive correlations between early stem elongation rate and seed mass suggest the possibility of accelerated, indirect selection programs breeding for early vigor traits in favorable conditions; however, future studies should incorporate branching architecture into their experimental design as scatterplot visualizations of the data indicate that branching architecture may be driving some of these correlations. The relatively high yield produced under water limitation by genotypes exhibiting early vigor as measured by growth rate but not phenology implies that these genotypes are employing a “drought avoidant” strategy, whereby plants avoid internal water stress by increasing access to water.

Percent reduction associates with GMP for biomass but not seed yield

For both R-stage and V-stage water limitation, genotypes with high biomass GMP are more likely to exhibit low percent reduction of biomass. However, seed count, seed mass, and 25-seed mass did not have a strong association between GMP and percent reduction. This suggests that genotypes which exhibit high GMPs across treatments are likely maintaining relatively high yields across treatments.

Early stem elongation rate predicts sunflower yield under water limitation

Under stress, early SELR under favorable conditions predicts absolute shoot mass, seed mass, and 25-seed mass, and GMP of plant and shoot biomass and 25-seed mass. Early SELR was not useful for determining percent reduction in any of these performance traits. These results in cultivated sunflower support Richards et al.’s strategy for improving water limitation resistance in wheat (2010). However, it should be noted that high SELR alone does not characterize all aspects of aboveground growth. For example, some genotypes could be growing taller at the expense of leaf area or robust stems. Indeed, when SEPR was assessed along with SELR in 2015, a strong negative association between SELR and SEPR was observed (Table 2.4A). Furthermore, scatterplot visualizations of the correlations between early vigor traits and biomass and/or yield traits for both R-stage and V-stage water limitation indicate that branching architecture may be driving some of these correlations (Figures S2.1-S2.4).

Faster phenology, as assessed by number of days to budding, is strongly correlated with biomass, with faster onset of reproduction leading to higher biomass (as assessed by GMP and absolute biomass under water limitation). However, number of days to budding does not strongly correlate with any seed yield metrics, suggesting that it does not provide any additional explanatory power for predicting performance under water limitation. However, our R-stage experimental design did not allow for successful drought escape via phenology because water limitation was imposed at budding for each genotype.

Broadly speaking, the suite of traits Richards et al. (2010) recommends selecting for to achieve drought resistance employ a drought avoidance strategy, whereby plants establish vigorous root systems to access deeper levels of ground water. However, in our study, deep rooting was made impossible by pots, and volumetric water content was held at a consistently low level for water limitation treatments via an automatic irrigation system. This irrigation regime worked on a feedback system whereby soils below the programmed moisture threshold triggered watering; thus, larger plants with larger transpiration demand were watered more often, and received larger absolute quantities of water compared to smaller plants in the water-limited treatments (though these plants were still subjected to lower soil moisture content than plants in the well-watered treatments). Furthermore, while root growth to deeper soil water was precluded, plants may have invested in root growth near each pot's irrigation source. In their 2017 study, Puertolas et al. compared the effects of a drought imposed using an automatic irrigation system to a more traditional dry-down/rewetting system and found that plants droughted with the automatic irrigation system had higher transpiration rates and stomatal conductance, and lower levels of ABA. Thus, the net plant water status in our water limitation treatment may have been similar to that of plants with deep root systems accessing groundwater below agricultural fields during climatic droughts, and the drought avoidance suite of traits may have been favored in these specific irrigation conditions.

Implications for breeding

The strong positive correlations between early SELR and seed mass imply that it may be possible to accelerate sunflower breeding by selecting for early SELR in favorable conditions, though more studies should be done in the field to examine the relationship between SELR and other early vigor traits as they relate to yield. These studies should also incorporate branching architecture into their experimental design to eliminate the potential for the presence/absence of branching to influence yield outcomes. We additionally suggest that automatic irrigation systems may represent an acceptable experimental environment for in-depth physiological studies of above-ground traits where drought avoidance is the preferred resistance strategy. Finally, we recommend genotype PI 597368 for additional research into drought-resistance at multiple ontogenetic stages, due to its high resistance to R-stage and V-stage water limitation.

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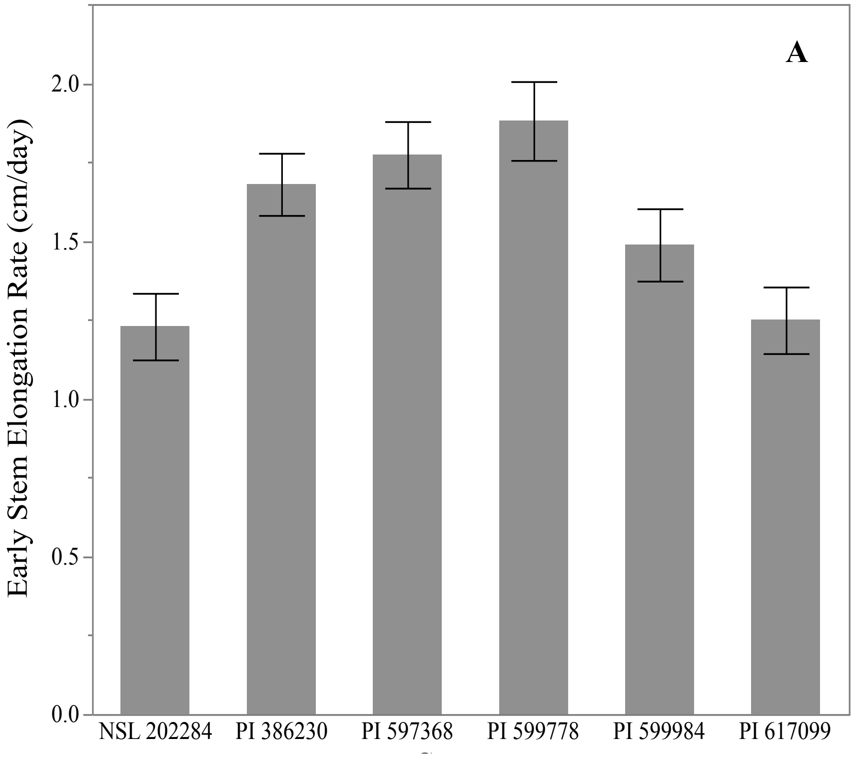
FIGURES



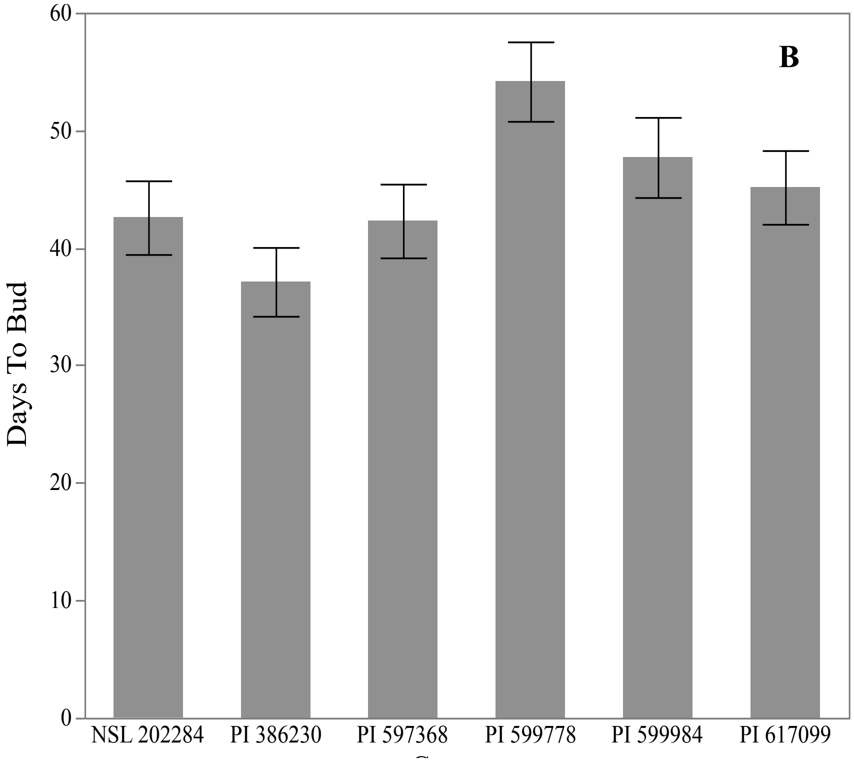
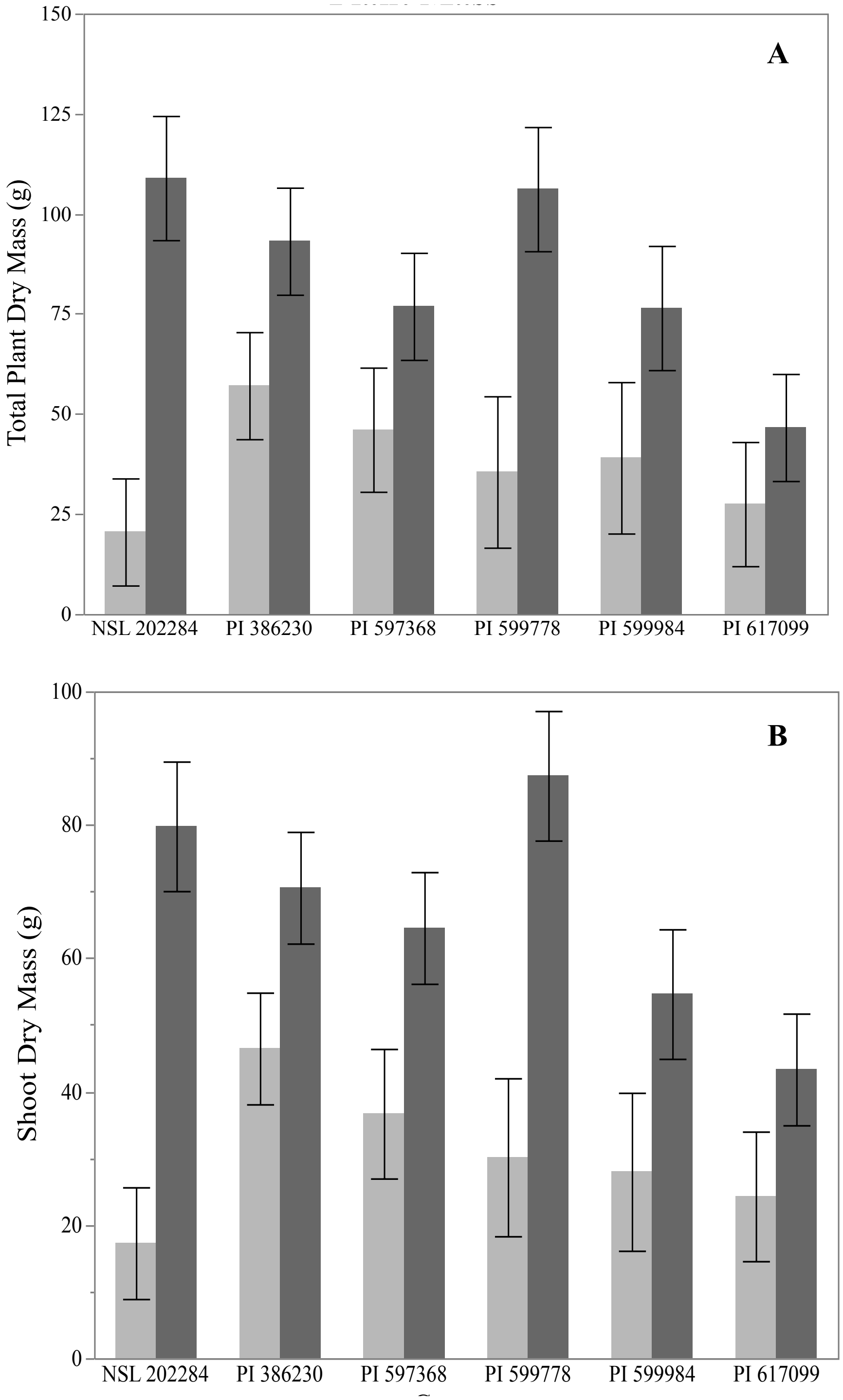
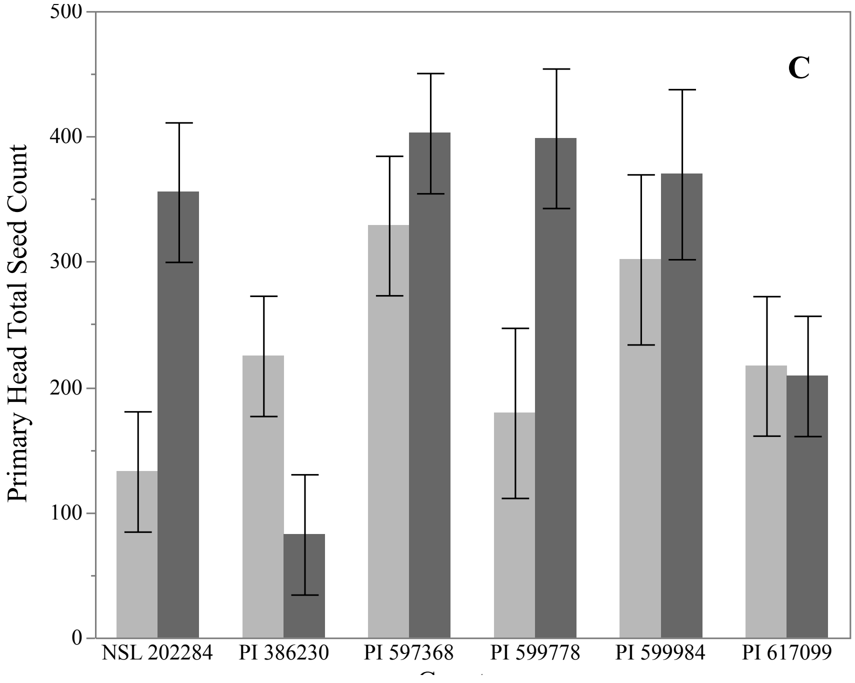
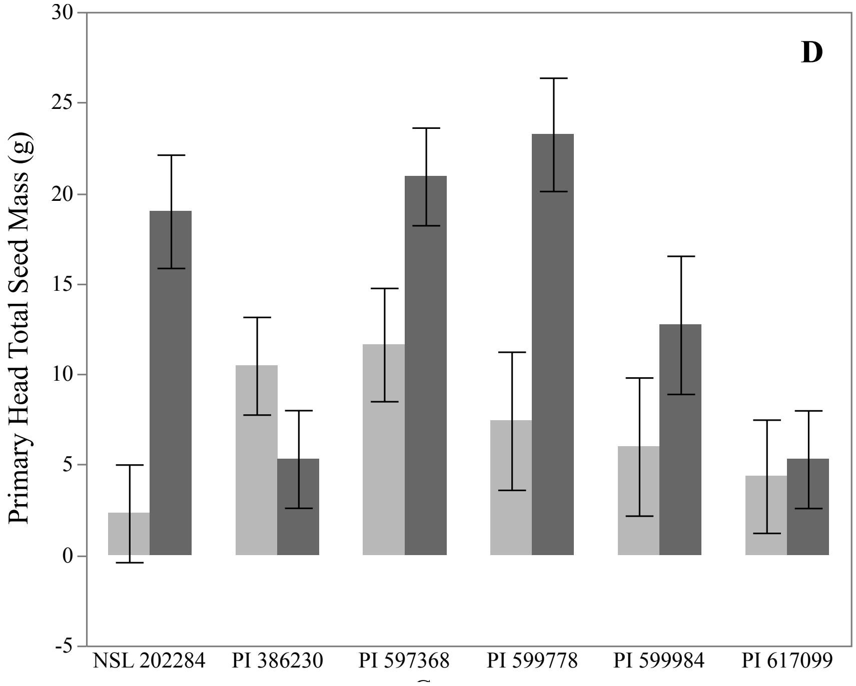


Figure 2.1. Genotype least-squares means for (A) early stem elongation rate and (B) days to bud for 2014 (R-stage) data. Error bars represent one standard deviation from mean.


A
Total Plant Dry Mass (g)
0
25
50
75
100
125
150
NSL 202284
PI 386230
PI 597368
PI 599778
PI 599984
PI 617099
B
Shoot Dry Mass (g)
0
20
40
60
80
100
NSL 202284
PI 386230
PI 597368
PI 599778
PI 599984
PI 617099


C
Primary Head Total Seed Count
0
100
200
300
400
500
NSL 202284
PI 386230
PI 597368
PI 599778
PI 599984
PI 617099


D
Primary Head Total Seed Mass (g)
-5
0
5
10
15
20
25
30
NSL 202284
PI 386230
PI 597368
PI 599778
PI 599984
PI 617099

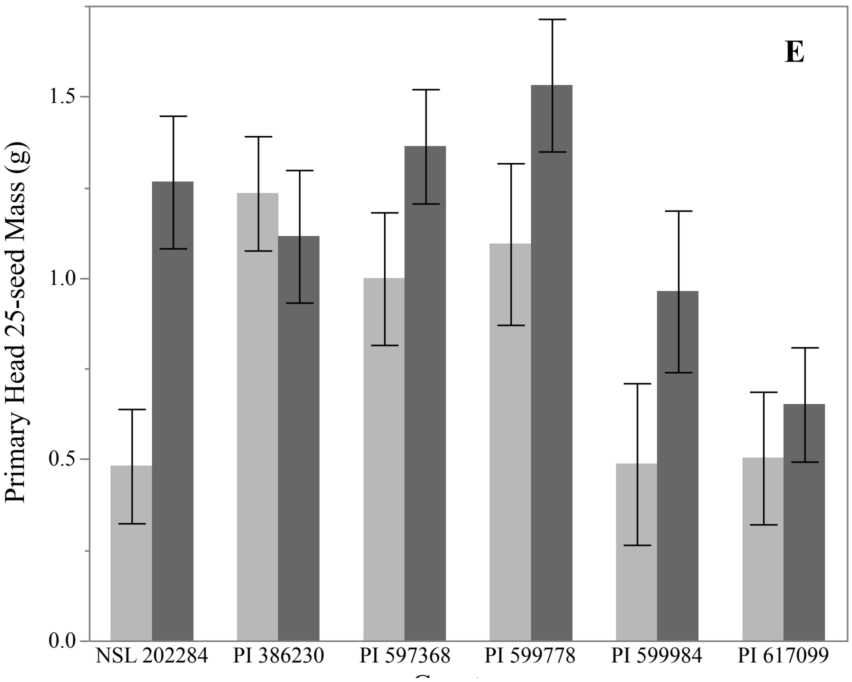
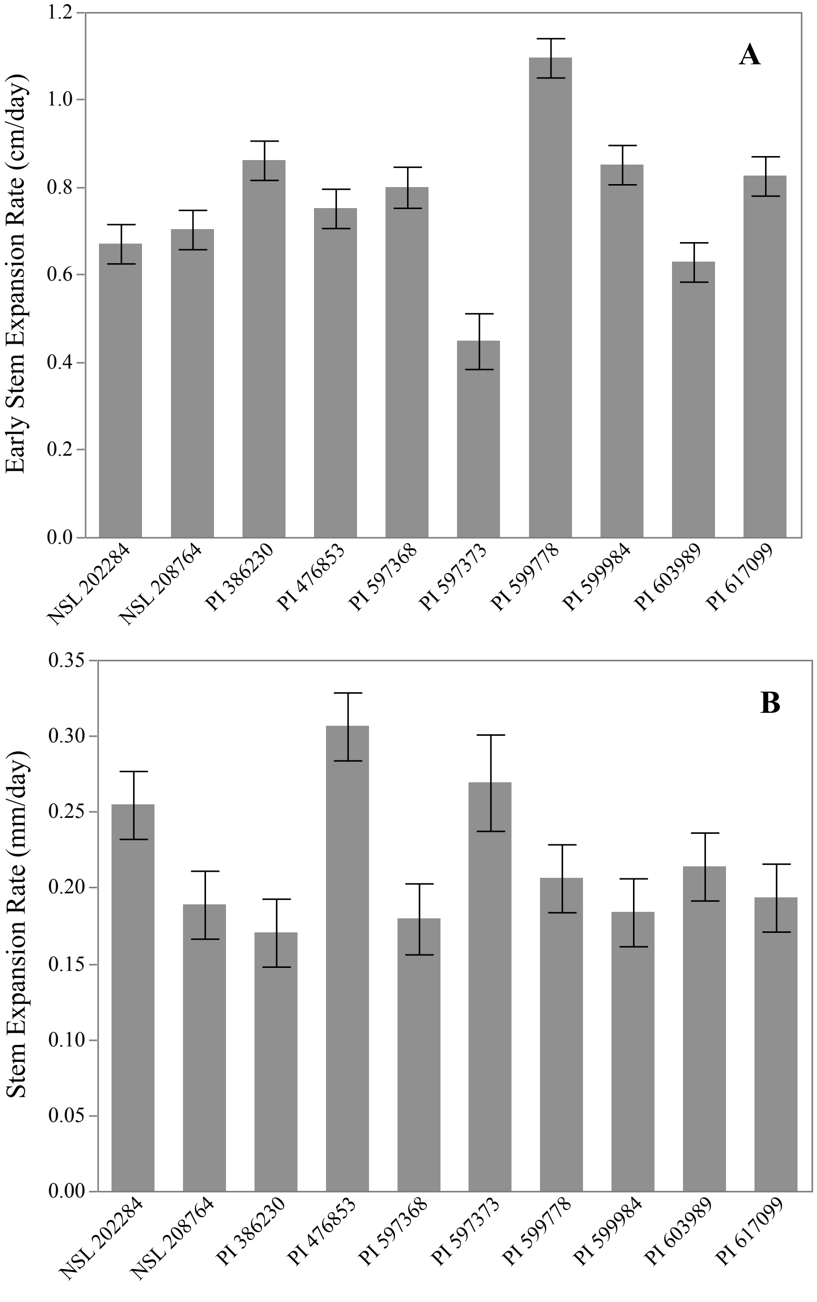


Figure 2.2: Genotype least-squares means for performance traits for 2014 (R-stage) data. Light gray signifies water-limited treatment. Dark gray signifies well-watered treatment. Error bars represent one standard deviation from the mean. Measured traits include (A) Plant biomass, (B) Shoot biomass, (C) Total seed count of the primary head, (D) Total seed mass of the primary head, and (E) 25-seed mass from the primary head.


A
Early Stem Expansion Rate (cm/day)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NSL 202284
NSL 208764
PI 386230
PI 476853
PI 597368
PI 597373
PI 599778
PI 599984
PI 603989
PI 617099
B
Stem Expansion Rate (mm/day)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
NSL 202284
NSL 208764
PI 386230
PI 476853
PI 597368
PI 597373
PI 599778
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PI 603989
PI 617099

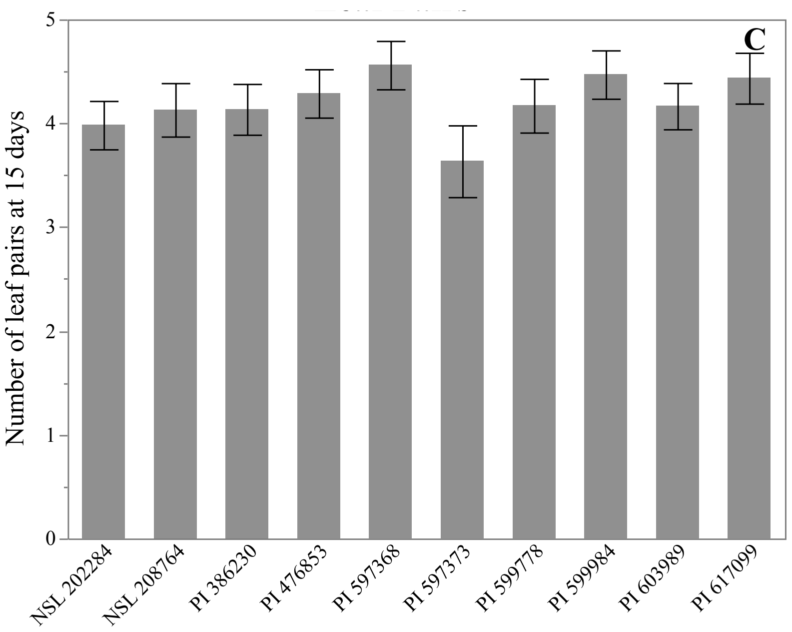


Figure 2.3: Genotype least-squares means for (A) early stem elongation rate, (B) early stem expansion rate, (C) early number of leaf pairs for 2015 (V-stage) data. Error bars represent one standard deviation from the mean.

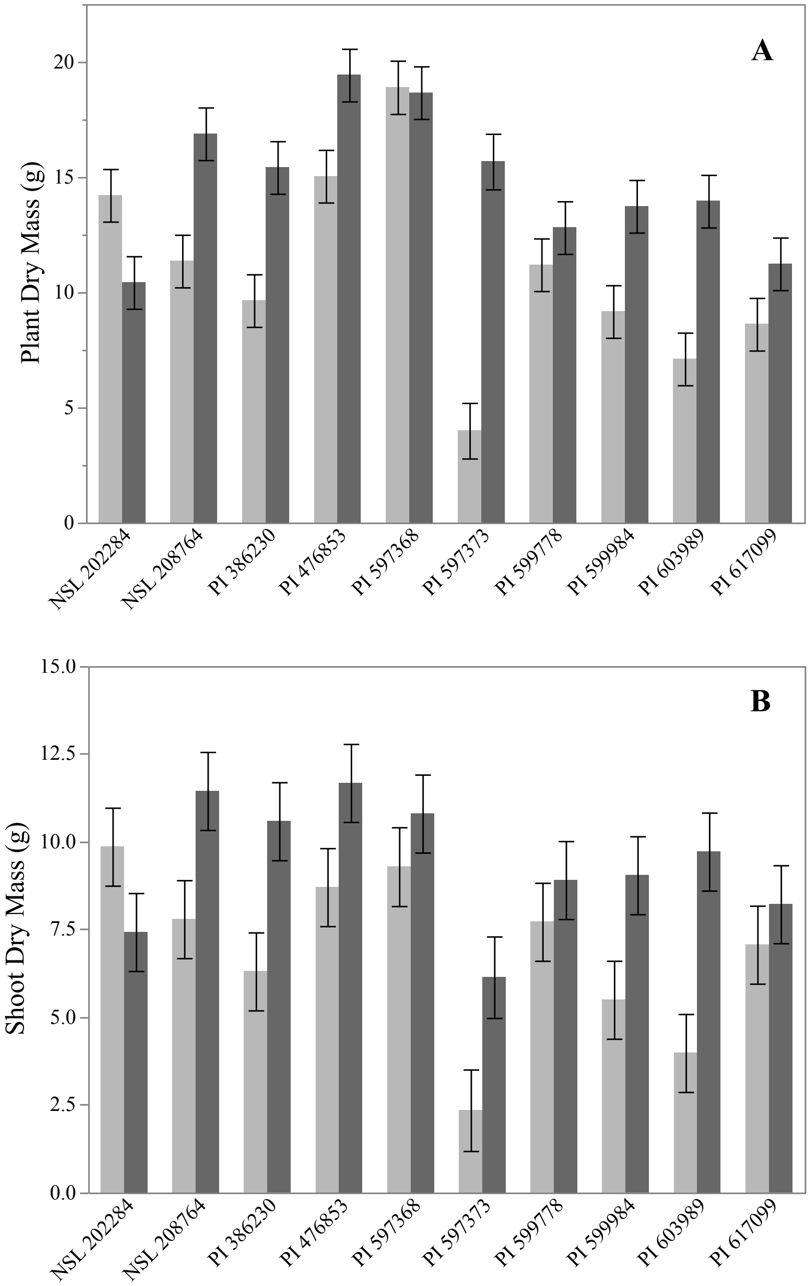
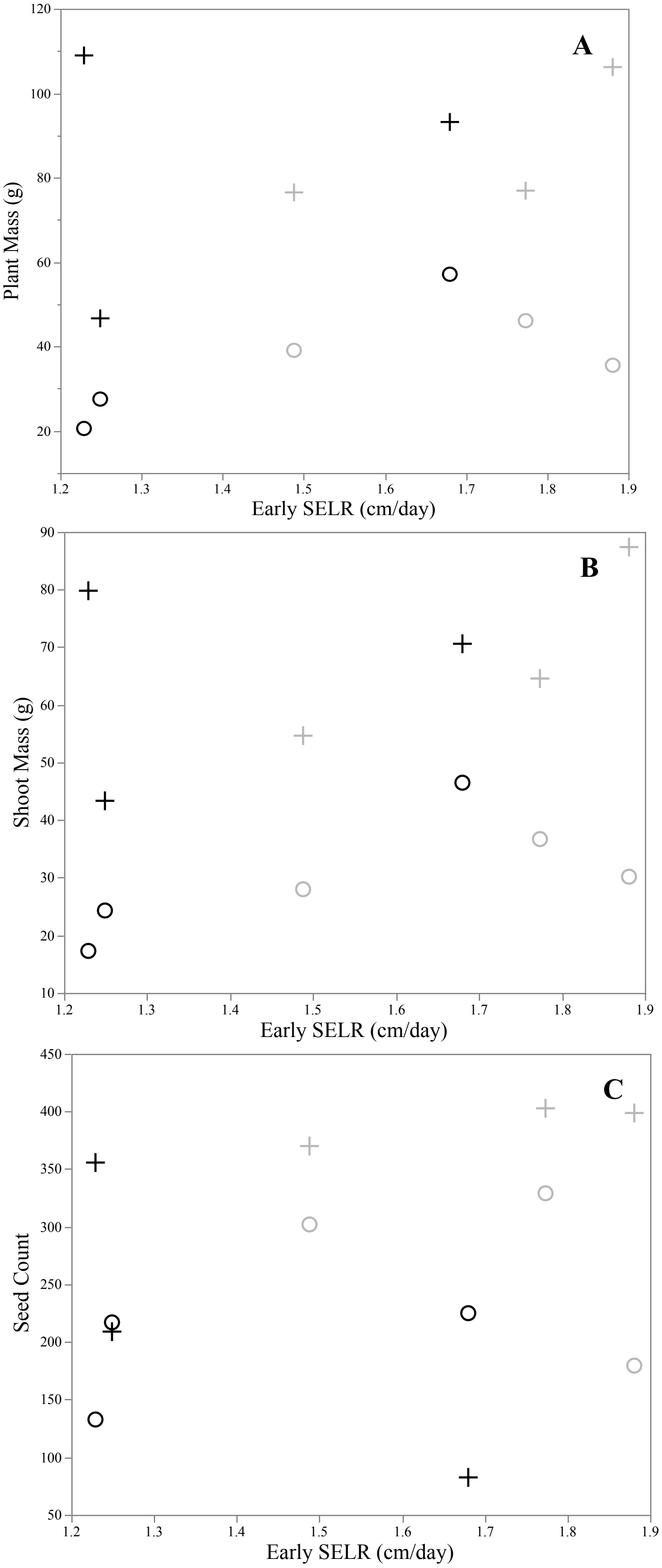


Figure 2.4: Genotype least-squares means for (A) plant and (B) shoot biomass for 2015 (V-stage) data. Light gray indicates water-limited treatment. Dark gray indicates well-watered treatment. Error bars represent one standard deviation from the mean.


A
Plant Mass (g)
120
100
80
60
40
20
Early SELR (cm/day)
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
B
Shoot Mass (g)
90
80
70
60
50
40
30
20
10
Early SELR (cm/day)
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
C
Seed Count
450
400
350
300
250
200
150
100
50
Early SELR (cm/day)
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9

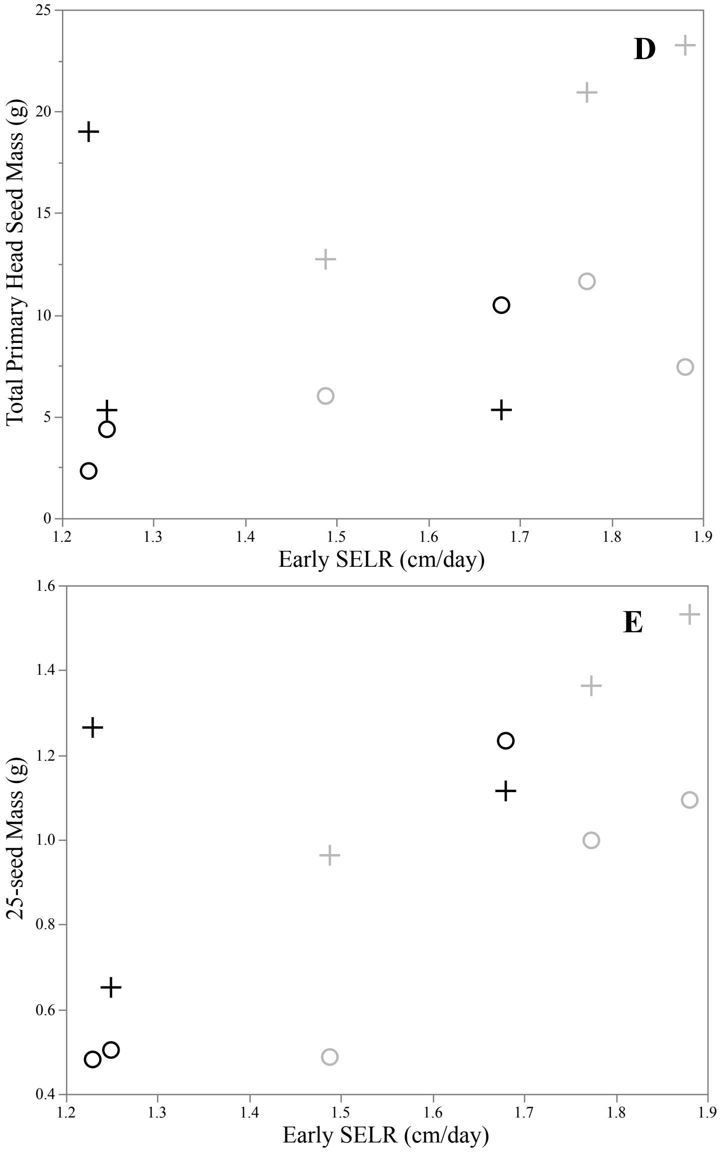
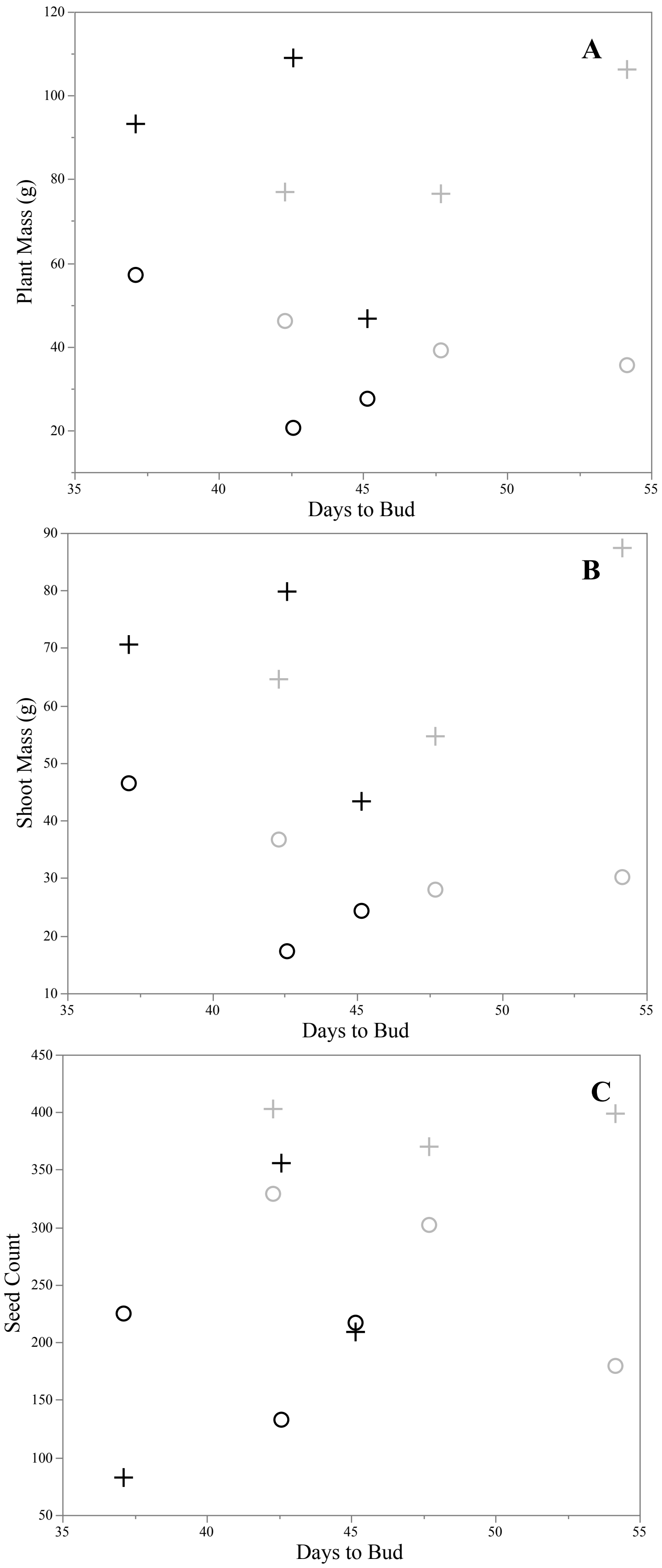


Figure S2.1: Scatterplot of R-stage least squares means of early stem elongation rate verse least squares means of (A) Plant mass, (B) Shoot mass, (C) Primary head total seed count, (D) Primary head total seed mass, and (E) Primary head 25-seed mass. Colors denote branching architecture (black indicated branching, gray indicates non-branching). Shapes denote treatment (open circles indicate water-limited treatment, crosses indicate well-watered treatment).


A
Plant Mass (g)
120
100
80
60
40
20
35
40
45
50
55
Days to Bud
B
Shoot Mass (g)
90
80
70
60
50
40
30
20
10
35
40
45
50
55
Days to Bud
C
Seed Count
450
400
350
300
250
200
150
100
50
35
40
45
50
55
Days to Bud

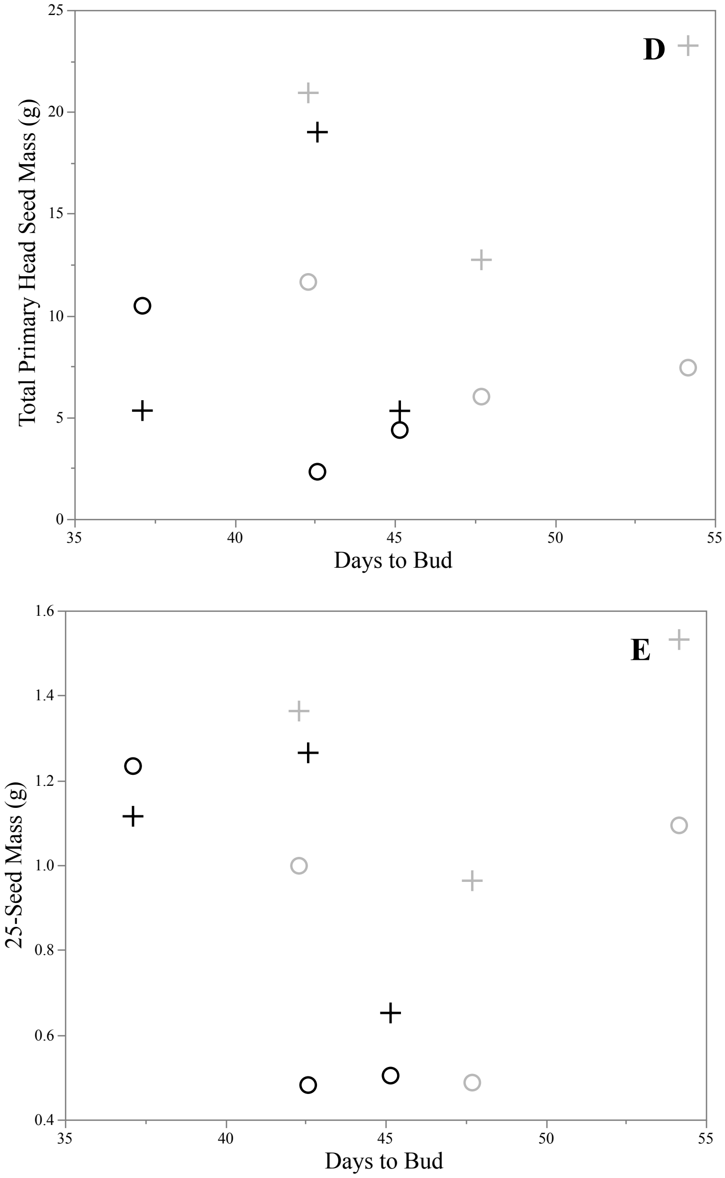


Figure S2.2: Scatterplot of R-stage least squares means of days to bud verse least squares means of (A) Plant mass, (B) Shoot mass, (C) Primary head total seed count, (D) Primary head total seed mass, and (E) Primary head 25-seed mass. Colors denote branching architecture (black indicated branching, gray indicates non-branching). Shapes denote treatment (open circles indicate water-limited treatment, crosses indicate well-watered treatment).

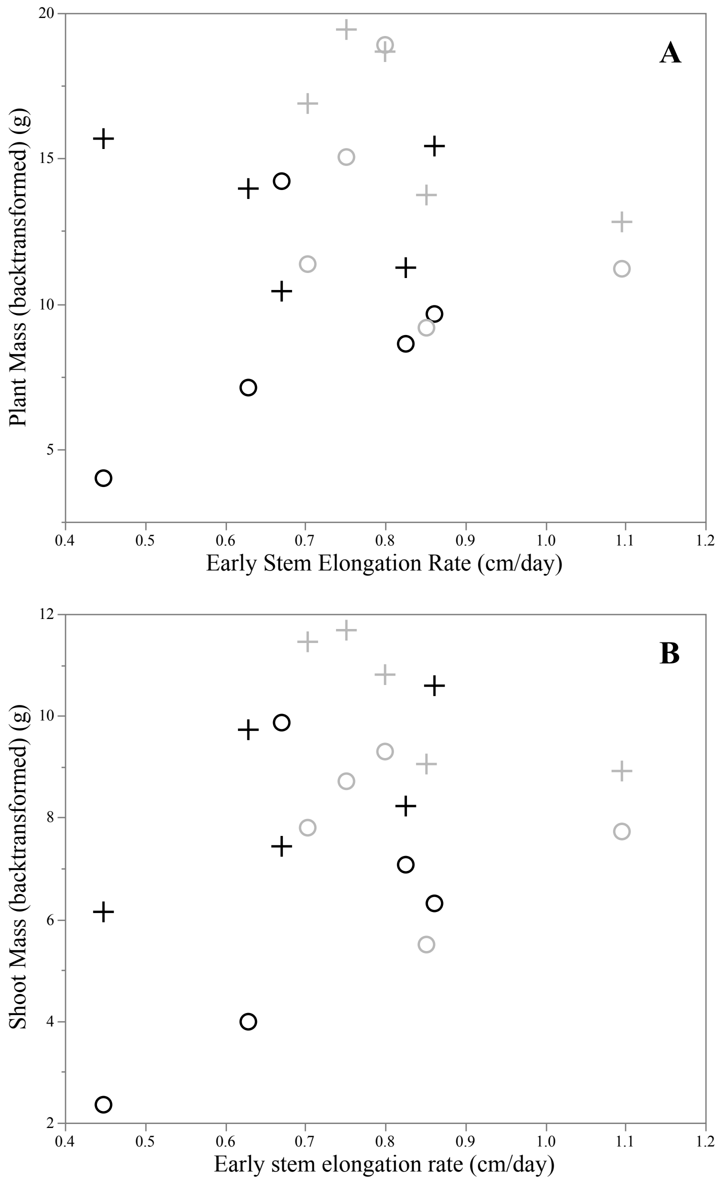


Figure S2.3: Scatterplot of V-stage least squares means of early stem elongation rate verse least squares means of (A) Plant mass, and (B) Shoot mass. Colors denote branching architecture (black indicated branching, gray indicates non-branching). Shapes denote treatment (open circles indicate water-limited treatment, crosses indicate well-watered treatment).

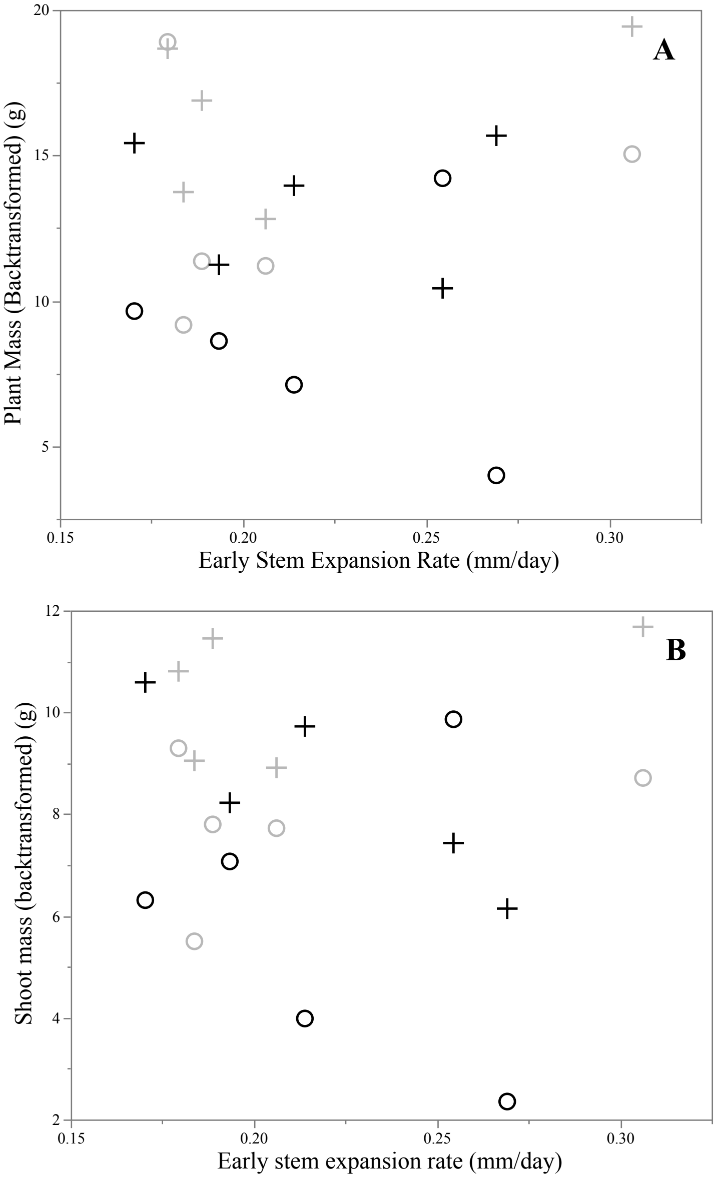


Figure S2.4: Scatterplot of V-stage least squares means of early stem expansion rate verse least squares means of (A) Plant mass, and (B) Shoot mass. Colors denote branching architecture (black indicated branching, gray indicates non-branching). Shapes denote treatment (open circles indicate water-limited treatment, crosses indicate well-watered treatment).

TABLES

Table 2.1: ANOVA tables for yield metrics under R-stage water limitation (Fall 2014) and V-stage water limitation (Summer 2015). Bold indicates significant p-values at $p \leq 0.05$.

		Genotype		Treatment		Genotype*Treatment	
		F	P	F	P	F	P
2014 (R-stage)	Total Plant Dry Mass	1.7130	0.1669	28.4683	**<0.0001**	1.5801	0.2007
	Shoot Dry Mass	2.1821	0.0871	42.9589	**<0.0001**	1.9262	0.1242
	Seed Count	4.5735	**0.0042**	4.9914	**0.0347**	3.5877	**0.0139**
	Total Seed Mass	4.2660	**0.0061**	16.5190	**0.0004**	4.2356	**0.0063**
	25-Seed Mass	5.0628	**0.0026**	10.5664	**0.0034**	1.5928	0.2001
2015 (V-Stage)	Total Plant Dry Mass	6.1486	**<0.0001**	34.0886	**<0.0001**	3.9448	**0.0003**
	Shoot Dry Biomass	9.8166	**<0.0001**	55.7722	**<0.0001**	5.27	**<0.0001**

Table 2.2: Spearman's ρ^2 with sign for correlations between percent reduction and geometric mean productivity for each trait. All correlations are significant at $p < 0.05$. "–" indicates no significance.

			GMP
Percent Reduction	2014 (R-stage)	Plant Mass	(-) 0.184
		Shoot Mass	(-) 0.184
		Seed Count	–
		Seed Mass	(+) 0.020
		25-Seed Mass	(-) 0.020
	2015 (V-Stage)	Plant Mass	(-) 0.381
		Shoot Mass	(-) 0.574

Table 2.3: Spearman's ρ^2 with sign for traits for the 2014 R-stage experiment. All ρ^2 are significant at $p < 0.05$. (A) Performance traits (biomass and seed traits) are measured under water-limited (below the diagonal line) or well-watered (above the diagonal line) conditions. (B) Performance traits are either GMP (below the diagonal line) or percent reduction (above the diagonal line). "–" indicates no significance.

(A)	Early SELR	Days to Bud	Plant Mass	Shoot Mass	Seed Count	Seed Mass	25-Seed Mass
Early SELR		–	–	–	–	–	–
Days to Bud	–		–	0.007	–	–	0.007
Plant Mass	–	(-) 0.236		0.889	–	–	–
Shoot Mass	0.595	(-) 0.184	0.889		–	–	–
Seed Count	–	(-) 0.099	0.595	–		–	–
Seed Mass	0.687	(-) 0.138	0.784	0.889	0.510		0.866
25-Seed Mass	0.595	(-) 0.066	0.510	0.784	–	0.595	
(B)	Early SER	Days to Bud	Plant Mass	Shoot Mass	Seed Count	Seed Mass	25-Seed Mass
Early SER		–	(-) 0.099	(-) 0.099	(-) 0.020	(-) 0.020	(-) 0.099
Days to Bud	0.020		–	–	–	–	–
Plant Mass	0.595	(-) 0.066		1.000	0.889	0.889	0.784
Shoot Mass	0.595	(-) 0.066	1.000		0.889	0.889	0.784
Seed Count	–	–	–	–		1.000	0.889
Seed Mass	–	–	–	–	–		0.889
25-Seed Mass	0.595	(-) 0.007	0.784	0.784	–	–	

Table 2.4: Spearman's ρ^2 with sign for traits measured in the 2015 V-stage experiment. All ρ^2 are significant at p 0.05. (A) Performance traits (biomass and seed traits) are measured under water limitation (below the diagonal line) or well-watered (above the diagonal line). (B) Performance traits are either GMP (below the diagonal line) or percent reduction (above the diagonal line). "–" indicates no significance.

(A)	Early SELR	Early SEPR	Early Leaf Pairs	Plant Mass	Shoot Mass
Early SELR		(-) 0.360	–	–	–
Early SEPR	(-) 0.360		–	–	–
Early Leaf Pairs	–	(-) 0.165		–	–
Plant Mass	–	–	–		–
Shoot Mass	–	–	–	0.860	

(B)	Early SELR	Early SEPR	Early Leaf Pairs	Plant Mass	Shoot Mass
Early SELR		–	–	–	–
Early SEPR	(-) 0.360		–	–	–
Early Leaf Pairs	–	–		–	–
Plant Mass	–	–	–		0.952
Shoot Mass	–	–	–	0.838	

Table S2.1: Percent reduction for each genotype. Negative values indicate the trait value increased under water limitation.

	2014					2015	
Genotype	Plant Mass	Shoot mass	Seed Count	Seed Mass	25-Seed Mass	Plant Mass	Shoot mass
NSL 202284	0.811	0.783	0.627	0.878	0.619	(-) 36.278	(-) 32.720
PI 386230	0.387	0.342	(-) 1.727	(-) 0.968	(-) 0.106	37.457	40.412
PI 597368	0.401	0.432	0.183	0.444	0.268	(-) 1.240	13.993
PI 599778	0.665	0.655	0.550	0.680	0.286	12.589	13.342
PI 599984	0.489	0.488	0.184	0.528	0.494	33.240	39.211
PI 617099	0.410	0.439	(-) 0.038	0.178	0.227	23.314	14.022
NSL 208764	NA					32.717	31.906
PI 476853						22.576	25.420
PI 597373						74.501	61.700
PI 603989						49.042	59.022

Table S2.2: Geometric mean productivity (GMP) for each genotype.

	2014					2015	
Genotype	Plant Mass	Shoot mass	Seed Count	Seed Mass	25-Seed Mass	Plant Mass	Shoot mass
NSL 202284	47.3278	37.1479	217.2960	6.6367	0.7801	12.1758	8.5613
PI 386230	72.9455	57.2840	136.2443	7.4645	1.1726	12.1963	8.1740
PI 597368	59.5442	48.6779	364.0238	15.6071	1.1664	18.7850	10.0220
PI 599778	61.4319	51.3469	267.5154	13.1424	1.2941	11.9795	8.2962
PI 599984	54.6720	39.1124	334.2753	8.7434	0.6848	11.2250	7.0554
PI 617099	35.8291	32.4589	212.9728	4.8150	0.5726	9.8421	7.6251
NSL 208764	NA					13.8499	9.4498
PI 476853						17.0968	10.0867
PI 597373						7.9164	3.8021
PI 603989						9.9647	6.2243

Table S2.3: List of genotypes

Genotype	Name	Type	Architecture
NSL 202284	HA 328	Non-oil	Branching
PI 386230	VIR 847	Non-oil	Branching
PI 597368	HA 404	Oil	Non-branching
PI 599778	HA 234	Oil	Non-branching
PI 599984	HA 821	Oil	Non-branching
PI 617099	RHA 426	Oil	Branching
NSL 208764	HA 316	Non-oil	Non-branching
PI 476853	Mammoth	Non-oil	Non-branching
PI 597373	RHA 396	Oil	Branching
PI 603989	RHA 408	Oil	Branching

CHAPTER 3

NITROGEN AND PHOSPHORUS USE EFFICIENCY IN CULTIVATED SUNFLOWER UNDER MULTI-NUTRIENT STRESS[1]

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[1] Rea AM, Bowsher AW, Donovan LA. To be submitted to *Journal of Agronomy and Crop Science*

ABSTRACT

Nitrogen and phosphorus strongly limit global crop production. Nutrient fertilizer is effective at relieving these limitations, but bears an economic and ecological cost. Improving crop nutrient use efficiency (i.e. production per fertilizer input) can improve crop production with less fertilization. Efforts to study the components of nitrogen and phosphorus use efficiency have generally used single-nutrient limitation experiments. However, the majority of global agricultural systems are subject to multiple concurrent nutrient stresses, which can influence both the magnitude of stress and the response relative to single-nutrient stresses. Building on previous work in cultivated sunflower by Bowsher et al. (2017), we here examine nitrogen and phosphorus use, acquisition, and utilization efficiency using a controlled, concurrent limitation of eleven nutrients across four fertilizer treatments.

Under multi-nutrient stress, we find that nitrogen and phosphorus acquisition and utilization efficiencies often display no relationship to use efficiency when literature on single-nutrient stresses predict a strongly positive relationship. We find that root use efficiency traits are generally independent from shoot use efficiency traits, suggesting that roots and shoots can be targeted separately in breeding programs. We further find that nitrogen use efficiency traits generally do not trade-off with phosphorus use efficiency traits, suggesting that crop improvement can increase nitrogen and phosphorus use efficiency simultaneously. Finally, we find that most green-leaf, biomass, and developmental traits do not associate with nutrient use efficiency components across treatments. However, we do suggest that improving fine root biomass may lead to increased shoot P use efficiency across variable nutrient supply. Our results highlight the

need for nutrient use efficiency studies to include multi-nutrient limitation scenarios to inform breeding efforts towards low-input sunflower varieties.

INTRODUCTION

Nitrogen (N) and phosphorus (P) are the two most limiting macronutrients plants require for survival, growth, and reproduction (Balemi & Negisho, 2012; Ha & lam-Son, 2014; Rose & Wissuwa, 2012). Plant nutrient limitation is often reduced via synthetic fertilizer application. However, fertilizer inputs are associated with a host of economic and ecological costs. For example, N fertilizer production is expensive, uses valuable fuel, and releases greenhouse gases as waste (Garnett, Conn, & Kaiser, 2009; *Sustainable Ammonia Synthesis*, 2016). P mining shares these costs, with the additional concern that P mining sources are depleting (Cordell & White, 2014; Lopez-Arrendondo, Leyva-Gonzalez, et al. 2014; Reijnders, 2014). Compounding these costs, most high-yielding cultivars developed in the last half-century were bred under conditions of supplemented nutrient availability (Gallais & Hirel, 2004; Kant, Bi, & Rothstein, 2011; Vandamme, Rose, et al., 2016; Wissuwa, Mazzola, & Picard, 2009), but often take up only a fraction of the fertilizer applied. For example, the global cereal crop takes up only 33% of N fertilizers applied due to a combination of factors such as low nutrient use efficiency by the plant, variable soil and climate factors, and poor fertilization management practices (Raun and Johnson 1999). To minimize costs associated with fertilization and maintain food security going forward, efforts to develop low-input crops are underway (Banziger, Betran, & Lafitte, 1997; Chen et al., 2016; Fan et al., 2016; Fang et l., 2013; Gallais & Coque, 2005; Hu et al., 2015; Presterl et al. 2002; Vear, 2016; Wissuwa et al., 2009). Current and future efforts to develop more nutrient efficient crops will require a better understanding of the traits that contribute to N and P fertilizer use efficiency (fUE; amount of dry matter (DM) produced per nutrients applied).

The overarching concept of fUE has been widely examined using different terminologies, calculations, and scales of study. These have been extensively reviewed by Fageria et al. (2008) and Good et al. (2004), with additional terms and calculations provided by others (e.g. Abbadi & Gerendas (2015), Moll et al. (1982)). Scales of study range from ecosystem-level to agricultural fields to individual plants (Reich et al., 2014), and include both realized (i.e., absolute) and potential (i.e., comparative) DM production and/or harvest yield. Our study focuses on fertilizer nutrient use efficiency (fUE), which we define as the realized amount of individual organ DM produced per unit applied fertilizer (Abbadi & Gerendas, 2015; Bayuelo-Jimenez & Ochoa-Cadavid, 2014; Cormier et al., 2016; Moll et al., 1982; Reich et al., 2014). Within fertilizer treatments, fUE is proportional to organ DM. fUE is the product of fertilizer acquisition efficiency and fertilizer utilization efficiency. We define fertilizer acquisition efficiency (fAqE), also known as "uptake efficiency", as the absolute amount of nutrients absorbed from the soil relative to the amount available in the soil. Within fertilizer treatments, fAqE is proportional to organ nutrient content. We define fertilizer utilization efficiency (fUtE), also known as "efficiency ratio" or "physiological efficiency", as the absolute organ DM the plant produces relative to the amount of nutrients within its tissues. (See Table 3.1 for a list of all nutrient use efficiency terms and calculations).

At different fertilization levels, fAqE or fUtE may have greater or lesser effect on fUE. However, the effect of nutrient supply on the relationship between fUE and its components has mostly been studied under single-nutrient limitation stresses (Table 3.2, Reich et al., 2014). In single-nutrient limitation studies, fAqE strongly (correlation coefficient ≥ 0.25, see Poorter et al., 2014) associates with fUE under both low and high nutrient supply (Table 3.2). fUtE strongly associates with fUE at high nutrient supply, and either strongly associates or has no relationship to fUE under low nutrient supply. However, relatively few studies have examined the relationships between fUE and its components under more complex soil nutrient conditions, such as low availability of multiple nutrients, which is common in agricultural settings (Dathe et al. 2013). This is a key point, because interactions (i.e. synergisms or antagonisms) between applied nutrients on crop performance are common (Rietra et al. 2017). Thus, understanding whether the relative contributions of fAqE and fUtE to fUE follow patterns established in single-nutrient stress studies could inform breeding strategies for complex soil nutrient conditions.

Future breeding efforts to improve crop fUE may find it advantageous to target root fUE, fAqE, and fUtE separately from shoot fUE, fAqE, and fUtE (Rose et al., 2011; Rose & Wissuwa, 2012; Vandamme et al., 2016). One of the few examples of studies that have analyzed roots and shoot separately is Rose et al.'s aquaponics study of phosphorus limitation in rice (2011). Rose et al. found evidence that shoot and root P-fUtE were genetically independent traits and concluded that they could be separately targeted for improvement. We here ask if root and shoot nutrient efficiency traits are physiologically independent, i.e., if roots and shoots are capable of labile responses to nutrient conditions. We therefore test whether root and shoot fUE, root and shoot fAqE, and root and shoot fUtE are strongly correlated within and across different fertilizer supply.

Efforts to breed low-fertilizer input crops may benefit from maximizing production under low fertilizer supply overall. However, studies of fUE components generally focus on single-nutrient limitation. We therefore ask if N-fAqE and P-fAqE are negatively correlated and if N-fUtE and P-fUtE are negatively correlated. We hypothesize that a trade-off between N and P fUE components may occur because root morphological responses to single-nutrient limitations of N or P are pronouncedly different. Low P leads to inhibition of primary root growth and promotion of lateral root growth and root hair growth (Ha & Lam-Son, 2014; Reich, Aghajanzadeh, & De Kok, 2014). Meanwhile, low N triggers deeper root growth with fewer lateral roots and less branching (Reich et al., 2014). Therefore, under soil conditions of low N and low P, it seems likely that fUE for one nutrient will correlate negatively with fUE for the other (Reich et al., 2014).

Plant traits contributing to fUE generally do so via associations with fAqE or fUtE (Bowsher et al., 2017; Lynch, 2007; Lynch, 2011). The relative influences of fAqE and fUtE on fUE are expected to shift with fertilizer supply (Table 3.2). It follows that understanding fUE physiology relies to some degree on elucidating how trait associations with fUE components change with fertilizer supply. As part of their 2017 study in cultivated sunflower, Bowsher et al. investigated the relationship between nutrient availability and metrics of nutrient stress resistance in cultivated sunflower. They found that shoot N-fUE (i.e. DM within each fertilizer level) did not associate with most green-leaf traits, but did associate with leaf dry matter content (LDMC). The association between this structural trait and shoot N-fUE was not due to any effect of leaf longevity, and they call for additional studies to address the relationship between LDMC and shoot N-fUE. We here ask what plant traits associate with fUE components across treatments, with particular emphasis on associations between LDMC and shoot N-fUE components.

We examined variation in root and shoot N- and P-fUE components in cultivated sunflower (*Helianthus annuus* L.). Cultivated sunflower is a globally important crop (Foreign Agricultural Service, 2016; Sheoran et al., 2016, FAOSTAT; http://faostat3.fao.org). The achenes are the dominant harvestable portion, with the rest of the shoot increasingly valued as feedmeal and roughage. Sunflower is native to North America and grown commercially throughout the world, with Russia, Ukraine, the European Union, and Argentina producing the majority of sunflower products (Foreign Agricultural Service, 2016; Heiser, 2001; Heiser, 1950). Due to its intensive root system and short-term drought-resistance relative to other crops, sunflower is often grown on fringe arable land (National Sunflower Association; *Limited Irrigation Farming*, 2001; Vear, 2016; FAOSTAT, http://faostat3.fao.org). As a result, sunflower potential as a low-input crop for fertilizers as well as water is being investigated (Škorić 2009). However, relatively few studies have assessed sunflower fUE under varying nutrient supply (Abbadi & Gerendas, 2015; Gerendas, Abbadi, & Sattelmacher, 2008; Massignam, Chapman, Hammer, & Fukai, 2009; Sheoran et al., 2016; Yuan et al., 2004), and none that we are aware of have studied sunflower fUE under multi-nutrient stress. As the majority of agricultural systems worldwide are subject to multiple nutrient stresses (Dathe et al., 2013), research on plant N- and P-fUE components under multiple macro- and micro-nutrient fertilizer levels is needed.

Our broad objective is to examine the relationship between N- and P- fUE, fAqE, and fUtE under multi-nutrient stress. Specifically, we ask: (1) Whether variation in fUE is more strongly associated with variation in fAqE or fUtE, and if this changes with fertilizer supply, (2) whether root and shoot fUE components (i.e. fAqE and fUtE) are associated, (3) whether N-fUE components trade-off with P-fUE components, and (4) what other plant traits associate with fAqE and fUtE.

METHODS

Plant selection and growth

Our study builds on a greenhouse study in which Bowsher et al. (2017) investigated the relationship between nutrient availability and metrics of nutrient stress resistance in cultivated sunflower. As part of that study, 12 diverse genotypes were grown under varying fertilization levels, which were imposed as general multi-nutrient limitations. Briefly, these included PI 597368 (oil, “HA 404”), PI 597373 (oil, “RHA 396”), PI 599783 (non-oil, “HA 314”), PI 386230ao (non-oil, “VIR 847”), PI 476853 (non-oil, “Mammoth”), PI 599778 (oil, “HA 234”), PI 599984 (oil, “HA 821”), PI 603989 (oil, “RHA 408”), PI 617099 (oil, “RHA 426”), NSL 202284 (non-oil, “RHA 328”), NSL 208764 (non-oil, “HA 316”), SF 230 (INRA HA, “SF 230”). They calculated shoot N-fUE (analyzed as shoot biomass within fertilizer treatments), and examined relationships between shoot N-fUE and other plant traits. For our study, we used plants from Bowsher et al. (2017) to examine the components of fUE physiology for N and P, and for roots and shoots, across varying nutrient supply.

We assessed root and shoot N and P concentration of a subset of treatments from Bowsher et al. to determine root, shoot, and whole-plant N and P fUE, fAqE, and fUtE. In that study, 12 diverse genotypes were grown at eight different fertilizer levels, harvested at the R-2 budding stage (when the bud is 0.5 cm above the nearest leaf pair), and measured for green-leaf, allocation, growth, and developmental traits (Bowsher et al., 2017). To maximize diversity of treatment response, we selected all budding-stage replicate plants grown at 4g, 8g, 12g, and 20g of Osmocote Plus time-release fertilizer for further investigation in this study. Each gram of Osmocote Plus contains 15% N, 9%P, and 12% potassium, with magnesium (1.3%), sulfur (6%), boron (.02%), copper (0.05%), iron (0.46%), manganese (0.06%), molybdenum (0.02%), and zinc (0.05%). Supplemental calcium was added as limestone. Osmocote and supplemental calcium were evenly mixed with the substrate.

Determination of fUE, fAqE, and fUtE

For this study, we re-dried samples in a forced-air furnace at 60°C for twenty-four hours. We ground root and shoot tissue separately using a Wiley-Thomas mill for coarse-level grinding and a ball mill for fine grinding. Tissue was analyzed for N concentration using LECO Combustion Analysis at Midwest Laboratories (13611 B Street, Omaha, Nebraska 68144-3693, USA). Tissue was analyzed for P concentration in the Donovan Lab (UGA, Athens, GA 30605, USA) using acid-molybdate spectrophotometry on ashed tissue powder (Varvel et al., 1976).

As part of the Bowsher et al. (2017) study, the MRFEL was harvested separately for each plant and analyzed for N. This left insufficient tissue for MRFEL P analysis. Ideally, shoot fUE, fAqE, and fUtE measurements would include MRFEL nutrient content in their calculations. However, analyses for N-fUE components with and without MRFEL N show no significant differences. For consistency across N and P, we here present analyses of nutrient use efficiency traits without the MRFEL. In addition to the

MRFEL, the plants used in our study had an average of 14.4 (± 3.2 SD) additional fully expanded leaves (minimum of 9, maximum of 32).

We multiplied organ nutrient (N or P) concentration by organ DM to obtain organ N and P content. We calculated fUE as the organ DM (g) relative to the nutrient fertilizer applied (g) (Bayuelo-Jimenez & Ochoa-Cadavid, 2014; Gourley, Allan, & Russelle, 1994; Moll et al., 1982; Sattelmacher, Horst, & Becker, 1994). We calculate fAqE as the nutrient (N or P) content of the plant tissue (g) relative to the nutrient content applied as fertilizer (g) (Abbadi & Gerendas, 2015; Bayuelo-Jimenez & Ochoa-Cadavid, 2014; Cormier et al., 2016; Moll et al., 1982; Sattelmacher et al., 1994). We calculate fUtE as the organ DM (g) relative to the tissue nutrient (N or P) content (g) (Abbadi & Gerendas, 2015; Coltman, Gerloff, & Gabelman, 1985; Rose et al., 2011; Rose & Wissuwa, 2012; Santa-Maria, Moriconi, & Oliferuk, 2015).

Statistical Analysis

We performed least-squares ANOVA using block, genotype, treatment, and genotype*treatment interaction for all traits using the lsmeans package in R (Lenth, 2016). Data was tested for normality of the residuals using the Shapiro-Wilk Goodness-of-fit test in R. For those trait residuals which were not normal at $p \leq 0.05$, we transformed to better approach normality. Tukey-adjusted lsmeans were calculated using the lsmeans package in R (Lenth, 2016; Mangiafico, 2016). We used these lsmeans and trait lsmeans from Table S2 of Bowsher et al. (2017) in Spearman's rank correlation tests to account for block-effects and missing data. These traits related to traits contributing to size, structure, and shape of the most recent fully expanded leaf, organ biomass and allocation, and plant developmental rate and size. We used Spearman’s rank correlations to determine the proportion of shared variance between ranked traits (ρ^2). Each Spearman’s test was performed using the Hmisc package in R (Harrell, 2017) within treatments. For our data, Spearman’s ρ was preferable to Pearson’s r because the XY plots rarely exhibited bivariate normality, thus failing an assumption of Pearson’s and making a non-parametric Spearman's tests more appropriate.

RESULTS

Root and shoot fUE, fAqE, and fUtE across treatments and genotypes

In general, shoot and root N-fUE and P-fUE decease as fertilizer level increases. Least squares means for shoot N- fUE range from 1.497 g DM g^{-1} soil P (PI 386230ao, 20g treatment) to 14.282 g DM g^{-1} soil P (PI 599783, 4g treatment), and for root N- fUE range from 0.611 g DM g^{-1} soil P (PI 386230ao, 20g treatment) to 3.623 g DM g^{-1} soil P (PI 599783, 4g treatment) (Table 3.3A). Least squares means for shoot P- fUE range from 2.495 g DM g^{-1} soil P (PI 386230ao, 20g treatment) to 23.782 g DM g^{-1} soil P (PI 599783, 4g treatment), and for root P- fUE range from 1.016 g DM g^{-1} soil P (PI 386230ao, 20g treatment) to 6.036 g DM g^{-1} soil P (PI 599783, 4g treatment) (Table 3.3B).

Shoot and root N-fAqE and P-fAqE generally decrease as fertilizer level increases. Least squares means for shoot N-fAqE range from 0.046 g tissue P g^{-1} soil P (NSL 208764, 8g treatment) to 0.172 g tissue P g^{-1} soil P (PI 599783, 4g treatment), and for root N-fAqE range from 0.011 g tissue P g^{-1} soil P (PI 386230ao, 20g treatment) 0.047 g tissue P g^{-1} soil P (PI 599783, 4g treatment) (Table 3.3A). Least squares means for shoot P-fAqE range from 1.83E^{-06}_ g tissue P g^{-1} soil P (PI 386230ao, 20g treatment) to 1.1E^{-05} g tissue P g^{-1} soil P (PI 599783, 4g treatment), and for root P-fAqE range from 4.93E^{-07} g tissue P g^{-1} soil P (PI 386230ao, 20g treatment) to 3.64E^{-06} g tissue P g^{-1} soil P (PI 603989, 4g treatment) (Table 3.3B).

Shoot and root N-fUtE decreases as fertilizer level increases. Least squares means for shoot N- fUtE range from 28.196 g DM g^{-1} tissue P (PI 386230ao, 20g treatment) to 104.895 g DM g^{-1} tissue P (NSL 208764, 8g treatment), and for root N- fUtE range from 47.467 g DM g^{-1} tissue P (PI 599778, 20g treatment) to 109.893 g DM g^{-1} tissue P (PI 476853, 4g treatment) (Table 3.3A). However, shoot and root P-fUtE generally increases with treatment. Least squares means for shoot P- fUtE range from 1,365,190 g DM g^{-1} tissue P (PI 386230ao, 8g treatment) to 2,633,146 g DM g^{-1} tissue P (PI 476853, 12g treatment), and for root P- fUtE range from 1,130,458 g DM g^{-1} tissue P (PI 603989, 4g treatment) to 3,055,764 g DM g^{-1} tissue P (PI 386230ao, 12g treatment) (Table 3.3B).

Associations of fAqE and fUtE with fUE at different nutrient supplies

In the highest fertilizer treatment, genotype rankings for fAqE are strongly ($\rho^2 \geq$ 0.25; i.e., over 25% of the variance explained by the relationship) positively associated with fUE for both organs and nutrients (Table 3.4). Shoot N-fUtE is only associated with shoot N-fUE at the 12g fertilizer supply, while shoot P-fUtE is strongly associated with shoot P-fUE at the 4g and 12g fertilizer treatments. For roots, N-fAqE is strongly positively associated with N-fUE at the 4g, 8g, and 20g fertilizer treatments, but root P-fAqE is not correlated with root P-fUE at any fertilizer treatments except the highest.

Root N-fUtE is associated with root N-fUE at only the 12g fertilizer treatment. Root P-fUtE strongly correlates with root P-fUE at the 4g and 12g fertilizer treatments.

For both nutrients, shoot and root fAqE, fUtE, fUE are strongly correlated, but only at high nutrient supply

At the low and intermediate fertilizer supplies, there were no significant associations between shoot and root N- and P-fAqE, -fUtE or fUE. At the highest fertilizer supply, genotype rankings for shoot fAqE are associated with higher root fAqE for both N and P (Table 3.5). Likewise, genotypes with higher shoot fUE also have higher root fUE for both N and P. For UtE, however, genotypes with higher shoot fUE also have higher root fUE for P but not N.

N- and P- fUE components are positively associated

More N in shoots (or roots) is generally associated with more P in shoots (or roots), regardless of nutrient supply (Table 3.6). For shoots, genotype rankings for N-fAqE and P-fAqE are strongly, positively associated for three of the four fertilizer treatments. Shoot N-fUtE positively correlates with shoot P-fUtE at all treatments. For roots, root N-fAqE is strongly positively associated with root P-fAqE at all but the lowest fertilizer treatment. Root N-fUtE is strongly positively associated with root P-fUtE at the 12g and 20g treatments.

LDMC and biomass traits consistently relate to fUE, fAqE, and fUtE across fertilizer supplies

Genotype rankings for every fUE component except root N-fUtE strongly associate with numerous green-leaf and biomass traits assessed for plants grown under the lowest fertilizer (Tables S3.1-S3.4). With the exception of MRFEL thickness, MRFEL fresh mass, and leaf area ratio, all strong associations are positive. However, of all the strong associations between traits assessed at the 4g fertilizer treatment and fUE components, only height, stem mass ratio, MRFEL LDMC, leaf area ratio, and many organ dry masses remain strong at higher levels of fertilization.

DISCUSSION

Impact of fAqE and fUtE on fUE at different nutrient supplies

Given the common occurrence of multi-nutrient scarcity in global agricultural systems, establishing if the relative contributions of fAqE and fUtE to fUE follow similar patterns under single- and multi-nutrient stress could inform breeding strategies for complex soil nutrient conditions. Our results suggest that changes in multi-nutrient supply impacts the associations between fUE and its components differently than changes in single-nutrient supply. For example, we find that shoot fAqE associates with fUE at the highest fertilizer supply, but not at the lower supply levels. This finding contradicts the growing body of single-nutrient limitation studies that suggests that fAqE and fUE are coupled at both high and low fertilizer supply (Table 3.2) (Erley et al., 2011; Manske et al., 2001; Mundim, Viana, & Maia, 2013; Presterl, Groh, et al., 2002; Sandana, 2016).

Shoot fUtE also associates differently with shoot fUE than in single-nutrient limitation studies. For example, we find that shoot N-fUtE does not associate with shoot N-fUE at the high (20g) or low (4g) treatment. These results differ greatly from those for winter oilseed rape where there is a strong association at high N supply, and for maize where there is a strong association at low and/or high N supply depending on whether inbred lines were developed under low or high N conditions (Erley et al., 2011, Preserl, Groh, et al., 2002). Additionally, we find that P-fUtE only strongly associates with P-fUE for shoot P at the lowest fertilizer supply. These results for P differ from wheat and potato, for which P-fUtE associates strongly with P-fUE at high P supply but not at low P supply (Sandana, 2016; Manske et al., 2001. They also differ from popcorn, which shows strong associations at both high and low P supply (Mundim et al., 2013).

These contrasts to the single-nutrient limitation literature may be the result of micronutrient supply changing N or P availability. Indeed, interactions between both macro and micronutrients on crop performance are common, where the effect of application of one nutrient depends on the application of another nutrient (reviewed by Rietra et al. 2017). Our results do not allow us to distinguish which nutrient(s) are limiting, but they suggest that the multi-nutrient fertilizer treatments lead to more complex associations between fUE and its components than typically observed under single-nutrient stresses. These findings highlight the need for more studies to determine the impact of agriculturally common multi-nutrient stresses on fUE, fAqE, and fUtE (Danthe et al., 2013).

Roots and shoot nutrient efficiencies are unrelated under nutrient stress

We found no relationship between root and shoot fUE, root and shoot fAqE, or root and shoot fUtE at low and intermediate nutrient supplies. Strong correlations between root and shoot biomass (i.e. fUE) at high nutrient supply, but not at lower nutrient supplies, indicates that root mass ratio shifts as nutrient availability drops, but in a genotype-dependent manner. Similarly, more nutrients in the root (i.e., higher fAqE) is associated with more shoot nutrients at high nutrient supply, but not at lower nutrient supplies. This indicates that nutrient allocation shifts with nutrient availability in a genotype-dependent manner. Finally, more efficient utilization of root P (i.e. higher fUtE) is associated with more efficient shoot P utilization at high nutrient supply, but not at lower nutrient supplies. Altogether, these results indicate genotype-dependent allocational shifts in biomass and nutrients across organs, as well as genotype-dependent shifts between P use and P storage within the organs, as nutrient availability declines. Overall, this genotypic variability in the response to decreasing nutrient supply suggests that roots and shoots can be targeted separately for breeding improved fAqE, fUtE, and fUE at low or intermediate nutrient levels.

The unrelated responses of root and shoot nutrient efficiency traits at lower nutrient supply may be due to their different growth responses to nutrient stress. Roots decrease their biomass less than shoots under nutrient stress, leading to an increased root:shoot ratio compared to optimal nutrient conditions. It is unclear whether this pattern is an adaptive response to nutrient stress or simply due to allometric shifts. Regardless, the increased root:shoot ratio, coupled with the overall lower nutrient concentration of roots relative to shoots (Rose & Wissuwa, 2012), leads to an overall higher measure of N-fUE in roots relative to shoots.

No evidence of a trade-off between N and P for fUE components

Genotypes that rank highly for N-fAqE also rank highly for P-fAqE, and genotypes that rank highly for N-fUtE also rank highly for P-fUtE. This positive association may be due to the multi-nutrient stress our plants were subjected to. The multiple limitation hypothesis postulates that plants avoid investing in growth that will not yield a fitness benefit. Instead, they adjust their allocation so growth is equally limited by all resources (Bloom, Chapin, & Mooney, 1985; Chapin, Bloom, Field, & Waring, 1987). While our experiment was not designed to test which nutrients are actually limiting or co-limiting, positive associations between N- and P-fUE components indicate these sunflowers may respond to multi-nutrient stress by constantly shifting their uptake and use to bring all resources into balance. For example, as plants acquire sufficient N, P may then become limiting for growth, causing plants to re-allocate resources to increase P-AqE. Thus, high N-AqE may lead to high P-AqE, and vice versa. Similarly, high N-UtE may be related to high P-UtE (and vice versa) because of the metabolic costs of uptake and storage of excess N or P. For example, high N-UtE but low P-UtE suggests uptake and storage of P that is not being used for growth, so high N-UtE in absence of high P-UtE may be energetically wasteful. In addition, the pot environment may limit the effect of differing root morphologies by capping the volume of soil available for exploration and the fertilizer method of slow-release Osmocote may have resulted in root mining for both N and P from the same pellet. While additional studies conducted in the field are needed to confirm if high N-fAqE genotypes also rank highly for P-fAqE, and likewise for fUtE, our data suggests that N -and P-fUE components can be improved simultaneously.

Traits associated with fAqE and fUtE at low fertilizer supply often lose their association as fertilization increases

While many traits measured in the MRFEL leaf associate with fAqE or fUtE in the lowest fertilizer treatment, almost none of these trait correlations with fAqE or fUtE are strong under higher fertilizer supplies. Often, these traits display no correlation with fUE components as fertilizer supply increases. The one exception was LDMC which strongly positively correlated to shoot N-fUtE and P-fUtE in the 4g, 8g, and 12g treatments. Bowsher et al. (2017) noted the association between shoot biomass (i.e. fUE) and LDMC. Our findings suggest that LDMC associates with fUE via fUtE, but not fAqE, across treatments.

Many organ dry masses are highly associated with N- and P-fUE components regardless of fertilization level. This finding is not surprising, since the fUE of a given organ is calculated from the biomass of that organ. However, fine root dry mass and total root dry mass associate with shoot P-fAqE and shoot P-fUtE across treatments. These relationships are likely not due to autocorrelations and instead may be the consequence of increased root growth leading to increased P mining. Thus, shoot P-fUE may be improved by targeted breeding to increase root and especially fine root biomass under both low and high nutrient supplies.

Overall, it appears that plant green-leaf and biomass traits contribute differently to fAqE and fUtE across treatments. Often, these traits show a strong relationship with fAqE or fUtE at one fertilizer level but no relationship at the next. For example, fine root fraction correlates strongly and positively with root P-fAqE at the 4g treatment, weakly and negatively at the 8g treatment, not at all at the 12g treatment, and very weakly (and positively) at the 20g treatment. While these patterns remain difficult to interpret, they underscore findings by Bowsher et al. that the specific traits contributing to fUE (or in our case, direct components of fUE) are nuanced and conditional, and that these relationships are extremely sensitive to changes in multi-nutrient fertilizer supply.

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TABLES

Table 3.1: Full terms and calculations for each nutrient use efficiency trait.

Abbreviation	Full term	Calculation
N-fUE	Nitrogen fertilizer use efficiency	g biomass ÷ g N fertilizer
N-fAqE	Nitrogen fertilizer acquisition efficiency	g tissue N ÷ g nitrogen N
N-fUtE	Nitrogen fertilizer utilization efficiency	g biomass ÷ g tissue N
P-fUE	Phosphorus fertilizer use efficiency	g biomass ÷ g P fertilizer
P-fAqE	Phosphorus fertilizer acquisition efficiency	g tissue P ÷ g P fertilizer
P-fUtE	Phosphorus fertilizer utilization efficiency	g biomass ÷ g tissue P

Table 3.2: Correlation coefficients between acquisition efficiency, utilization efficiency, and use efficiency in other crop species. n.s. = not significant.

Correlation between UE and UtE	Correlation between UE and AqE	Correlation calculation	AqE calcuation	UtE calcuation	Nutrient	Species	Source
High N: 0.68 Low N: n.s.	High N: 0.65 Low N: 0.78	Simple linear coefficient	Shoot N ÷ N supply	kg seed yield ÷ kg shoot N	N	Winter oilseed rape	(Erley et al., 2011)
High N: 0.59 Low N: 0.67	High N: 0.62 Low N: 0.65	Estimated coefficients of phenotypic correlation	Total N in grain and stover (separately for each treatment)	Total N in grain and stover ÷ aboveground N yield	N	Maize lines (inbred under low N)	(Presterl, Groh, et al., 2002)
High N: n.s. Low N: .86	High N: 0.50 Low N: 0.85	Estimated coefficients of phenotypic correlation	Total N in grain and stover (separately for each treatment)	Total N in grain and stover ÷ aboveground N yield	N	Maize lines (inbred under high N)	(Presterl, Groh, et al., 2002)
High P: 0.60 Low P: n.s.	High P: 0.67 Low P: 0.84-0.92	Pearson correlation coefficients	Shoot P ÷ P available	Grain production ÷ shoot P	P	Wheat	(Manske et al., 2001)
High P: 0.49 Low P: 0.45	High P: 0.95 Low P: 0.97	Pearson's correlation coefficient	Shoot P ÷ P applied	Shoot dry weight ÷ plant P content	P	Popcorn	(Mundim et al., 2013)
High P: 0.29 Low P: n.s.	High P: 0.77 Low P: 0.92	Pearson's correlation coefficient	Plant P content ÷ P supply	Tuber yield ÷ plant P content	P	Potato	(Sandana, 2016)

Table 3.3A: Least-squares means of nitrogen nutrient use efficiency traits for roots and shoots.

		Shoot			Root		
Genotype	Osmocote	N-fAqE	N-fUtE	N-fUE	N-fAqE	N-fUtE	N-fUE
NSL 202284	4g	0.09911404	61.2402481	6.11788882	0.02843333	96.2288041	2.55376413
NSL 208764	4g	0.09274271	93.4752927	8.60257262	0.03193333	87.4827648	2.67181169
PI 386230ao	4g	0.08856867	92.9631584	8.22081057	0.04333333	74.1829163	3.39793291
PI 476853	4g	0.1019387	94.3696509	9.48532268	0.03003333	109.892628	3.26910661
PI 597368	4g	0.11130905	73.529549	8.10854273	0.03163333	89.0254907	2.80529649
PI 597373	4g	0.09456266	55.1270222	5.40424444	0.03433333	65.2322055	2.22605673
PI 599778	4g	0.10765449	84.2819768	9.12234185	0.02823333	84.3121413	2.42628985
PI 599783	4g	0.17195635	80.9383869	14.2822324	0.04733333	76.1548144	3.62273289
PI 599984	4g	0.13198555	61.0709116	7.80151635	0.0407	73.3735184	2.97459151
PI 603989	4g	0.07368842	91.7974343	6.82011068	0.03076667	80.9111107	2.46516166
PI 617099	4g	0.07771523	63.3636743	5.16209231	0.0237	85.2538826	2.0552644
SF 230	4g	0.13535369	64.9740273	8.88431096	0.03026667	92.8986269	2.81627885
NSL 202284	8g	0.08435384	70.5949427	5.85459816	0.02133333	82.430335	1.73388222
NSL 208764	8g	0.04617371	104.895477	6.68023661	0.01756667	89.7201703	1.6841977
PI 386230ao	8g	0.0639535	47.5179418	3.11256429	0.02163333	64.2434887	1.39262866
PI 476853	8g	0.08382263	93.7793991	7.88675485	0.03933333	69.9348296	2.72233024
PI 597368	8g	0.07577478	72.1273618	5.48794411	0.02543333	66.7489608	1.65158223
PI 597373	8g	0.0572112	60.001781	3.39647796	0.02493333	60.1250157	1.4458758
PI 599778	8g	0.08008378	70.5982747	5.64609431	0.02036667	70.772392	1.44022403
PI 599783	8g	0.11750573	91.1915593	10.6393653	0.03273333	92.5971851	3.01663807
PI 599984	8g	0.09056749	68.8158161	6.19007632	0.03046667	66.5722063	2.06243867
PI 603989	8g	0.0705563	71.146595	5.03264126	0.02686667	69.8707026	1.83216858
PI 617099	8g	0.07915529	70.589971	5.57938045	0.02683333	70.610821	1.91062128
SF 230	8g	0.10408996	58.0290164	5.99420569	0.02133333	71.9682005	1.50458053
NSL 202284	12g	0.09092143	51.1128205	4.66029935	0.0215	73.0001178	1.55168347
NSL 208764	12g	0.08131921	68.4388459	5.73051506	0.02506667	69.3747943	1.77752963
PI 386230ao	12g	0.08768362	64.9849106	5.54173881	0.02713333	95.2709264	2.58724881
PI 476853	12g	0.08296661	88.5197845	7.22525934	0.0258	82.4314557	2.1388351
PI 597368	12g	0.05964374	95.7380697	6.20343929	0.02316667	70.9390199	1.61740242
PI 597373	12g	0.06199998	46.8883558	2.93099327	0.02463333	50.9360918	1.25169104
PI 599778	12g	0.07694039	73.1711834	5.60949381	0.02356667	66.680356	1.54556271
PI 599783	12g	0.08744989	71.4322339	6.25421295	0.02283333	75.7616472	1.666268
PI 599984	12g	0.09213395	68.1892897	6.28994749	0.0223	80.0231802	1.66204934
PI 603989	12g	0.10074878	48.8714787	5.01561454	0.02556667	75.6946978	1.93187196
PI 617099	12g	0.10075121	39.3915688	3.96718917	0.0181	68.8093778	1.2444245
SF 230	12g	0.09147714	60.2481716	5.53154315	0.02523333	62.9189651	1.55401768

NSL 202284	20g	0.05150381	72.9738532	3.68141562	0.01963333	53.6691769	1.04899501
NSL 208764	20g	0.0482055	69.7889979	3.41779541	0.0221	47.7151454	1.06678547
PI 386230ao	20g	0.05303184	28.1962006	1.49692904	0.01106667	54.9523103	0.61126028
PI 476853	20g	0.07378123	65.394207	4.79914009	0.02483333	57.8114143	1.43716201
PI 597368	20g	0.07305613	61.1047267	4.4753237	0.02133333	58.2774025	1.24927937
PI 597373	20g	0.06047508	35.2094735	2.19353073	0.01473333	48.8716851	0.70415464
PI 599778	20g	0.10339629	45.9979363	4.71062707	0.03153333	47.4673979	1.36769087
PI 599783	20g	0.07252638	65.5085581	4.74970417	0.01879486	55.3187878	0.99966295
PI 599984	20g	0.06211571	68.140433	3.96158345	0.0247497	60.6403369	1.35652418
PI 603989	20g	0.06892184	50.9953231	3.62230182	0.02046667	59.6117471	1.23326367
PI 617099	20g	0.07272243	54.7456513	3.93049928	0.01973333	58.9404635	1.16406998
SF 230	20g	0.07811132	49.3005255	3.93766021	0.02276667	50.7639337	1.15562246

Table 3.3B: Least-squares means of phosphorus nutrient use efficiency traits for roots and shoots.

		Shoot			**Root**		
Genotype	Osmocote	P-fAqE	P-fUtE	P-fUE	P-fAqE	P-fUtE	P-fUE
NSL 202284	4g	0.0000064	1623444	10.1879779	0.0000027	1702416.21	4.24074586
NSL 208764	4g	0.0000078	1907145	14.3310112	0.00000197	2360053.33	4.44436834
PI 386230ao	4g	0.00000668	2281960	13.5881191	0.00000282	2208486.78	5.63808027
PI 476853	4g	0.00000765	2132722	15.8034129	0.00000307	1781487.41	5.43689611
PI 597368	4g	0.00000793	1716962	13.5064451	0.00000328	1456738.85	4.67207285
PI 597373	4g	0.00000615	1487044	8.99827091	0.00000253	1500834.18	3.69576009
PI 599778	4g	0.00000737	2109816	15.2004583	0.00000273	1486588.29	4.04105342
PI 599783	4g	0.000011	2246586	23.7816603	0.00000267	2372525.11	6.03627816
PI 599984	4g	0.00000811	1720943	12.9883471	0.00000307	1849152.65	4.93883145
PI 603989	4g	0.00000596	2090833	11.3585295	0.00000364	1130458.48	4.10702049
PI 617099	4g	0.00000479	1852187	8.56809327	0.00000179	1898872.35	3.41511073
SF 230	4g	0.00000732	2058177	14.8064958	0.00000221	2129074.19	4.69370943
NSL 202284	8g	0.00000525	1877275	9.75041179	0.00000172	1695843.39	2.88833433
NSL 208764	8g	0.00000455	2527338	11.0987611	0.00000114	2556217.66	2.80013828
PI 386230ao	8g	0.00000387	1365190	5.17264499	0.00000123	1891645.58	2.3186198
PI 476853	8g	0.00000533	2470068	13.144386	0.0000023	2068191.7	4.5350736
PI 597368	8g	0.00000452	2036978	9.14618087	0.00000161	1715259.16	2.74572078
PI 597373	8g	0.00000331	1748392	5.65622663	0.00000166	1489171.47	2.39505176
PI 599778	8g	0.00000478	2030562	9.41001368	0.00000152	1614829.71	2.39724212
PI 599783	8g	0.00000844	2128662	17.7235287	0.00000213	2372617.98	5.02673506
PI 599984	8g	0.00000521	2075560	10.3115	0.0000017	2036783.12	3.43266795
PI 603989	8g	0.00000416	2030455	8.38337559	0.00000149	2047975.65	3.04684434
PI 617099	8g	0.00000489	1911979	9.29482812	0.0000013	2461720.96	3.18345787

SF 230	8g	0.00000626	1614942	9.98707528	0.00000125	2012430.01	2.50557207
NSL 202284	12g	0.00000449	1744932	7.76572819	0.00000118	2256705.09	2.5848988
NSL 208764	12g	0.00000394	2445712	9.54778819	0.00000138	2232445.19	2.96142739
PI 386230ao	12g	0.00000378	2563650	9.23077421	0.00000143	3055764.34	4.30213035
PI 476853	12g	0.00000481	2633146	12.0390845	0.00000124	2929117.89	3.56062433
PI 597368	12g	0.00000508	2048435	10.3330864	0.00000126	2140029.68	2.69366496
PI 597373	12g	0.00000318	1576530	4.88215007	0.00000152	1364029.25	2.08230209
PI 599778	12g	0.00000424	2271508	9.3456409	0.00000154	1678775.96	2.5733734
PI 599783	12g	0.0000053	1997791	10.422443	0.00000116	2437770.53	2.76707355
PI 599984	12g	0.0000061	1747302	10.4808304	0.00000104	2728002.98	2.75477139
PI 603989	12g	0.00000501	1689024	8.35594586	0.0000014	2306661.21	3.20958904
PI 617099	12g	0.00000425	1666442	6.59793196	0.00000085	2437175.6	2.07274768
SF 230	12g	0.00000438	2123831	9.21504773	0.00000124	2049565.43	2.57538983
NSL 202284	20g	0.00000263	2526365	6.13486073	7.33E-07	2429438.01	1.74741649
NSL 208764	20g	0.00000269	2221301	5.69470123	0.0000008	2230071.59	1.77679488
PI 386230ao	20g	0.00000183	1531211	2.49464287	4.93E-07	2036121.95	1.01642605
PI 476853	20g	0.00000331	2443015	7.99775368	0.00000095	2550262.18	2.39506036
PI 597368	20g	0.000004	1897497	7.45582332	8.33E-07	2211428	2.07583192
PI 597373	20g	0.0000025	1397133	3.65368947	6.93E-07	1712733.32	1.17181386
PI 599778	20g	0.00000435	1870989	7.84397895	0.00000182	1603827.77	2.27042655
PI 599783	20g	0.00000415	1942297	7.91590023	0.00000073	2292977.59	1.66499255
PI 599984	20g	0.00000352	2040941	6.59847257	0.00000132	1978184.44	2.25516195
PI 603989	20g	0.00000296	2140678	6.017023	0.00000081	2621167.86	2.02179849
PI 617099	20g	0.00000309	2134305	6.54486894	8.07E-07	2450432.95	1.938701
SF 230	20g	0.00000325	2043989	6.55038331	0.00000116	1728405.93	1.92267618

Table 3.4: Spearman's ρ^2 with sign. Correlations are between fAqE or fUtE and fUE for the same organ and nutrient. Each column represents a different fertilizer treatment (4g, 8g, 12g, or 20g Osmocote applied). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

			4g	**8g**	**12g**	**20g**
Shoot	fAqE	N-fAqE : N-fUE	–	–	–	**0.4858**
		P-fAqE : P-fUE	–	**0.6771**	0.2761	**0.7967**
	fUtE	N-fUtE : N-fUE	–	–	**0.716**	–
		P-fUtE : P-fUE	**0.4747**	–	0.37	**(-) 0.0681**
Root	fAqE	N-fAqE : N-fUE	**0.3312**	**0.5275**	–	**0.7694**
		P-fAqE : P-fUE	–	–	–	**0.783**
	fUtE	N-fUtE : N-fUE	–	–	**0.6022**	–
		P-fUtE : P-fUE	0.2167	–	0.4107	**(-) 0.0743**

Table 3.5: Spearman's ρ^2 with sign. Correlations are between root N and P fUE, fAqE, and fUtE and shoot N and P fUE, fAqE, and fUtE. Each column represents a different fertilizer treatment (4g, 8g, 12g, or 20g Osmocote applied). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

Shoot vs Roots		**4g**	**8g**	**12g**	**20g**
fAqE	Shoot N : Root N	–	–	–	**0.2761**
	Shoot P : Root P	–	–	–	**0.4316**
fUtE	Shoot N : Root N	–	–	–	–
	Shoot P : Root P	–	–	–	**0.4858**
fUE	Shoot N : Root N	–	–	–	**0.4107**
	Shoot P : Root P	–	–	–	**0.4107**

Table 3.6: Spearman's ρ^2 with sign. Correlations are between organ N-fAqE and N-fUtE to equivalent organ P-fAqE and P-fUtE. Each column represents a different fertilizer treatment (4g, 8g, 12g, or 20g Osmocote applied). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

N vs P		**4g**	**8g**	**12g**	**20g**
Shoot	N-fAqE : P-fAqE	**0.5426**	**0.783**	–	**0.4969**
	N-fUtE : P-fUtE	**0.531**	**0.7559**	**0.4747**	**0.4638**
Root	N-fAqE : P-fAqE	−	0.3471	**0.3232**	**0.8669**
	N-fUtE : P-fUtE	−	–	**0.8245**	**0.2416**

Table S3.1: Spearman's ρ^2 with sign for correlations between traits and organ N-fAqE. Columns represent fertilizer treatments (4g, 8g, 12g, or 20g Osmocote). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

	Root N-fAqE				Shoot N-fAqE			
	4g	8g	12g	20g	4g	8g	12g	20g
Seed Mass	**0.088**	–	–	**0.114**	**0.341**	**(-) 0.040**	**(-) 0.095**	**0**
Days to Harvest	**(-) 0.079**	–	–	**0.663**	**0.214**	**0.153**	**0.074**	**0.106**
Chlorophyll Content	–	–	–	-	**(-) 0.049**	**(-) 0.076**	-	-
Height	**(-) 0.001**	0.025	–	**0.170**	**0.351**	**0.201**	**0.079**	**0.234**
Stem Diameter	**(-) 0.006**	–	**0.059**	**(-) 0.036**	**0.421**	**(-) 0.001**	**0.063**	**0.027**
MRFEL Fresh Mass	–	–	0.065	**0.088**	**0.018**	-	**(-) 0.077**	**(-) 0.027**
MRFEL Thickness	–	–	**(-) 0.182**	**(-) 0.122**	**(-) 0.370**	**0.091**	**(-) 0.032**	**0.073**
MRFEL Toughness	**0.048**	**(-) 0.261**	-	**0.229**	**(-) 0.075**	-	-	**(-) 0.075**
MRFEL Dry Mass	–	–	**0.169**	-	**0.078**	-	-	-
Dead Leaves Dry Mass	**(-) 0.237**	–	–	**0.505**	**0.038**	**0.285**	**(-) 0.118**	**0.599**
Cotyledons Dry Mass	**0.255**	–	–	**0.089**	**0.141**	**(-) 0.091**	**(-) 0.065**	**(-) 0.062**
Stem Dry Mass	**(-) 0.058**	0.048	–	**0.285**	**0.361**	**0.234**	**(-) 0.095**	**0.294**
Fine Root Dry Mass	**0.411**	**0.516**	**0.380**	**0.486**	**0.170**	**0.076**	-	**0.322**
Coarse Root Dry Mass	**(-) 0.055**	**0.139**	**0.070**	**0.333**	**(-) 0.049**	**0.300**	(-) 0.053	**(-) 0.060**
Buds Dry Mass	**(-) 0.062**	–	–	**0.055**	**0.017**	**(-) 0.000**	-	**(-) 0.039**
Total Live Leaf Dry Mass	**0.092**	0.006	**(-) 0.001**	**(-) 0.052**	**0.083**	**0.121**	-	**0.012**
Total Root Dry Mass	**0.332**	**0.507**	**0.348**	**0.770**	**0.209**	**0.206**	-	**0.351**
Total Shoot Dry Mass	**(-) 0.040**	–	–	**0.242**	**0.285**	**0.313**	**0.029**	**0.543**
Whole Plant Dry Mass	**0.022**	0.0140	**0.076**	**0.421**	**0.259**	**0.268**	**0.052**	**0.590**
MRFEL Leaf Area	–	0.0350	–	**0.006**	**0.033**	-	**(-) 0.046**	**0.014**
MRFEL Perimeter	–	–	0.037	**0.088**	**0.091**	-	-	**(-) 0.082**
MRFEL Length	–	–	–	**(-) 0.017**	**0.029**	-	**(-) 0.055**	**(-) 0.070**
MRFEL Width	–	(-) 0.049	–	**0.060**	**0.068**	-	-	-
MRFEL Circularity	–	–	–	**(-) 0.379**	-	-	-	**0.060**
Total Live Leaf Area	**0.134**	(-) 0.075	(-) 0.046	-	**0.048**	**0.076**	-	-
MRFEL SLA	**0.036**	–	**(-) 0.053**	**(-) 0.615**	**(-) 0.128**	**(-) 0.008**	**(-) 0.033**	**(-) 0.141**
MRFEL LDMC	**(-) 0.031**	–	**0.135**	**0.171**	**0.130**	**(-) 0.033**	**(-) 0.168**	**0.119**
Leaf N	**(-) 0.105**	0.059	**(-) 0.443**	**(-) 0.027**	-	**0.000**	**(-) 0.063**	**0.063**
Whole Plant SLA	**0.082**	–	–	**(-) 0.017**	**(-) 0.091**	**0.081**	**(-) 0.040**	**(-) 0.003**
Fine Root Fraction	**0.030**	–	**0.038**	**(-) 0.067**	**(-) 0.043**	**0.038**	-	**0.103**
Leaf Mass Ratio	–	(-) 0.013	–	**(-) 0.402**	**(-) 0.074**	**(-) 0.053**	**0.030**	**(-) 0.111**
Stem Mass Ratio	**(-) 0.083**	–	–	**0.378**	**0.475**	**0.171**	**(-) 0.069**	**0.244**
Shoot Mass Ratio	–	(-) 0.136	–	**(-) 0.090**	**0.206**	**0.125**	**0.071**	**0.128**
Root Mass Ratio	–	0.136	–	**0.090**	**(-) 0.206**	**(-) 0.125**	**(-) 0.071**	**(-) 0.128**
Leaf Area Ratio	–	–	–	**(-) 0.665**	**(-) 0.017**	**0.055**	**0.156**	**(-) 0.304**
Dead Leaf Fraction	**(-) 0.223**	–	–	**0.521**	**0.039**	**0.170**	**(-) 0.074**	**0.580**

Table S3.2: Spearman's ρ^2 with sign for correlations between traits and organ P-fAqE. Columns represent fertilizer treatments (4g, 8g, 12g, or 20g Osmocote). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

	Root P-fAqE				Shoot P-fAqE			
	4g	8g	12g	20g	4g	8g	12g	20g
Seed Mass	–	**(-) 0.081**	–	**(-) 0.001**	**0.867**	**0.027**	**0.017**	**0.201**
Days to Harvest	**(-) 0.041**	**(-) 0.086**	–	**0.589**	**0.086**	**0.168**	**(-) 0.033**	**0.257**
Chlorophyll Content	**(-) 0.083**	–	**0.168**	–	–	**(-) 0.277**	–	–
Height	**(-) 0.036**	**0.178**	–	**0.259**	**0.508**	**0.134**	**0.128**	**0.783**
Stem Diameter	–	**0.053**	–	**(-) 0.046**	**0.411**	**0.095**	**(-) 0.026**	**0.234**
MRFEL Fresh Mass	**(-) 0.328**	–	–	**0.090**	**0.052**	–	**0.072**	**(-) 0.003**
MRFEL Thickness	–	**(-) 0.092**	**(-) 0.035**	**(-) 0.154**	**(-) 0.303**	**0.089**	–	**(-) 0.093**
MRFEL Toughness	–	**(-) 0.085**	–	**0.123**	**0.007**	–	**(-) 0.017**	**(-) 0.055**
MRFEL Dry Mass	**(-) 0.176**	–	(-) 0.044	**0.041**	**0.088**	**0.003**	–	–
Dead Leaves Dry Mass	**(-) 0.084**	(-) 0.072	–	**0.545**	–	**0.543**	**(-) 0.070**	**0.423**
Cotyledons Dry Mass	–	–	–	**(-) 0.089**	**0.641**	**(-) 0.056**	**0.194**	**0.239**
Stem Dry Mass	**(-) 0.036**	**0.277**	–	**0.304**	**0.380**	**0.391**	**0.121**	**0.743**
Fine Root Dry Mass	**0.153**	**0.042**	–	**0.615**	**0.443**	**0.201**	(-) 0.063	**0.361**
Coarse Root Dry Mass	–	**0.655**	–	**0.139**	**(-) 0.046**	**0.387**	–	**0.071**
Buds Dry Mass	**(-) 0.009**	**(-) 0.025**	–	**0.152**	**(-) 0.015**	**(-) 0.015**	**0.069**	**0.152**
Total Live Leaf Dry Mass	–	**0.012**	–	**(-) 0.058**	**0.260**	**0.486**	**0.178**	**0.053**
Total Root Dry Mass	**0.006**	**0.278**	–	**0.783**	**0.432**	**0.348**	**(-) 0.040**	**0.391**
Total Shoot Dry Mass	**(-) 0.079**	**0.095**	–	**0.285**	**0.421**	**0.677**	**0.310**	**0.867**
Whole Plant Dry Mass	**(-) 0.074**	**0.134**	–	**0.443**	**0.351**	**0.639**	**0.053**	**0.839**
MRFEL Leaf Area2	**(-) 0.099**	–	–	**0.026**	**0.072**	–	**(-) 0.091**	**(-) 0.059**
MRFEL Perimeter	**(-) 0.038**	–	–	**0.000**	**(-) 0.003**	–	–	**(-) 0.012**
MRFEL Length	**(-) 0.035**	–	–	**(-) 0.008**	**0.058**	–	**0.091**	**(-) 0.070**
MRFEL Width	**(-) 0.040**	–	–	**0.072**	**0.081**	–	(-) 0.091	–
MRFEL Circularity	**(-) 0.052**	–	(-) 0.012	**(-) 0.420**	**0.003**	**0.028**	–	**0.011**
Total Live Leaf Area	–	**(-) 0.072**	(-) 0.057	–	**0.285**	**0.065**	**0.148**	**0.017**
MRFEL SLA	–	0.087	–	**(-) 0.432**	**(-) 0.163**	**(-) 0.242**	**0.077**	**(-) 0.178**
MRFEL LDMC	**0.074**	**(-) 0.058**	–	**0.050**	**0.342**	**0.143**	**0.075**	**0.096**
Leaf N	**(-) 0.087**	–	**(-) 0.046**	**0.060**	–	**0.001**	–	**(-) 0.065**
Whole Plant SLA	–	–	–	**0.055**	**0.089**	**(-) 0.076**	**0.076**	**0.026**
Fine Root Fraction	**0.536**	**(-) 0.169**	–	**0.036**	**0.083**	–	–	**(-) 0.017**
Leaf Mass Ratio	**(-) 0.131**	**(-) 0.101**	–	**(-) 0.439**	**(-) 0.108**	**0.001**	**0.091**	**(-) 0.377**
Stem Mass Ratio	**(-) 0.048**	**0.108**	–	**0.415**	**0.411**	**0.128**	**0.081**	**0.657**
Shoot Mass Ratio	–	–	(-) 0.128	**(-) 0.082**	**0.235**	**0.261**	**0.454**	**0.242**
Root Mass Ratio	–	–	0.128	**0.082**	**(-) 0.235**	**(-) 0.261**	**(-) 0.454**	**(-) 0.242**
Leaf Area Ratio	**(-) 0.001**	**0.070**	–	**(-) 0.555**	**0.052**	**(-) 0.095**	**0.083**	**(-) 0.531**
Dead Leaf Fraction	**(-) 0.071**	(-) 0.088	–	**0.630**	–	**0.408**	**(-) 0.070**	**0.510**

Table S3.3: Spearman's ρ^2 with sign for correlations between traits and organ N-fUtE. Columns represent fertilizer treatments (4g, 8g, 12g, or 20g Osmocote). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

	Root N-fUtE				Shoot N-fUtE			
	4g	8g	12g	20g	4g	8g	12g	20g
Seed Mass	–	**0.022**	(-) 0.052	–	**(-) 0.040**	**0.121**	**0.531**	**0.156**
Days to Harvest	–	**0.046**	–	–	**0.074**	**(-) 0.020**	**0.234**	**(-) 0.027**
Chlorophyll Content	–	**(-) 0.217**	–	–	–	–	–	**(-) 0.351**
Height	–	**0.022**	**0.037**	**0.012**	**(-) 0.033**	**0.178**	**0.370**	**(-) 0.006**
Stem Diameter	0.032	**0.121**	**0.101**	–	**(-) 0.058**	**0.304**	**0.095**	**0.141**
MRFEL Fresh Mass	–	–	–	**0.055**	**0.058**	–	**(-) 0.059**	–
MRFEL Thickness	0.021	**0.024**	**(-) 0.010**	–	**0.052**	**(-) 0.385**	**(-) 0.084**	**0.018**
MRFEL Toughness	–	–	–	–	**0.280**	**(-) 0.070**	–	**0.126**
MRFEL Dry Mass	–	**0.277**	–	**(-) 0.273**	–	**0.088**	–	–
Dead Leaves Dry Mass	0.116	**0.411**	–	–	–	**(-) 0.074**	**0.528**	–
Cotyledons Dry Mass	–	**0.164**	**(-) 0.008**	–	–	**0.210**	**0.309**	–
Stem Dry Mass	–	**0.178**	**0.032**	(-) 0.043	**0.193**	**0.401**	**0.896**	**0.027**
Fine Root Dry Mass	–	**0.017**	**0.341**	**0.294**	**0.065**	**0.268**	**(-) 0.018**	**(-) 0.033**
Coarse Root Dry Mass	(-) 0.046	**0.006**	**0.234**	–	**0.039**	**(-) 0.031**	**0.156**	**0.171**
Buds Dry Mass	–	**0.038**	0.043	**0.073**	**(-) 0.061**	**0.004**	**0.025**	**(-) 0.039**
Total Live Leaf Dry Mass	0.068	**0.475**	**0.411**	–	**0.102**	**0.304**	**0.128**	**0.134**
Total Root Dry Mass	–	**0.057**	**0.674**	**0.022**	**0.065**	**0.218**	**0.092**	**(-) 0.055**
Total Shoot Dry Mass	–	**0.443**	**0.198**	–	**0.332**	**0.268**	**0.719**	**(-) 0.033**
Whole Plant Dry Mass	–	**0.401**	**0.555**	–	**0.313**	**0.304**	**0.543**	**(-) 0.043**
MRFEL Leaf Area	–	–	–	**(-) 0.008**	**0.068**	–	**(-) 0.042**	–
MRFEL Perimeter	–	–	–	0.083	**(-) 0.059**	–	**0.089**	–
MRFEL Length	–	–	–	0.088	**(-) 0.042**	–	**(-) 0.070**	–
MRFEL Width	–	–	–	**(-) 0.234**	**0.087**	–	**(-) 0.091**	–
MRFEL Circularity	–	0.091	–	**(-) 0.039**	**0.003**	–	–	**0.028**
Total Live Leaf Area	–	**0.201**	**0.783**	**0.294**	**(-) 0.052**	**(-) 0.001**	–	**0.209**
MRFEL SLA	–	**(-) 0.313**	–	–	**(-) 0.059**	**(-) 0.114**	**(-) 0.128**	**0.014**
MRFEL LDMC	–	**0.285**	**(-) 0.041**	–	**0.244**	**0.462**	**0.484**	**0.064**
Leaf N	–	**(-) 0.259**	**0.063**	–	**(-) 0.089**	**(-) 0.351**	**0.033**	**(-) 0.304**
Whole Plant SLA	–	**(-) 0.082**	–	**0.401**	**(-) 0.088**	**(-) 0.027**	**0.046**	–
Fine Root Fraction	–	–	**(-) 0.011**	**0.229**	**(-) 0.016**	**0.038**	**(-) 0.090**	–
Leaf Mass Ratio	–	–	**0.019**	**(-) 0.035**	**(-) 0.001**	**(-) 0.065**	**(-) 0.415**	**0.020**
Stem Mass Ratio	–	**(-) 0.036**	–	(-) 0.023	**0.042**	**0.089**	**0.725**	**0.038**
Shoot Mass Ratio	(-) 0.039	**0.572**	–	–	**0.079**	**0.017**	**0.350**	**(-) 0.035**
Root Mass Ratio	0.039	**(-) 0.572**	–	–	**(-) 0.079**	**(-) 0.017**	**(-) 0.350**	**0.035**
Leaf Area Ratio	–	**(-) 0.037**	–	–	**(-) 0.027**	**(-) 0.209**	**(-) 0.520**	**0.049**
Dead Leaf Fraction	0.092	**0.205**	–	–	–	**(-) 0.071**	**0.399**	–

Table S3.4: Spearman's ρ^2 with sign for correlations between traits and organ P-fUtE. Columns represent fertilizer treatments (4g, 8g, 12g, or 20g Osmocote). All ρ^2 are significant at $p < 0.05$. Bold signifies significant correlations under FDR multiple comparisons. "—" indicates no significant correlation.

	Root P-fUtE				Shoot P-fUtE			
	4g	8g	12g	20g	4g	8g	12g	20g
Seed Mass	**0.095**	**(-) 0.026**	–	(-) 0.008	**(-) 0.072**	**0.432**	**0.217**	–
Days to Harvest	**(-) 0.077**	–	–	–	**0.397**	**0.062**	**0.225**	(-) 0.041
Chlorophyll Content	**(-) 0.059**	**(-) 0.053**	0.022	**(-) 0.370**	–	–	–	**(-) 0.520**
Height	–	–	–	–	**0.148**	**0.285**	**0.070**	–
Stem Diameter	**0.037**	**(-) 0.010**	**0.022**	**0.048**	**(-) 0.063**	**0.268**	**0.065**	**0.037**
MRFEL Fresh Mass	–	**0.076**	–	–	**0.010**	–	**0.046**	–
MRFEL Thickness	**0.090**	–	0.075	–	**0.061**	**(-) 0.074**	**(-) 0.043**	0.053
MRFEL Toughness	**0.317**	–	–	–	**0.284**	**(-) 0.079**	–	**0.133**
MRFEL Dry Mass	**0.062**	**0.361**	–	–	–	**0.022**	–	–
Dead Leaves Dry Mass	**0.057**	**(-) 0.049**	–	(-) 0.030	–	**(-) 0.026**	**0.477**	–
Cotyledons Dry Mass	**0.012**	**(-) 0.024**	–	(-) 0.037	–	**0.209**	**(-) 0.042**	**(-) 0.274**
Stem Dry Mass	**(-) 0.001**	**0.053**	–	–	**0.520**	**0.690**	**0.464**	–
Fine Root Dry Mass	**0.065**	**0.590**	**0.209**	–	**0.313**	**0.370**	**0.250**	0.017
Coarse Root Dry Mass	**0.377**	**(-) 0.081**	**0.042**	–	**0.189**	**0.048**	**0.217**	**0.222**
Buds Dry Mass	–	–	–	–	**0.223**	**0.126**	–	(-) 0.057
Total Live Leaf Dry Mass	**0.328**	**0.391**	**0.361**	**0.201**	**0.032**	**0.225**	**0.268**	**0.268**
Total Root Dry Mass	**0.217**	**0.387**	**0.408**	–	**0.268**	**0.352**	**0.300**	(-) 0.001
Total Shoot Dry Mass	**0.065**	**0.234**	0.074	–	**0.475**	**0.475**	**0.348**	–
Whole Plant Dry Mass	**0.141**	**0.304**	**0.380**	–	**0.555**	**0.486**	**0.578**	–
MRFEL Leaf Area	–	**(-) 0.001**	–	–	**(-) 0.003**	–	**0.018**	–
MRFEL Perimeter	–	**(-) 0.026**	–	–	**0.040**	**(-) 0.003**	**0.074**	–
MRFEL Length	–	**(-) 0.033**	–	–	**(-) 0.030**	**0.029**	**(-) 0.003**	0.022
MRFEL Width	–	**(-) 0.008**	–	–	**0.049**	–	**(-) 0.090**	–
MRFEL Circularity	–	**(-) 0.092**	–	–	–	–	–	0.040
Total Live Leaf Area	**0.076**	**0.037**	**0.652**	**0.639**	–	**(-) 0.018**	**0.053**	**0.341**
MRFEL SLA	**(-) 0.101**	**(-) 0.156**	–	–	**(-) 0.070**	**(-) 0.259**	**(-) 0.114**	0.006
MRFEL LDMC	**(-) 0.061**	**0.047**	–	–	**0.538**	**0.653**	**0.653**	**0.233**
Leaf N	**(-) 0.040**	**(-) 0.268**	–	**(-) 0.095**	**(-) 0.068**	**(-) 0.193**	**(-) 0.095**	**(-) 0.313**
Whole Plant SLA	**(-) 0.053**	**(-) 0.082**	–	**0.193**	**(-) 0.193**	**0.006**	**0.079**	–
Fine Root Fraction	–	**0.207**	(-) 0.021	–	**0.010**	–	**(-) 0.023**	–
Leaf Mass Ratio	–	–	–	–	**(-) 0.236**	**(-) 0.225**	**(-) 0.349**	–
Stem Mass Ratio	**(-) 0.063**	–	–	–	**0.259**	**0.243**	**0.233**	–
Shoot Mass Ratio	**(-) 0.061**	**(-) 0.079**	–	–	**0.082**	**0.012**	**(-) 0.061**	–
Root Mass Ratio	**0.061**	**0.079**	–	–	**(-) 0.082**	**(-) 0.012**	**0.061**	–
Leaf Area Ratio	–	**(-) 0.121**	–	–	**(-) 0.421**	**(-) 0.443**	**(-) 0.351**	–
Dead Leaf Fraction	**0.030**	**(-) 0.027**	–	**(-) 0.139**	–	**0.034**	**0.311**	–

CHAPTER 4

EVOLUTION OF NUTRIENT RESORPTION ACROSS THE HERBACEOUS GENUS *HELIANTHUS*[1]

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[1] **Rea AM, Mason CM, Donovan LA**. 2018. *Plant Ecology*. http://doi.org/10.1007/s11258-018-0841-3. Reprinted here with permission of the publisher.

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 pre-selected 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 concentration). 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.

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 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 towards 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. 2013; Sack et al. 2012; 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, Brandt 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 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 Villemereuil et al. 2016; Donovan et al. 2014). We examined nutrient resorption variation through evolutionary time in 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 4.1). 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, non-hybrid 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 4.1 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 pre-designated 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 concentration for this study.

All leaf economic traits, including green-leaf N and P concentration, 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 hours before being finely ground witha 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:

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

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

$$Resorption\ proficiency = nutrients_{SL}$$

Environmental data

Source habitat soil 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 hours, 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 (Blombert 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 2004; Goolsby 2016; Martins and Garland 1991). To assess correlations between nutrient resorption and the LES, we 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 concentration) (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 Hochbert 1995). Ancestral state reconstructions were performed using restricted maximum likelihood on the most recent *Helianthus* phylogeny with the *phylopars* function (Paradis 2004; Goolsby 2016; Stephens et al. 2015). To perform phylogenetic least squares ANOVA, we used the *gls* function in the *nlme* package (Martins and Garland 1991; Pinheiro 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 efficiency and proficiency. Resorption efficiency varies almost two-fold for P (57—93%) and less so for N (69.5—90%). N and P resorption proficiency varies more than efficiency for both nutrients, with mass-based senesced-leaf N varying three-fold (0.42—1.5%), mass-based senesced-leaf P varying seven-fold (0.03—0.21%), area-based senesced-leaf N varying four-fold (18.3—70.7 g/m^2) and area-based senesced-leaf P varying nine-fold (1.06—9.16 g/m^2). N and P resorption efficiency 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 4.2).

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 4.2).

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 concentration) 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 (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 4.2b). 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 4.2b).

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 4.2). 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 concentration regardless of normalization (Table 4.2). Tannin activity is negatively correlated with senesced-leaf N concentration under mass normalization (Table 4.2a). 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 4.2).

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 efficiency and area-based normalizations of N and P resorption proficiency (senesced-leaf N and P) do not correlate with any native habitat soil fertility measurement (Table 4.3). Mass-based senesced-leaf P concentration only correlates with soil pH, though this correlation loses significance under FDR correction; mass-based senesced-leaf N concentration does not correlate with any measure of fertility (Table 4.3).

In general, senesced-leaf nutrient concentration decreases in warmer habitats with more precipitation (Table 4.3). Under mass-based normalization, senesced-leaf N decreases as mean annual temperature increases (Table 4.3a). 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 4.3).

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 concentration) 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 bi-variate 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, Goolsby, et al. 2017). 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 resorption 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 shows that neither tannin activity (as measured by protein-precipitation capacity) nor total phenolics (as measured by Folin-Ciocalteau 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%—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 suggests that phenolics may not influence resorption at concentrations lower than 2.5%. Our data supports 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 does 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 shows *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. 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 congenerics.

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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TABLES

Table 4.1: Literature base on which the dataset was compiled for analysis with N and P resorption efficiency and proficiency.

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)

Table 4.2: r^2 of evolutionary trait-trait correlations on population-weighted species means for **(a)** mass- and **(b)** area-normalized data. 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, $N_{\mathbf{GL}}$: green-leaf N concentration, $P_{\mathbf{GL}}$: green-leaf L P concentration, Tannin: green-leaf tannin activity, VD: green-leaf vein density, LESPC1: principle components 1 of leaf economics spectrum traits.

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	–	–	–	–	–	–	–	–	–
LL	–	–	–	(+) 0.15	–	–	–	–	(+) 0.19
N_{GL}	–	–	–	–	–	–	–	–	–
P_{GL}	–	–	–	–	–	–	–	–	–
N_{GL}:P_{GL}	–	–	–	–	–	–	–	–	–
C_{GL}:N_{GL}	–	–	–	–	–	–	–	–	–
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	–	–	–	–	–	–	–	–	–
LESPC1	–	**(+) 0.33**	–	**(+) 0.33**	–	–	–	–	–

Table 4.3: r^2 of evolutionary trait-environment correlations on population-weighted species means for **(a)** mass- and **(b)** area-normalized data. 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.

3 (a)	P_{eff}	P_{SL}	N_{eff}	N_{SL}	N_{eff}:P_{eff}	N_{SL}:P_{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_{GL}:P_{GL}	–	–	–	–	–	–	–	–	–
C_{GL}:N_{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_{eff}:P_{eff}	N_{SL}:P_{SL}	Flavonoid	Phenol	LSR
Latitude	–	–	–	–	–	–	–	–	–
Longitude	–	–	–	(–) 0.27	–	–	–	–	–
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	–	–	–	–	–	–	–	–	–

CHAPTER 5

CONCLUSIONS

The purpose of this dissertation research was to use a traits-based approach to investigate water and nutrient use across scales in *Helianthus*. At the ecological scale, I used cultivated sunflower (*H. annuus* L.) to investigate traits associated with performance under controlled water and nutrient regimes. At the evolutionary scale, I used 27 species of wild *Helianthus* of known phylogenetic relationship and one species of *Phoebanthus* as an outgroup to examine patterns of correlated trait evolution between one nutrient-use trait (nutrient resorption) and other suites of leaf traits, as well as native habitat.

First, I examined variation in drought resistance at different ontogenetic stages and with different indices at the ecological scale in cultivated sunflower. Using an automatic irrigation system where volumetric soil moisture was maintained, I investigated the relationship between early vigor traits and yield in diverse cultivated sunflower genotypes. I used two separate experiments to examine trait-trait relationships at two ontogenetic stages: reproductive stage (early budding) and vegetative stage (4-6 leaf pairs). I found genotypic variation for seed yield traits in response to R-stage water limitation, and for biomass traits in response to V-stage water limitation. I also found that two indices, GMP and percent reduction, are moderately or strongly associated for biomass traits, but not seed yield traits. Importantly, I found that early stem elongation rate predicts absolute shoot biomass and seed mass traits and GMP of plant and shoot biomass and 25-seed mass under reproductive stage water limitation. The strong positive correlations between early stem elongation rate and seed mass suggest the possibility of accelerated, indirect selection programs breeding for early vigor traits in favorable conditions; however, future studies should incorporate branching architecture into their experimental design as scatterplot visualizations of the data indicate that branching architecture may be driving some of these correlations. The relatively high yield produced under water limitation by genotypes exhibiting early vigor as measured by growth rate but not phenology implies that these genotypes are employing a “drought avoidant” strategy, whereby plants avoid internal water stress by increasing access to water.

Second, I examined variation in nitrogen and phosphorus use, acquisition, and utilization efficiency at the ecological scale in cultivated sunflower. Building on a previous multi-nutrient stress greenhouse study by Bowsher et al. (2017), I investigated the relationship between nutrient availability and metrics of nutrient stress resistance. I sought to identify potential trade-offs within and among organs regarding nitrogen and phosphorus acquisition and utilization. I also aimed to identify traits corresponding to nutrient acquisition and utilization across nutrient levels. I found that acquisition and utilization efficiencies often related differently to performance than predicted by single-nutrient limitation studies, suggesting that multi-nutrient stress influences plant traits differently than single-nutrient stress. I further found that root and shoot nutrient use efficiency did not correlate at lower fertilizer levels, and neither did root and shoot acquisition or utilization efficiencies. I also found that nitrogen and phosphorus acquisition efficiencies did not trade-off, nor did nitrogen and phosphorus utilization efficiencies. Finally, I found that leaf dry matter content positively associates with shoot

N utilization efficiency, and fine root dry mass positively associates with shoot P acquisition and utilization efficiency, suggesting that these traits can be targeted to improve nutrient use efficiency metrics. Overall, our results identify traits worth targeting, and suggests that root and shoot nutrient efficiency traits can be targeted separately in breeding programs. Furthermore, nitrogen and phosphorus efficiency traits can likely be improved simultaneously in breeding efforts. I recommend that field nutrient use efficiency studies move towards incorporating multi-nutrient stress in their experimental design.

Finally, I explored variation of one nutrient utilization efficiency trait (nutrient resorption) at the evolutionary scale in light of physiological trade-offs and resource availability in native habitats. Building on a common garden experiment by Mason and Donovan (2015), I captured patterns of trait expression in the *Helianthus* genus by measuring foliar nutrient resorption under carefully controlled environmental and ontogenetic conditions. Using phylogenetically-informed analyses, I found that nutrient resorption capacity is greater in slower-growing species exhibiting a relatively more resource-conservative leaf functional strategy. Leaves with high nutrient resorption capacity were also better protected with higher concentrations of chemical defense compounds, and evolved in warmer, rainier habitats. However, native habitat soil fertility did not predict nutrient resorption capacity. This suggests that nutrient resorption evolution is more closely tied to resource economic strategy than native habitat, confirming that physiological trade-offs impact trait evolution in ways I would not predict from looking at environmental conditions alone.

This dissertation has explored trait-trait relationships across multiple scales in the context of variation in water and nutrient availability. It has expanded knowledge of how individual traits predict yield and productivity across resource supply in the globally important sunflower crop, and elucidated the evolutionary relationship between nutrient resorption, other leaf traits, and native habitat. These insights, paired with further research investigating resource limitation response to at the genomic or metabolomic level, may help determine the genetic or physiological basis of the trait relationships I describe in this dissertation. Additional research investigating our findings using more genotypes and ecological stresses will be important for the development of sunflower as a valuable and sustainable oilseed crop.

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