

Soil type and species diversity influence selection on physiology in *Panicum virgatum*

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Abstract Species diversity influences the productivity and stability of plant communities, but its effect on the evolution of species within those communities is poorly understood. In this study, we tested whether species diversity and soil type influence selection on physiology in switchgrass (*Panicum virgatum*). Plants were grown in 0.33–0.55 ha plots in eight full-factorial treatment combinations: four diversity treatments (1 species—switchgrass monoculture; 5 species—a mix of C₄ grasses; 16 species—a mix of grasses, forbs, and legumes; 32 species—a mix of grasses, forbs, legumes, and sedges) and two soil types (Waukee loam “loam” and Spillville–Coland alluvial complex “clay”). We measured selection on photosynthetic rate, chlorophyll concentration, and specific leaf area in each treatment combination and compared the strength of selection between soil types and diversity treatments. When significant, selection favored increased photosynthesis, increased chlorophyll concentration, and decreased specific leaf area in all treatment combinations. Selection for these attributes was stronger in the faster-draining loam soil than the slower-draining clay soil. Selection rarely differed significantly between diversity treatments; however, most instances in which selection differed significantly between soil types occurred in the high-diversity mixes suggesting that diversity alters the impact of soil type as an agent of selection. Selection may have been stronger in the loam soil because of

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its lower available water capacity. There was a lengthy summer drought during our experiment. Under these conditions, plants with high photosynthesis and chlorophyll concentration would have more resources to invest in their root system for water uptake. Increased capacity for water uptake would benefit plants in both soil types during drought but would have greater adaptive significance in the faster-draining loam soil. Our results suggest that species diversity is a weak agent of selection and only influences physiological evolution by modifying the pressures exerted by other environmental factors.

Keywords Bioenergy · Biomass crop · Chlorophyll concentration · Phenotypic selection · Photosynthetic rate · Selection differentials · Selection gradients · Specific leaf area · Switchgrass

Introduction

Plant physiological traits control the uptake, use, and allocation of resources and ultimately affect growth rate, development, and reproductive output. Because of the functional significance of these traits, physiological variation is often thought to be a product of natural selection (Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003). Inter- and intraspecific comparisons support these hypotheses by showing that physiological variation is often correlated with environmental resources, such as soil fertility (reviewed in Lambers and Poorter 1992) and water availability (e.g., Sandquist and Ehleringer 2003); however, these comparative experiments are not specifically designed to identify the agents (the environmental causes) or targets (the phenotypic traits upon which selection acts) of selection (Wade and Kalisz 1990; Ackerly et al. 2000). A better method for identifying the environmental causes and adaptive significance of physiological variation is by comparing the strength and direction of selection on traits in contrasting environments (Lande and Arnold 1983; Wade and Kalisz 1990).

Manipulative experiments have identified several abiotic factors that act as agents of selection on plant physiology. For example, selection favors increased water-use efficiency (WUE; the ratio of carbon assimilation to water loss) in dry environments but not wet environments in *Cakile edentula* (Dudley 1996). Similarly, selection favors increased specific leaf area in high-nutrient soil but not low-nutrient soil in *Hordeum spontaneum* (Verhoeven et al. 2004). In contrast to these abiotic factors, the role of biotic factors as potential agents of selection remains largely unexplored (Abdala-Roberts and Marquis 2007). One biotic factor that could influence selection on plant physiology is the diversity of the surrounding community (Parachnowitsch et al. 2014). Species diversity influences primary productivity (e.g., Tilman et al. 1996, 2001; Hector et al. 1999; Balvanera et al. 2006; Cardinale et al. 2007; Isbell and Wilsey 2011), community stability (Tilman et al. 2006), nutrient retention (e.g., Tilman et al. 1996), susceptibility to invasion (Levine 2000; Balvanera et al. 2006) and other ecosystem processes (Yachi and Loreau 1999; Loreau et al. 2001; Balvanera et al. 2006; Cardinale et al. 2007). Diverse plant communities also foster diversity at higher trophic levels (Myers et al. 2012). Because species diversity affects many processes in plant communities, it likely influences the selection pressures exerted on plants within those communities as well.

Diversity–productivity experiments highlight two mechanisms through which species diversity influences productivity and could also influence selection on plant physiology:

niche differentiation and facilitation (the ‘complementarity effects’; Loreau and Hector 2001; Cardinale et al. 2007; but see Huston 1997; Hooper et al. 2005 for discussion on alternative mechanisms for the positive effect of diversity on productivity). Niche differentiation proposes that high diversity communities capture more of the total resource pool than low diversity communities because resource acquisition varies across space and time. Facilitation is an interspecific interaction that increases the performance of species within the community. For example, the presence of legumes, which form symbiotic associations with nitrogen-fixing rhizobia, increases nitrogen availability for the community (Fargione et al. 2007). Similarly, deeply rooted perennials can increase water and nutrient availability for shallowly rooted plants through hydraulic redistribution and nutrient pumping (reviewed in Callaway 1995). Plants within a community compete for resources such as nitrogen and water (Chapin III 1980; Grime 2001) and selection should favor individuals that best acquire these resources. If plants in high-diversity communities have greater access to resources because of niche differentiation and/or facilitation, then plants in contrasting high- versus low-diversity communities may incur different selection pressures on traits that influence resource capture.

Traits that influence growth rate and plant size, such as photosynthetic rate and specific leaf area, are likely candidates for physiological traits that would incur contrasting selection pressures in communities of different diversity. High photosynthesis and high specific leaf area should be adaptive attributes in productive environments with intense competition because they confer rapid growth and enable plants to occupy space and capture resources (Grime 2001; Verhoeven et al. 2004). Further, some of the factors that influence selection on growth-related traits, such as nutrients (Verhoeven et al. 2004; Lovelock et al. 2004), water (Dudley 1996; Heschel et al. 2004; Sherrard and Maherali 2006; Donovan et al. 2007, 2009), and light (Heschel et al. 2002; Sims and Kelley 1998; Arntz et al. 2000), are resources that would likely vary in communities of different diversity. If rapid growth provides individuals with better access to nutrients, water, and light, then selection for increased photosynthesis and increased specific leaf area should be strongest in environments where competition for these resources is most intense. More specifically, selection for increased photosynthesis and increased specific leaf area should be stronger in low-diversity communities that lack legume facilitation than high-diversity communities with legume facilitation and better niche differentiation.

Soil properties, such as nutrient availability and available water capacity, are another aspect of the environment that will likely influence selection on plant physiology. For example, species from high-nutrient environments typically have high carbon assimilation, high specific leaf area, and rapid growth, which increase their ability to compete with neighboring plants for resources, whereas species from low-nutrient environments typically have low carbon assimilation, low specific leaf area, and slow growth, allowing them to better conserve resources (Bradshaw et al. 1964; Grime and Hunt 1975; Chapin III 1980; Poorter and Remkes 1990; Lambers and Poorter 1992; Grime 2001). These contrasting functional strategies suggest that soil fertility has helped shape the evolution of plant physiology. Water availability also influences selection on traits associated with carbon assimilation. For example, in *Avena barbata*, selection favours high photosynthetic capacity in wet environments but low photosynthetic capacity in dry environments because of the respiratory costs associated with maintaining excess enzyme and substrate capacity (Sherrard and Maherali 2006). Collectively, these results suggest that soils with different nutrient availability and available water capacity should exert contrasting selection pressures on plant physiology.

In this study, we test whether species diversity and soil type influence selection on two photosynthetic traits (photosynthetic rate and chlorophyll concentration) and one trait associated with growth rate (specific leaf area) in a perennial, rhizomatous grass. In addition to their effect on plant growth, these traits are important elements of the worldwide leaf economic spectrum (Wright et al. 2004): a consistent pattern of correlations among leaf traits observed across species that promote distinct functional strategies. In brief, fast-growing species tend to produce structurally inexpensive thin leaves (high SLA) with high nitrogen content and high photosynthesis while slow-growing species tend to produce structurally expensive thick leaves (low SLA) with low nitrogen content and low photosynthesis (Wright et al. 2004). We estimated selection on populations grown in eight full-factorial treatment combinations: four communities with contrasting species diversity and two soil types with different characteristics (nutrient availability, available water capacity, percentage of sand, silt, and clay). We hypothesize that selection on these traits will vary between treatment combinations because of differences in resource availability. This study addresses the need for estimates of phenotypic selection on plant physiological traits in natural environments highlighted in several reviews (Kingsolver et al. 2001, 2012; Geber and Griffen 2003; Hereford et al. 2004).

Materials and methods

Study species

To test whether soil type and species diversity influence selection on plant physiology, we used the perennial, C₄ grass *Panicum virgatum* L. (switchgrass). Switchgrass occurs throughout most of the United States and is a central component of the tallgrass prairie ecosystem (Beatty et al. 1978). Switchgrass produces an open, spreading panicle and flowers between late July and early September. It is wind pollinated and obligately outcrossing but also reproduces clonally through an underground rhizome. Switchgrass is a popular bioenergy crop for cellulosic ethanol production (Schmer et al. 2008). The switchgrass seed used to establish our populations was purchased from Custom Seed Services (Panama, IA), whose source plants originated from remnant prairies in Iowa.

Research site

This experiment was conducted at a 40 ha research site located in the Cedar River Natural Resource Area in Blackhawk County, Iowa (42°23N, 92°13W; Fig. 1). The site was used for row crop (corn and soybean) production from the late 1980s until 2007 and leased to University of Northern Iowa's Tallgrass Prairie Center in 2008. Their goal was to create a biomass production research site for investigating the feasibility of using native perennial vegetation for bioenergy. The site is on marginal farmland with a flat slope (0–2 %) and a corn suitability rating of 50–79 (NRCS 2014). There are three soil types at the site: (1) an excessively drained Flagler sandy loam; (2) a well-drained Waukee loam; and (3) a somewhat poorly-drained Spillville–Coland alluvial complex (NRCS 2014; Fig. 1). The relative amounts of sand, silt, and clay vary between soils as follows: Flagler sandy loam—73.8 % sand, 17.0 % silt, and 9.2 % clay; Waukee loam—66.2 % sand, 20.9 % silt, and 12.8 % clay; Spillville–Coland alluvial complex—42.1 % sand, 35.9 % silt, and 22.0 % clay (NRCS 2014). Because of these compositional differences, the Spillville–Coland

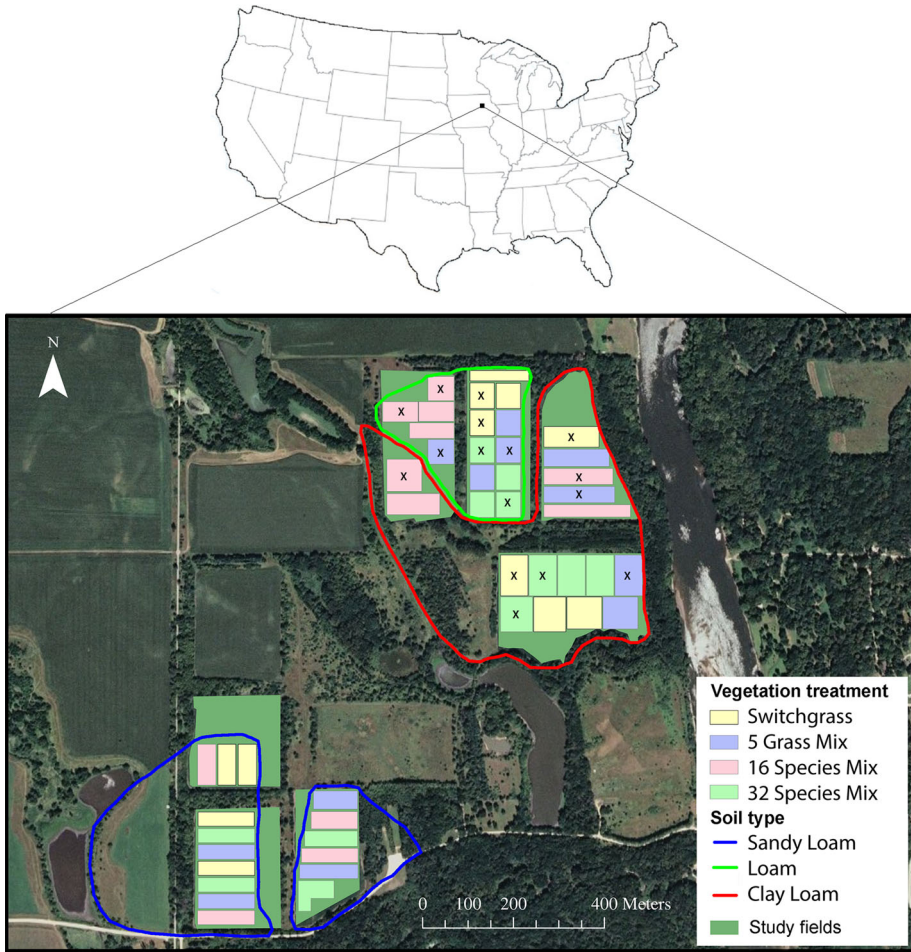


Fig. 1 Map of the study site in the Cedar River Natural Resource Area, Black Hawk County, Iowa, USA. Plots used in this study are indicated with *cross mark*

alluvial complex has the greatest available water capacity, followed by the Waukee loam, and Flagler sandy loam (NRCS 2014).

In 2009, 48 research plots (16 plots per soil type; plot sizes = 0.33–0.56 ha each; Fig. 1) were established at the site. Each plot was drill-seeded with one of four crop mixtures of differing species richness: (1) one species—a switchgrass monoculture; (2) five species—a mix of C_4 grasses; (3) 16 species—a mix of grasses, forbs, and legumes; or (4) 32 species—a mix of grasses, forbs, legumes, and sedges (see Table S1 in supplementary materials for species list and seeding rates). For consistency with other diversity–productivity experiments, we will refer to these four crop mixtures as ‘diversity treatments’ throughout this manuscript. Each diversity treatment contains the entire species composition of lower diversity treatments plus additional species. The seeding rate of the switchgrass monoculture and five warm season grass mix was 561 pure live seeds/m², which was based on recommendations for establishing switchgrass for bioenergy (NRCS

2009). The seeding rate of the 16 and 32 species mixes was 829 and 869 pure live seeds/m², respectively; each treatment was seeded with the same number of graminoid seeds as the switchgrass monoculture and five warm season grass mix (561 pure live seeds/m²) plus seeds from other functional groups. Although the seeding rate was higher in the 16 and 32 species mixes, aboveground primary productivity varied little between diversity treatments over a 5-year study at the site (Jess Abernathy unpublished ms) suggesting that plant density is comparable between treatments (also see Myers et al. 2012 for establishment data). Four replicate plots of each diversity treatment were randomly assigned and established on each of the three soil types (4 reps × 4 diversity treatments × 3 soil types = 48 research plots; Fig. 1).

To ensure a uniform management history, all plots were managed for soybean production in 2008, just before the onset of treatment establishment. Soybeans were harvested in fall 2008 and all plots were treated with a broad leaf herbicide. Other management which occurred at the site prior to summer 2012 (the field season for this experiment) included: establishment mowing on June 16, 2009 to reduce competition with annual weeds; burning on April 5, 2011; and all research plots were hayed March 26–30, 2012. No fertilization, herbicides, pesticides, or irrigation have been applied since the treatment plots were established.

The species composition of the four diversity treatments was selected based on their potential utility as bioenergy feedstocks. Switchgrass was selected as the perennial monoculture because it is a leading candidate crop for bioenergy in the Midwest. The five C₄ grass mix was selected because it has the same functional diversity as a switchgrass monoculture but higher species diversity and because all five are highly productive species in tallgrass prairies. The 16 species mix was selected based on 9 criteria: (1) a statewide distribution—to ensure this mix could be successfully grown anywhere in Iowa; (2) high rates of aboveground biomass production; (3) availability of source identified ‘Iowa Yellow Tag’ seed—to ensure that the genotype of all seed originated from Iowa remnant prairies; (4) ease of establishment from seed; (5) ability to maintain standing vegetation throughout winter; (6) ability to grow in a variety of soil moisture conditions; (7) variable phenologies and life histories—species that produce biomass at different times in the growing season and legumes to increase nitrogen fixation; (8) long life span; and (9) ability to co-exist with other species. Many of the species in the 32 species mix were selected based on the above criteria; however, some were selected because they provide other ecosystem services and are commonly used species in prairie restoration in the Midwest (Dave Williams pers. comm.). Based on this design, the 16 and 32 species mixtures have higher functional diversity than the one and five species mixtures, in addition to higher species diversity.

Soil sampling and analysis

Previous phenotypic selection studies have shown that water and nutrient availability are both important agents of selection on plant physiology (e.g., Dudley 1996; Verhoeven et al. 2004). To evaluate the potential role of soil type as an agent of selection in this study, we measured several soil characteristics: total nitrogen (N), extractable phosphorus, and percent soil moisture, and compared them between soil types. We also measured soil organic carbon (C) because it comprises 50–58 % of soil organic matter on a mass basis (Broadbent 1965), which is positively correlated with available water holding capacity (Hudson 1994).

In May 2008, one soil core was collected to a depth of 90 cm using a 4.5 cm diameter hydraulically-driven soil probe at three to eight locations in each research plot. The cores were frozen, cut into 4 depth increments (0–15, 15–30, 30–60, and 60–90 cm) and stored at -20°C . Frozen core increments were thawed at 4°C immediately prior to processing. Because of severe flooding at the site in 2008, the experiment was delayed and research plots were not established until 2009. To ensure that we had accurate baseline nutrient data at the start of our experiment, we re-sampled the shallowest soil depth before seeding in May 2009. At that time, we returned to the deep core sampling locations and collected soil cores to 15 cm depth using a 3.2 cm diameter hand-held soil probe. Three shallow cores were collected from soil directly adjacent to each deep core sampling location. The cores from each location were cut into two depth increments (0–7.5 and 7.5–15 cm).

Field-moist soil samples were pushed through an 8 mm-diameter sieve and a portion of the 8 mm sieved soil was pushed through a 2 mm sieve and air-dried. Soil water content was determined gravimetrically after oven drying overnight at 105°C . Mehlich-III extractable macro- (P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, and Zn) were quantified using inductively coupled plasma-optical emission spectroscopy (Tran and Simard 1993; Whitney 1998; we present P data only). A sub-sample of air-dried, 2 mm sieved soil was pulverized prior to quantification of total soil C and N using dry combustion. Total soil C is equivalent to soil organic C because no inorganic C was detected. Soil properties are expressed per kg of oven-dry soil.

Because we were interested in characterizing the soil properties at the site for this and other ongoing studies, we present data on all three soil types for reference purposes; however, we restrict the phenotypic selection analysis to plots on the Waukee loam and the Spillville–Coland alluvial complex only.

The 2012 field season

Our experiment was conducted in summer 2012. During this time, Iowa experienced a lengthy summer drought. Annual precipitation in 2012 was 29.5 % lower than the 30-year average (61.14 vs. 86.72 cm) and growing season (April–September) precipitation was 45 % lower than the 30-year average (34.52 vs. 62.81 cm) for the area (data from nearest weather station: Waterloo Airport, 15.5 km; NOAA 2014). Mean annual temperature in 2012 was 2.17°C higher than the 30-year average (10.78 vs. 8.61°C ; NOAA 2014).

Experimental design

To examine the effect of soil type and species diversity on selection for plant physiology, we selected a subset of plots at the research site (Fig. 1). Because of time constraints we limited our analysis to two of the three soil types at the site: the Waukee loam and the Spillville–Coland alluvial complex (these soils will henceforth be referred to as the “loam” and “clay” soil, respectively). We chose to focus on these two soil types, rather than the excessively drained Flagler sandy loam because we anticipated higher plant mortality in response to drought in the sandy soil. Within each soil type, we randomly selected two of the four replicate plots of each diversity treatment. In each plot, we established five 30 m transects. Transects ran west to east and were evenly distributed (5–10 m apart) across the width of each plot. To reduce sampling in edges, we did not place transects within 5 m of any plot edge. Along the length of each transect, we systematically tagged the closest switchgrass tiller at 3 m intervals (10 plants per transect; 50 plants per plot). The goal of the 3 m systematic sampling was to minimize the possibility of selecting two tillers from

the same genet (Beatty et al. 1978). In total, there were 800 plants: 8 treatment combinations (2 soil types \times 4 diversity treatments) and 100 plants per treatment combination (2 plots/diversity treatment \times 50 plants/plot).

Physiological traits

To assess plant investment toward the light-harvesting complex for photosynthesis, we measured apparent chlorophyll concentration using a portable chlorophyll meter (SPAD 502, Minolta Inc., Ramsey, NJ). Three measurements were taken at different positions along the length of the youngest fully expanded leaf on the culm and values are reported as the average of those measurements. Because selection on physiology can vary with development (e.g., Sherrard and Maherali 2006), we measured chlorophyll concentration on the same leaf twice during the growing season: June 11–22, 2012 and July 12–19, 2012.

We measured the photosynthetic rate (A) of all plants using an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE). Measurements were made on the youngest fully expanded leaf on the culm at a controlled cuvette temperature of 29 °C, a vapor pressure deficit of 2.8–3.0 kPa, and a saturating irradiance of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. All measurements were made between 900 and 1300 CST, June 26–July 16, 2012. Leaf area was measured using a digital caliper.

To assess plant growth rate and investment in leaf tissue, we measured specific leaf area (SLA) as the ratio of wet tissue area (cm^2) to dry tissue weight (grams). SLA was measured on the youngest fully expanded leaf on the culm. Tissue was collected at the same time as aboveground biomass harvest (September 5–24, 2012) to avoid destructive sampling during the growing season. To account for potential variation in leaf carbohydrate levels (Garnier et al. 2001), leaves were harvested between 900 and 1300 CST. We photographed the leaves immediately after sampling and measured their area in Adobe Photoshop © CS 5.1 Extended Edition. After photographing the leaves, they were dried to a constant mass at 70 °C (min. 48 h) and weighed. Plants with a senescing youngest fully expanded leaf were excluded from this analysis.

Fitness measures

We estimated selection on physiology using two different fitness metrics: (1) the glume number and (2) aboveground biomass of individual switchgrass tillers. We chose to use two fitness measures to account for the different modes of reproduction in switchgrass, sexual and vegetative. Glume number provided our best estimate of sexual reproduction and was chosen preferentially over seed number to account for seed loss prior to harvest. Aboveground biomass was used to assess potential for clonal reproduction because larger individuals have more photosynthetic tissue and should be capable of greater rhizome investment. As a fitness measure, aboveground biomass can also be useful for quantifying indirect effects of physiology on fitness based on the expectation that leaf traits primarily affect seed production by influencing plant size (Farris and Lechowicz 1990; Arntz et al. 1998, 2000; Ludwig et al. 2004). Some studies of phenotypic selection on physiology have preferentially used vegetative biomass as the fitness measure for this reason (e.g., Donovan et al. 2007). We chose individual tillers as the unit of study, rather than genets, because we could not confidently delineate all tillers of a single connected genet without destructively excavating the rhizome. Switchgrass produces both long and short rhizomes (Beatty et al. 1978) and individuals do not always form distinct clumps. We acknowledge that annual biomass production and glume number are not lifetime fitness measures in a perennial

plant, but they are good metrics for comparing fitness between individuals for several reasons. First, the rate of clonal reproduction was low in our plots (mean = 0.62; median = 0; estimated as the number of additional tillers within 10 cm² of the focal tiller on 150 individuals in the 5, 16, and 32 species diversity mixes) suggesting that annual glume production of one tiller was equivalent to the annual glume production of the genet for many individuals. Second, individuals with high fitness in 2012 (our experimental field season) may have invested more resources into their rhizome in previous years and should be capable of higher clonal reproduction in the future.

Aboveground biomass of all plants was harvested September 5–24, 2012 (loam soil: September 5–15; clay soil: September 18–24), which is the time of maximum yield in switchgrass biomass crops (Heaton et al. 2004). Plant tissue was dried to a constant mass at 70 °C (min. 48 h) and weighed. The inflorescence was separated from the remaining biomass during harvest and glume number was counted by hand. The weight of the inflorescence and tissue used to estimate SLA were included in our final measure of aboveground biomass.

Statistical analysis

We compared physiology and fitness between treatment combinations using 2-way analysis of variance (ANOVA), with soil type and diversity treatment as fixed factors. Within each soil type, we compared values among diversity treatments using Tukey's HSD test. For each ANOVA, we tested the assumption of homogenous residual variance using Levene's test and the assumption of normally distributed residual variance using the Shapiro–Wilk test. When these assumptions were violated, we re-ran the ANOVA on log-transformed data. If log-transformation corrected heteroscedasticity or non-normality, we calculated the *F*-ratios and *P* values using transformed data. If log-transformation did not correct heteroscedasticity or non-normality, we present the *F*-ratios and *P* values using untransformed data and note the assumption violation in our results. Transformation did not change the significance associated with any *F*-ratio. We present *P* values and *F*-ratios, and indicate significance both before and after Holm-Bonferroni correction for multiple tests (Sokal and Rohlf 1995).

We measured phenotypic selection on all traits using univariate and multivariate approaches. Univariate selection differentials (*S*), which estimate both direct selection on a phenotypic trait and indirect selection via correlated traits, were calculated as the linear regression between each phenotypic trait (standardized to mean of 0 and standard deviation of 1) and fitness (relativized by dividing the fitness value of each plant by the mean fitness value; Lande and Arnold 1983; Conner 1988). Multivariate selection gradients (β) estimate only direct selection on a trait, assuming that all traits relevant to fitness are included in the model. We calculated selection gradients for each trait as the partial regression coefficient from a multiple regression of all the physiological traits against fitness (Lande and Arnold 1983). In multivariate selection analyses on physiology, developmental traits such as biomass are sometimes removed to account for indirect effects of physiology on fitness (Dudley 1996). To account for these possible indirect effects, we regressed glume number on physiology in models with and without aboveground biomass. In models with aboveground biomass, selection on physiology was never significant in any treatment combination. Consequently, we present two multivariate selection analyses: (1) we regressed biomass on the four physiological traits (with glume number excluded from model), and (2) we regressed glume number on the four physiological traits (with aboveground biomass

excluded from model). Fitness was relativized and traits were standardized separately within each treatment combination.

To test whether soil type influenced the strength of selection on physiology, we used paired t tests and analyses of covariance (ANCOVA). First, we tested whether the strength of selection differed on average between soil types by comparing the selection differentials from each diversity treatment in the clay versus loam soil. We ran separate paired t tests on the differentials measured with each measure of fitness: (1) glume number; and (2) aboveground biomass. Because differentials can either be positive or negative, we used the absolute value of each differential for this comparison. Second, we analyzed whether the selection differentials for each trait within a given diversity treatment differed between soil types using ANCOVA. Within each diversity treatment, the standardized phenotypic data from both soil types were combined and analyzed in a model with a continuous term for the trait, a categorical term for soil type, and a trait \times soil type interaction. Relative fitness (either glume number or aboveground biomass) was the dependent variable. In this analysis, a significant trait \times soil type interaction indicates that the relationship between the trait and fitness (i.e., selection on the trait) differs between soil types. We also analyzed whether selection gradients differed between soil types within a given diversity treatment using ANCOVA. Within each diversity treatment, the standardized phenotypic data from both soil types were combined and analyzed in a model with continuous terms for each trait, a categorical term for soil type, and every trait \times soil type interaction. Relative fitness (either glume number or aboveground biomass) was the dependent variable. In this analysis, a significant trait \times soil type interaction indicates that the relationship between the trait and fitness differs between soil types.

To test whether species diversity influenced the strength of selection on physiology, we used ANCOVA. Within a soil type, the standardized phenotypic data from each diversity treatment were combined and analyzed in a model with a continuous term for the trait, a categorical term for the diversity treatment, and a trait \times diversity treatment interaction. Relative fitness (either glume number or aboveground biomass) was the dependent variable. In this analysis, a significant trait \times diversity treatment interaction indicates that the relationship between the trait and fitness (i.e., selection on the trait) differs between diversity treatments. We also analyzed whether selection gradients differed between diversity treatments using ANCOVA. Within a soil type, the standardized phenotypic data from each diversity treatment were combined and analyzed in a model with continuous terms for each trait, a categorical term for diversity treatment, and every trait \times diversity treatment interaction. Relative fitness (either glume number or aboveground biomass) was the dependent variable. In this analysis, a significant trait \times diversity treatment interaction indicates that the relationship between the trait and fitness differs between diversity treatments.

For each univariate regression, we tested the assumption of homogeneous residual variance by calculating the Spearman rank correlation between the absolute value of the residual variance in fitness and each standardized trait (Neter et al. 1989) and the assumption of normally distributed residual variance by visually inspecting the residuals from the normal probability plot. All residuals appeared to be normally distributed; however, 6 of the 64 regressions violated the assumption of homoscedastic residual variance and these regressions are indicated in our results. For each multivariate regression, the assumptions of normally distributed residual variance and homogeneous residual variance using the Anderson–Darling test and by calculating the Spearman rank correlation between the absolute value of the residual variance in fitness and each standardized trait (Neter et al. 1989), respectively. No significant deviations in normality were detected;

however, 4 of the 64 traits violated the assumption of homoscedastic residual variance and these traits are indicated in our results.

Because physiology is sensitive to environmental fluctuation and selection estimates can be biased by site heterogeneity (Rausher 1992; Stinchcombe et al. 2002), we assessed the significance of several potential covariates in the selection analyses. We tested whether time of day, day of measure, volumetric soil water content (VWC) and plot (nested within treatment combination) covaried with photosynthetic rate. VWC was measured in the soil adjacent to each plant with a handheld moisture probe at the time of the photosynthetic measurement (Hydrasense II, Model HS2-12; Campbell Scientific, Logan, UT). We also tested whether VWC and plot (nested with treatment combination) covaried with chlorophyll concentration and SLA. When one or both of these factor(s) covaried significantly with a physiological trait, we removed variation in the trait caused by the covariate and performed the selection analyses on residual trait variation (standardized). We also tested whether our two fitness metrics covaried with VWC and plot (nested within treatment combination). If either covariate was significant, they were included as a factor in the regression between physiology and fitness (the selection analyses).

To help identify potential causes of indirect selection on physiology, we calculated phenotypic correlations (Pearson product-moment correlations) between all physiological traits and fitness measures in each treatment combination. For this analysis, we calculated correlations using the standardized residuals after removing significant covariates from all physiological and fitness measures (see above for list of potential covariates). We present *P* values for all correlations after Bonferroni correction for multiple tests (Sokal and Rohlf 1995).

To compare total nitrogen, extractable phosphorus, soil moisture, and organic carbon content between soil types within each depth increment, we used one-way ANOVA and Tukey's HSD test.

All statistics were performed in SYSTAT v 13.1 (Systat Software Inc., San Jose, CA) except the soil property comparison, which was performed in SAS v9.2 (SAS Institute Inc., Cary, NC).

Results

Soil properties

We observed significant differences in all soil properties between soil types at every depth interval (Table 1). Soil moisture was higher in the clay soil than loam soil at all depths, but the difference was statistically significant in the two surface soil increments and the deepest depth increment only. Soil organic carbon (SOC) and total N concentrations were consistently higher in the clay soil than the loam soil, but this effect was only significant for SOC at the two deepest soil layers. Extractable phosphorus concentrations were significantly higher in the clay soil than loam soil in the two surface soil increments but this effect was not observed below 30 cm.

Variation in physiology

All physiological traits varied between soil types (significant soil type term; Table 2). Photosynthetic rate and chlorophyll concentration were higher in the clay soil and specific

Table 1 Properties of the three soil types at the study site in the Cedar River Natural Resource Area, Black Hawk County, Iowa, USA. Selection on physiology was estimated in Spillville/Coland clay and Waukee loam soils only

	Spillville/Coland clay	Waukee loam	Flagler sandy loam
Soil moisture (%)			
0–7.5 cm [†]	21.13a	20.01b	13.69c
7.5–15 cm [†]	19.01a	18.05b	12.36c
15–30 cm	19.87a	18.92a	13.29b
30–60 cm	18.63a	17.39a	12.10b
60–90 cm	15.51a	13.45b	7.21c
Soil organic carbon (g kg ⁻¹)			
0–7.5 cm [†]	24.90a	23.37a	14.24b
7.5–15 cm [†]	21.00a	20.03a	11.36b
15–30 cm	19.62a	18.27a	12.03b
30–60 cm	13.38a	11.23b	7.83c
60–90 cm	6.80a	5.57b	2.20c
Total nitrogen (g kg ⁻¹)			
0–7.5 cm [†]	2.29a	2.14a	1.44b
7.5–15 cm [†]	1.97a	1.86a	1.12b
15–30 cm	1.75a	1.67a	1.13b
30–60 cm	1.20a	1.08a	0.77b
60–90 cm	0.68a	0.67a	0.31b
Phosphorus (mg kg ⁻¹)			
0–7.5 cm [†]	85.0a	57.9b	99.4a
7.5–15 cm [†]	41.0b	24.9c	53.0a
15–30 cm	18.0b	21.0b	36.7a
30–60 cm	14.6a	15.5a	18.8a
60–90 cm	13.4a	14.7a	12.6a

Values represent means and letters represent significant differences between soil types within each depth increment based on one-way ANOVA and Tukey's HSD test. All ANOVAs remained significant after Holm–Bonferroni correction for multiple comparisons

[†] Samples collected in May 2009; all other samples collected in May 2008

leaf area (SLA) was higher in the loam soil (Fig. 2a–d). The physiological traits also varied between diversity treatments (significant diversity treatment term; Table 2) and the relative rank of the four diversity treatments differed between soil types (significant soil × treatment term; Table 2). In the clay soil, photosynthetic rate was higher in the 16 species mix than the switchgrass monoculture and five warm season grass mix (Fig. 2a); chlorophyll concentration was highest in the 16 species mix and lowest in the five warm season grass mix (Fig. 2b, c); and SLA was lower in the 16 species mix than the switchgrass monoculture and five warm season grass mix (Fig. 2d). In the loam soil, photosynthetic rate was higher in the 32 species mix than the five warm season grass mix and 16 species mix (Fig. 2a); chlorophyll concentration was lowest in the five warm season grass mix (Fig. 2b, c); and SLA was highest in the switchgrass monoculture and 16 species mix and lowest in the five warm season grass mix (Fig. 2d).

Table 2 Two-way ANOVA table comparing four physiological traits and two fitness metrics between treatment combinations

	<i>df</i>	MS	<i>F</i>	<i>P</i>
Photosynthetic rate [†]				
Soil type (S)	1	1420.23	38.30	<0.001
Diversity treatment (T)	3	276.67	7.46	<0.001
S × T	3	264.81	7.14	<0.001
Chlorophyll concentration—June				
Soil type (S)	1	759.14	41.56	<0.001
Diversity treatment (T)	3	1781.04	97.51	<0.001
S × T	3	221.61	12.13	<0.001
Chlorophyll concentration—July				
Soil type (S)	1	838.01	41.05	<0.001
Diversity treatment (T)	3	2547.77	124.79	<0.001
S × T	3	49.66	2.43	0.064
Specific leaf area [‡]				
Soil type (S)	1	0.49	63.33	<0.001
Diversity treatment (T)	3	0.15	20.15	<0.001
S × T	3	0.37	47.72	<0.001
Aboveground biomass [‡]				
Soil type (S)	1	1.29	15.81	<0.001
Diversity treatment (T)	3	1.17	14.34	<0.001
S × T	3	0.46	5.69	<0.001
Glume number [‡]				
Soil type (S)	1	0.05	0.20	0.658
Diversity treatment (T)	3	2.45	10.34	<0.001
S × T	3	0.33	1.39	0.246

Reported values are: degrees of freedom (*df*), mean squares (MS), *F*-statistics (*F*) and associated *P* values (*P*). See Fig. 2 for trait units. All terms remained significant after Holm–Bonferroni correction

[†] ANOVA violates the assumption of homoscedastic residual variance

[‡] Data was log₁₀-transformed

Variation in fitness

Aboveground biomass differed between soil types but glume number did not (Table 2). Plants were larger in the clay soil than the loam soil (Fig. 2e). Both fitness measures varied between diversity treatments within a soil type. In the clay soil, plants were larger in the switchgrass monoculture and 16 species mix than the five warm season grass mix (Fig. 2e) and plants in the five warm season grass mix produced the fewest glumes (Fig. 2f). In the loam soil, plants were largest in the 32 species mix, smallest in the five warm season grass mix (Fig. 2e) and plants produced more glumes in the 16 and 32 species mixes than the switchgrass monoculture and five warm season grass mix (Fig. 2f).

Univariate selection on physiology (glume number)

Of the 32 selection differentials measured across treatment combinations (four traits × eight treatment combinations), 11 were significant when glume number was the fitness metric (four in the clay soil and seven in the loam soil; Table 3). When significant, selection favored increased photosynthetic rate, increased chlorophyll concentration, and decreased SLA in all treatment combinations. Selection on physiology was stronger on average in the loam soil than the clay soil ($t = -3.491$; $P = 0.003$). There was one

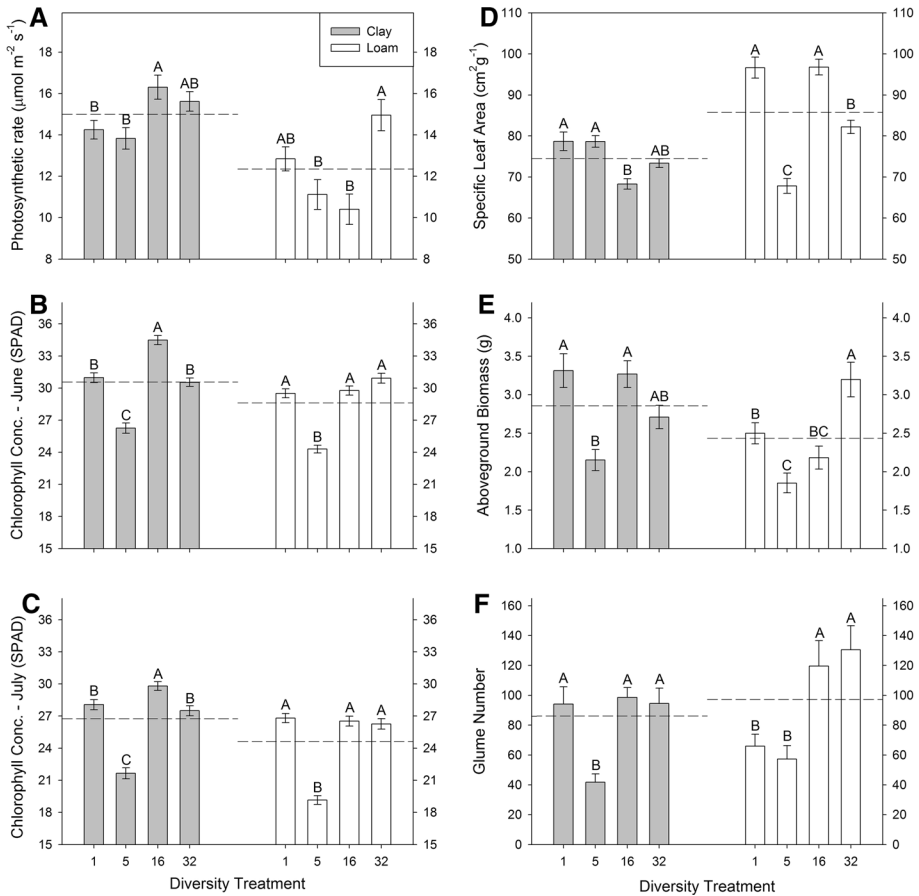


Fig. 2 Variation in physiology and fitness between treatment combinations. *Bars* represent trait means (± 1 SE) within treatment combinations and *dashed lines* represent means within each soil type. Tukey's HSD test was used to assess whether physiology and fitness differed between diversity treatments within a soil type and significant differences are indicated with *letters*. All traits differed significantly ($P < 0.05$) between soil types except glume number

instance in which the strength of selection on a particular trait differed significantly between soil types in a given diversity treatment. Selection for increased chlorophyll concentration was stronger in the loam soil than the clay soil in the 16 species mix (Table 3). Selection did not differ significantly between diversity treatments for any trait in either soil type.

Univariate selection on physiology (aboveground biomass)

Of the 32 selection differentials measured across treatment combinations (four traits \times eight treatment combinations), 30 were significant when aboveground biomass was the fitness metric (15 in the clay soil and 15 in the loam soil; Table 3). When significant, selection favored increased photosynthetic rate, increased chlorophyll concentration, and decreased SLA in all treatment combinations (Table 3). On average, selection was marginally stronger in the loam soil than the clay soil ($t = -2.073$; $P = 0.056$). There

Table 3 Phenotypic selection on physiology in each treatment combination

	Soil	1		5		16		32		<i>F</i>
		N	<i>S</i> (SE)	N	<i>S</i> (SE)	N	<i>S</i> (SE)	N	<i>S</i> (SE)	
Glume number										
Photosynthetic rate	Clay	62	0.247 (0.14)	44	0.239 (0.14)	76	0.128 (0.07)	57	0.076 (0.12)	1.39
	Loam	62	0.496 (0.11)***	28	0.320 (0.15)*	41	0.187 (0.12)	63	0.326 (0.15)	0.52
Chlorophyll—June	Clay	62	0.167 (0.12)	44	0.326 (0.14)*	76	0.078 (0.07)	57	0.195 (0.11)	0.08
	Loam	62	0.161 (0.14)	28	0.340 (0.19)	43	0.292 (0.15)	63	0.454 (0.12)***	2.55
Chlorophyll—July	Clay	62	0.137 (0.12)	44	0.463 (0.15)**	76	0.105 (0.07)	57	0.241 (0.12)*	0.01
	Loam	61	0.242 (0.12)	28	0.566 (0.17)**	43	0.482 (0.14)***	63	0.486 (0.11)***	1.83
Specific leaf area	Clay	54	-0.219 (0.16)	32	-0.177 (0.16)	69	-0.174 (0.06)**	56	-0.191 (0.14)	0.01
	Loam	47	-0.102 (0.21)	25	-0.105 (0.17)	42	-0.171 (0.15)	57	-0.366 (0.13)**	1.95
Aboveground biomass										
Photosynthetic rate	Clay	99	0.287 (0.06)***	100	0.358 (0.05)***†	99	0.130 (0.05)*	100	0.231 (0.05)***	4.08*
	Loam	100	0.294 (0.05)***	95	0.352 (0.06)***†	96	0.280 (0.07)***	98	0.269 (0.07)***	0.63
Chlorophyll—June	Clay	99	0.157 (0.061)*	100	0.366 (0.05)***†	99	0.151 (0.05)**	100	0.149 (0.05)**	3.15
	Loam	100	0.223 (0.053)***	96	0.310 (0.07)***†	99	0.285 (0.06)***	98	0.291 (0.07)***	0.41
Chlorophyll—July	Clay	99	0.123 (0.06)*	100	0.355 (0.05)***†	99	0.137 (0.05)**	100	0.158 (0.05)**	1.52
	Loam	93	0.226 (0.05)***	96	0.326 (0.06)***†	96	0.352 (0.06)***	97	0.339 (0.07)***	1.19
Specific leaf area	Clay	87	-0.111 (0.07)	70	-0.198 (0.08)*	90	-0.168 (0.06)**	97	-0.181 (0.05)***	0.061
	Loam	71	-0.185 (0.06)**	82	-0.012 (0.07)	94	-0.215 (0.07)**	86	-0.152 (0.08)*	0.37

Data presented are standardized linear selection differentials (*S*) ± (1 SE)

Significant selection differentials are indicated with an * (* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001) and values in bold remained significant after Holm-Bonferroni Correction within each treatment combination

Selection differentials were compared between soil types using ANCOVA. Solid boxes indicate a significant difference in selection between soil types (*P* < 0.05). Dashed boxes indicate a marginally significant difference in selection between soil types (*P* < 0.10). Selection differentials were compared between diversity treatments using ANCOVA. We report the *F*-statistics (*F*) for the trait × diversity treatment interaction term and indicate significance using *

† Regression violates the assumption of homoscedastic residual variance

were two instances in which the strength of selection on a particular trait differed significantly between soil types in a given diversity treatment: selection for increased chlorophyll concentration (July) was stronger in the loam soil than the clay soil in the 16 and 32 species mixes. There were four instances in which the strength of selection differed marginally between soil types (*P* < 0.10): selection for increased chlorophyll concentration (June) was marginally stronger in the loam soil than the clay soil in the 16 and 32 species mixes; selection for increased photosynthetic rate was marginally stronger in the loam soil than the clay soil in the 16 species mix; and selection for decreased SLA was marginally stronger in the clay soil than the loam soil in the five warm season grass mix (Table 3). In addition to differences between soil types, selection also differed significantly between diversity treatments for one trait (photosynthetic rate) in the clay soil (Table 3). Selection for increased photosynthesis was weaker in the 16 species mix than the other three diversity treatments.

Table 4 Directional selection gradients (β) \pm (1SE) on physiology in each treatment combination

	Soil	1	5	16	32
		β (SE)	β (SE)	β (SE)	β (SE)
Glume number					
Photosynthetic rate	Clay	0.312 (0.17)	-0.124 (0.27)	0.122 (0.07)	0.011 (0.13)
	Loam	0.550 (0.14)***	0.199 (0.13)	0.011 (0.13)	0.196 (0.13)
Chlorophyll—June	Clay	0.133 (0.15)	0.228 (0.23)	-0.045 (0.09)	0.036 (0.16)
	Loam	0.000 (0.19)	0.156 (0.18)	0.177 (0.17)	0.254 (0.14)
Chlorophyll—July	Clay	-0.064 (0.16)	0.348 (0.31)	0.052 (0.10)	0.237 (0.17)
	Loam	0.064 (0.18)	0.398 (0.19)*	0.468 (0.16)**	0.364 (0.15)*†
Specific leaf area	Clay	-0.254 (0.15)	-0.125 (0.16)	-0.167 (0.07)*	-0.216 (0.15)
	Loam	-0.170 (0.19)	-0.205 (0.15)	0.023 (0.15)	-0.241 (0.12)*
Aboveground biomass					
Photosynthetic rate	Clay	0.391 (0.07)***	0.102 (0.10)	0.118 (0.05)*	0.168 (0.05)**
	Loam	0.247 (0.05)***	0.238 (0.06)***	0.164 (0.06)**	0.166 (0.07)*
Chlorophyll—June	Clay	0.098 (0.07)	0.245 (0.10)*†	0.063 (0.08)	0.025 (0.06)
	Loam	0.119 (0.07)†	0.140 (0.07)*	0.131 (0.07)	0.100 (0.08)†
Chlorophyll—July	Clay	-0.116 (0.08)	0.074 (0.12)	0.056 (0.08)	0.056 (0.06)
	Loam	0.026 (0.07)	0.151 (0.07)*	0.234 (0.07)***	0.281 (0.09)**
Specific leaf area	Clay	-0.147 (0.06)**	-0.160 (0.07)*	-0.138 (0.05)*	-0.142 (0.05)**
	Loam	-0.169 (0.06)**	0.005 (0.05)	-0.112 (0.06)	-0.053 (0.06)

Significant selection gradients are indicated with an * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$) and values in bold remain significant after Holm–Bonferroni correction within each treatment combination

Selection gradients were compared between soil types using ANCOVA. Dashed boxes indicate a marginally significant difference in selection between soil types ($P < 0.10$)

Selection gradients were compared between diversity treatments using ANCOVA; no significant differences were detected

† Term within multiple regression violates assumption of homoscedastic residual variance

Multivariate selection on physiology (glume number)

The direction of selection on photosynthesis, chlorophyll concentration, and SLA was the same in both univariate and multivariate approaches (Tables 3, 4). In the loam soil, there was direct selection on five traits across the four diversity treatments: selection favored

increased photosynthesis in the switchgrass monoculture; increased chlorophyll concentration (July) in the five warm season grass mix, 16 species mix, and 32 species mix; and decreased SLA in the 32 species mix (Table 4). In the clay soil, selection favored decreased SLA in the 16 species mix.

Multivariate selection on physiology (aboveground biomass)

There were more significant selection gradients when aboveground biomass was the fitness measure than when glume number was the fitness measure (17 vs. 6; Table 4). When significant, selection favored increased photosynthetic rate, increased chlorophyll concentration, and decreased SLA in all treatment combinations. There were two instances in which the strength of selection on a particular trait differed marginally between soil types in a given diversity treatment: selection for increased chlorophyll concentration (July) was marginally stronger in the loam soil than the clay soil in the 16 and 32 species mixes (Table 4).

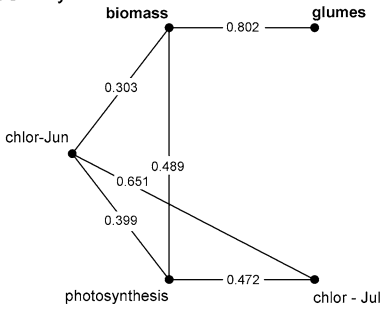
Phenotypic correlations

Significant correlations between chlorophyll concentration (June), chlorophyll concentration (July), and photosynthesis were common across treatment combinations (Fig. 3). For example, chlorophyll concentration (June) and chlorophyll concentration (July) were positively correlated in all eight treatment combinations, and photosynthesis was positively correlated with both chlorophyll concentration (June) and chlorophyll concentration (July) in five of eight treatment combinations. By contrast, SLA was not significantly correlated with photosynthesis or chlorophyll concentration in any treatment combination. Correlations between biomass and physiology occurred more frequently (25 of 32 possible correlations) than correlations between glume number and physiology (6 of 32 possible correlations). Biomass was significantly correlated with photosynthesis, chlorophyll concentration (June), chlorophyll concentration (July), and SLA in 7, 8, 6, and 4 treatment combinations respectively, while glume number was significantly correlated with photosynthesis, chlorophyll concentration (June), chlorophyll concentration (July), and SLA in 1, 1, 4, and 0 treatment combinations, respectively (Fig. 3). Biomass and glume number were positively correlated in every treatment combination.

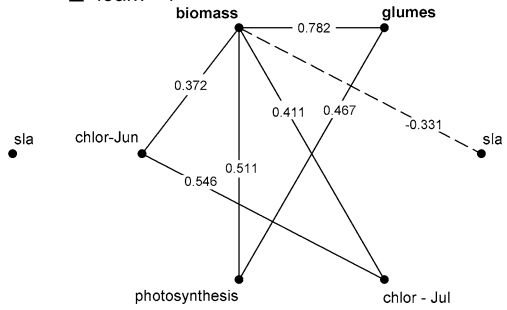
Discussion

In this study, we tested whether species diversity and soil type influence the strength of selection on physiology in switchgrass. While previous research has shown that soil factors influence selection on physiology in other species (e.g., Dudley 1996; Heschel et al. 2002, 2004; Verhoeven et al. 2004; Sherrard and Maherali 2006; reviewed in Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003), to our knowledge this is the first study to examine soil type as an agent of selection in switchgrass and the first to examine the role of species diversity as an agent of selection on physiology in any plant species. Selection favored high photosynthesis, high chlorophyll concentration, and low specific leaf area (SLA) in all treatment combinations (Tables 3, 4). The adaptive significance of high photosynthesis and high chlorophyll concentration are consistent with expectations of a fast-growing species in high-nutrient, competitive environments but selection favoring low

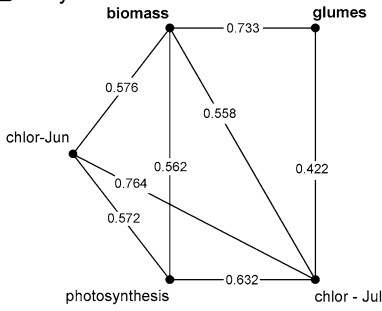
A clay - 1



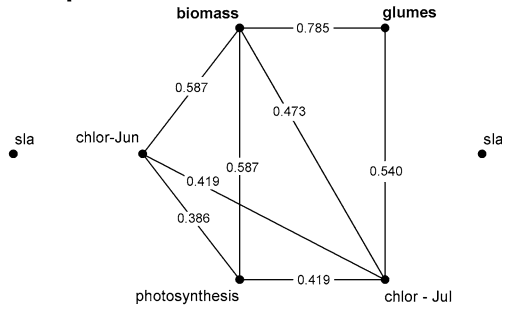
E loam - 1



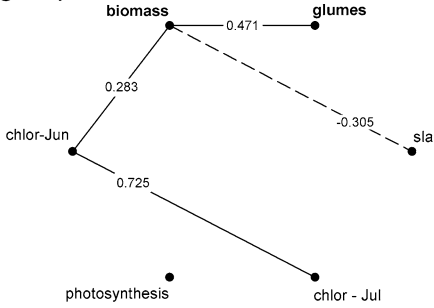
B clay - 5



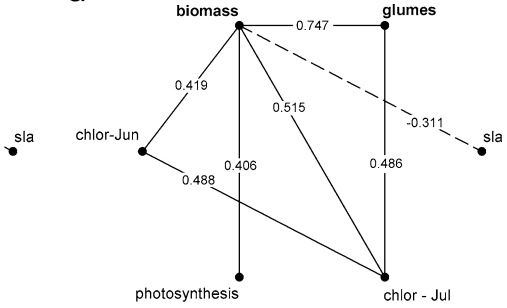
F loam - 5



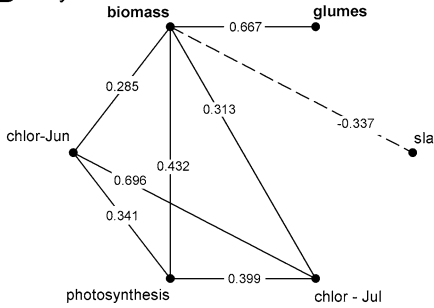
C clay - 16



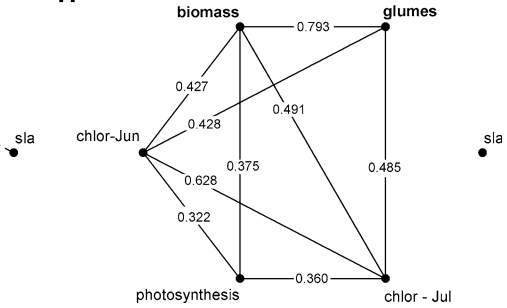
G loam - 16



D clay - 32



H loam - 32



◀ **Fig. 3** Phenotypic correlations between physiology and fitness in each treatment combination. Significant positive correlations ($P < 0.05$ after Bonferroni correction) are indicated with *solid lines* and significant negative correlations are indicated with *dashed lines*. Correlation coefficients are indicated. See Fig. 2 for trait units

SLA is contrary to adaptive expectations (Bradshaw et al. 1964; Grime and Hunt 1975; Chapin III 1980; Poorter and Remkes 1990; Lambers and Poorter 1992; Grime 2001). Selection was stronger on average in the loam soil than clay soil and most instances in which selection on a particular trait differed significantly between soil types were due to stronger selection in the loam soil (Tables 3, 4). Species diversity did not influence selection on physiology in a consistent manner as selection rarely differed significantly between diversity treatments in either soil type (Tables 3, 4).

Selection may have been stronger in the loam soil because plants experienced greater drought stress in that soil type. Water availability can be a strong agent of selection on plant physiology (e.g., water-use efficiency: Dudley 1996; Heschel and Riginos 2005; Donovan et al. 2009; Franks 2011; photosynthetic rate and photosynthetic capacity: Sherrard and Maherali 2006) and our research site in Iowa experienced a lengthy summer drought in 2012. Loam soils drain faster and have lower available water capacity than clay soils (NRCS 2014) because of differences in soil characteristics, such as percentage of organic matter and to a lesser extent, percentage of sand and clay (Hudson 1994). Consistent with its lower available water capacity, we found that soil moisture and soil organic carbon were both lower in the loam soil than clay soil (Table 1). Plants also produced less aboveground biomass in the loam soil than clay soil (Fig. 2), which is indicative of greater drought stress. In some species, drought reduces selection for high photosynthesis and increases selection for conservative water use (Dudley 1996; Sherrard and Maherali 2006) but this did not occur in our switchgrass populations. Instead, selection favored increased photosynthesis and chlorophyll concentration (Tables 3, 4), which indicates that individuals with higher photosynthetic function had higher fitness. These attributes may have been adaptive because they provided individuals with more photosynthate to invest in their root systems for water uptake. Increased capacity for water uptake would benefit plants in both soil types during drought but would have the greatest adaptive significance in the faster-draining loam soil. Future research could test this hypothesis more directly by comparing the strength of selection on traits associated with water uptake, such as root hydraulic conductivity, in environments of contrasting diversity. Although nutrient availability also varied between soil types (phosphorus; Table 1), we do not think that selection was stronger in the loam soil because of differences in fertility. Theoretical predictions (Grime 2001) and experimental data (Verhoeven et al. 2004) suggest that selection for increased photosynthesis, chlorophyll concentration, and SLA should all be stronger in higher-nutrient soil than lower-nutrient soil, which contrasts with our observed patterns of selection.

Our inability to detect differences in selection between diversity treatments could be due to the stronger influence of drought as an agent of selection in this particular year. We predicted that selection on physiology would be weaker in the high-diversity mixtures because legume facilitation and better niche differentiation would reduce competition for water and nutrients (Loreau and Hector 2001; Cardinale et al. 2007). In contrast, selection rarely differed between diversity treatments in either soil type (Tables 3, 4). Two lines of evidence support our interpretation that drought overwhelmed the potential effect of species diversity as an agent of selection. First, most instances in which selection differed significantly or marginally between soil types occurred in the high-diversity mixes (16 and

32 species; Tables 3, 4), where niche differentiation may have influenced water dynamics. Second, differences in the relative strength of selection between diversity treatments only occurred on the clay soil (photosynthetic rate; Table 3), which was the soil type with greater available water capacity (NRCS 2014; Table 1). Species diversity might have a greater impact on plant evolution in benign years; however, based on the results of this study, we conclude that species diversity is a weak agent of selection and only influences the evolution of physiology by altering the impact of other agents.

Specific leaf area (SLA), or its inverse ‘leaf mass area (LMA)’, is a trait that reflects a plant’s ecological strategy and has been identified as a key element of the worldwide leaf economics spectrum (Reich et al. 1999; Wright et al. 2004). Because fast-growing species tend to produce thin leaves (high SLA) with high nitrogen content and high photosynthesis, we predicted that selection would favor high SLA in switchgrass because it would allow individuals to grow fast and monopolize resources (Grime 2001). Instead, selection favored reduced SLA in all treatment combinations (Tables 3, 4). Previous studies have shown that selection on SLA is altered by environmental variation (Caruso et al. 2006; Agrawal et al. 2008; Donovan et al. 2011). For example, selection favors reduced SLA in dry environments but not wet environments in *Lobelia siphilitica* (Caruso et al. 2006). For switchgrass, low SLA may have increased fitness because it promotes longer tissue persistence and greater internal shading of leaf chloroplasts, which can be beneficial in dry environments (Reich et al. 1999; Wright et al. 2004). A recent review on the evolution of the worldwide leaf economic spectrum found that selection, not genetic constraint, is the most likely cause of the correlations among leaf traits (Donovan et al. 2011). One reason for their conclusion was that genetic correlations between LMA and other leaf traits are positive, negative, and non-significant with equal frequency in the literature (Donovan et al. 2011). Consistent with this conclusion, SLA was not phenotypically correlated with photosynthetic rate or chlorophyll concentration in any treatment combination in our study (Fig. 3).

Our research site provided a rare opportunity to study the effect of species diversity as an agent of selection. Communities with identical environmental characteristics but differing species diversity do not exist in the wild, which makes this a difficult question to test. Our switchgrass populations were established in the same year, with the same source seed, on multiple soil types, in communities of contrasting diversity, at a scale appropriate for phenotypic selection analysis. With this design, species diversity (and the associated differences in functional diversity) and soil type should be the main factors responsible for any differences in selection between treatment combinations (Wade and Kalisz 1990). We do not anticipate that the differences we observed in selection between treatment combinations will actually cause phenotypic divergence at the site because of the close proximity of the treatment combinations, the short-term tenure of the project, and annual site management. Nevertheless, our data still provide interesting perspective into the roles of species diversity and soil type as agents of selection in natural communities. In particular, our results suggest that soil characteristics are an important driver of physiological divergence in dry years because they influence available water capacity. Further, our results suggest that species diversity has little direct influence on the evolution of physiology despite its clear effect on productivity and other ecosystem properties (e.g., Tilman et al. 1996; Hector et al. 1999; Balvanera et al. 2006; Cardinale et al. 2007).

The two fitness metrics used in this study (glume number and aboveground biomass) revealed similar patterns of selection on physiology. Plants with high photosynthesis, high chlorophyll concentration, and low SLA produced more glumes and had greater aboveground biomass. Selection was also stronger in the loam soil with both fitness metrics. Attributes that increase both sexual reproduction and asexual reproduction should increase

in frequency within populations at a faster rate than attributes that only influence one mode of reproduction. This could be part of the reason why invasive species that reproduce both sexually and vegetatively adapt so rapidly to novel environments (e.g., St John's wort; Maron et al. 2004; reviewed in Buswell et al. 2011). Consistent with some previous studies of phenotypic selection (e.g., Farris and Lechowicz 1990; Arntz et al. 1998, 2000; Ludwig et al. 2004), our results suggest that physiological traits primarily influence seed production indirectly, via their effect on plant size. We found no direct selection on physiology when biomass was included in the multivariate selection model (data not shown) and far fewer significant selection gradients when glume number was the fitness measure (with biomass excluded from model) than when biomass was the fitness measure (with glume number excluded from model; Table 4). Nevertheless, selection should still act to increase photosynthesis and chlorophyll concentration and decrease SLA in these populations because of an overall association between physiology and both fitness measures (Table 3), direct associations between physiology and biomass (Table 4), and strong correlations between biomass and glume number in every treatment combination (Fig. 3). Although the strength of selection on physiology can vary with development (e.g., Sherrard and Maherali 2006), we found little difference in the strength of selection on chlorophyll concentration between June and July. This suggests that high photosynthetic function is important for fitness throughout switchgrass development.

Conclusion

Species diversity influences many aspects of ecosystem function (reviewed in Cardinale et al. 2007), but its effect on natural selection for physiology has not been tested. Similarly, with the exception of water availability (Dudley 1996; Heschel et al. 2002; Heschel and Riginos 2005; Sherrard and Maherali 2006), many of the hypothesized agents of selection on plant physiology have not been examined (Wade and Kalisz 1990; Ackerly et al. 2000; Arntz and Delph 2001). Our data indicate that soil characteristics influence selection on physiology via differences in available water capacity. This suggests that soil type will be a strong driver of phenotypic divergence in climatically extreme years (e.g., droughts, floods) and in regions with low annual precipitation. In contrast to its well-established influence on productivity, the role of species diversity as an agent of selection on physiology in switchgrass was indirect, and minor relative to other agents.

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References

- Abdala-Roberts L, Marquis R (2007) Local adaptation based on biotic interactions and soil abiotic conditions in the ant-tended *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 154:315–326
- Ackerly DD, Dudley SA, Sultan SE et al (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* 50:979–995

- Agrawal AA, Erwin AC, Cook SC (2008) Natural selection and predicted response for ecophysiological traits of swamp milkweed (*Asclepias incarnata*) in the field. *Ecology* 96:536–542
- Arntz M, Delph L (2001) Pattern and process: evidence for evolution of photosynthetic traits in natural populations. *Oecologia* 127:455–467
- Arntz AM, DeLucia EH, Jordan N (1998) Contribution of photosynthetic rate to growth and reproduction in *Amaranthus hybridus*. *Oecologia* 117:323–330
- Arntz M, DeLucia D, Jordan N (2000) From fluorescence to fitness: variation in photosynthetic rate affects fecundity and survivorship. *Ecology* 81:2567–2576
- Balvanera P, Pfisterer AB, Buchmann N et al (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol Lett* 9:1146–1156
- Beatty E, Engel J, Powell J (1978) Tiller development and growth in switchgrass. *J Range Manag* 31:361–365
- Bradshaw AD, Chadwick MJ, Jowett D et al (1964) Experimental investigations into the mineral nutrition of several grass species, IV. Nitrogen level. *Ecology* 52:665–676
- Broadbent FE (1965) Organic matter. In: Black CA (ed) *Methods of soil analysis*. Agron. Monogr. 9. American Society of Agronomy, Madison, pp 1397–1400
- Buswell JM, Moles AT, Hartley S (2011) Is rapid evolution common in introduced plant species? *J Ecol* 99:214–224
- Callaway R (1995) Positive interactions among plants. *Bot Rev* 61:306–349
- Cardinale BJ, Wright JP, Cadotte MW et al (2007) Impacts of plant diversity on biomass production increases through time because of species complementarity. *Proc Natl Acad Sci* 104:18123–18128
- Caruso CM, Maherali H, Sherrard ME (2006) Plasticity of physiology in *Lobelia*: testing for adaptation and constraint. *Evolution* 60:980–990
- Chapin FS III (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Conner JK (1988) Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 42:736–749
- Donovan LA, Dudley S, Rosenthal DM et al (2007) Phenotypic selection on leaf WUE and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia* 52:13–25
- Donovan LA, Ludwig F, Rosenthal DM et al (2009) Phenotypic selection on leaf ecophysiological traits in *Helianthus*. *New Phytol* 183:868–879
- Donovan LA, Maherali H, Caruso CM et al (2011) The evolution of the worldwide leaf economics spectrum. *Trends Ecol Evol* 26:88–95
- Dudley S (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102
- Fargione J, Tilman D, Dybzinski R et al (2007) From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proc R Soc* 274:871–876
- Farris MA, Lechowicz MJ (1990) Functional interactions among traits that determine reproductive success in a native annual plant. *Ecology* 71:548–557
- Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytol* 190:249–257
- Garnier E, Shipley B, Roumet C et al (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct Ecol* 15:688–695
- Geber M, Griffen L (2003) Inheritance and natural selection on functional traits. *Int J Plant Sci* 164:521–542
- Grime JP (2001) *Plant strategies, vegetation processes, and ecosystem properties* (2E). Wiley, Chichester
- Grime JP, Hunt R (1975) Relative growth-rate: its range and adaptive significance in a local flora. *Ecology* 63:393–422
- Heaton E, Voigt T, Long SP (2004) A quantitative review comparing the yields of two candidate C₄ perennial biomass crops in relation to nitrogen, temperature and water. *Biomass Bioenergy* 27:21–30
- Hector A, Schmid B, Beierkuhnlein C et al (1999) Plant diversity and productivity experiments in European grasslands. *Science* 286:1123–1127
- Hereford J, Hansen TF, Houle D (2004) Comparing strengths of directional selection: how strong is strong? *Evolution* 58:2133–2143
- Heschel MS, Riginos C (2005) Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *Am J Bot* 92:37–44
- Heschel MS, Donohue K, Hausmann N et al (2002) Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *Int J Plant Sci* 163:907–912
- Heschel MS, Sultan S, Glover S et al (2004) Population and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. *Int J Plant Sci* 165:817–824

- Hooper DU, Chapin FS III, Ewel JJ et al (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35
- Hudson B (1994) Soil organic matter and available water capacity. *J Soil Water Conserv* 49:189–194
- Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460
- Isbell FI, Wilsey BJ (2011) Increasing native, but not exotic, biodiversity increases aboveground productivity in ungrazed and intensely grazed grasslands. *Oecologia* 165:771–781
- Kingsolver JG, Hoekstra HE, Hoekstra JM et al (2001) The strength of phenotypic selection in natural populations. *Am Nat* 157:245–261
- Kingsolver JG, Diamond SE, Siepielski AM et al (2012) Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evol Ecol* 26:1101–1118
- Lambers H, Poorter H (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Adv Ecol Res* 23:187–261
- Lande R, Arnold S (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Levine J (2000) Species diversity and biological invasions: relating local process to community pattern. *Science* 288:852–854
- Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76
- Loreau M, Naem S, Inchausti P et al (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804–808
- Lovelock C, Felle I, McKee M et al (2004) The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Funct Ecol* 18:25–33
- Ludwig F, Rosenthal DM, Johnston JA et al (2004) Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early generation hybrids. *Evolution* 58:2682–2692
- Maron JL, Vilà M, Bommarco R et al (2004) Rapid evolution of an invasive plant. *Ecol Monogr* 74:261–280
- Myers M, Hoksich B, Mason J (2012) Butterfly response to floral resources during early establishment at a heterogeneous prairie biomass production site in Iowa, USA. *J Insect Conserv* 16:457–472
- Natural Resource Conservation Service (2009) Planting and managing switchgrass as a biomass energy crop. Technical Note No. 3. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1042293.pdf
- Natural Resource Conservation Service (2014) Web soil survey. <http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx> (accessed August 2014)
- Neter J, Wasserman W, Kutner MH (1989) Applied linear statistical models. Irwin, Homewood
- Parachnowitsch A, Cook-Patton SC, McArt SH (2014) Neighbours matter: natural selection on plant size depends on the identity and diversity of the surrounding community. *Evol Ecol* 28:1139–1153
- Poorter H, Remkes C (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* 83:553–559
- Rausher MD (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626
- Reich PB, Ellsworth DS, Walters MB et al (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955–1969
- Sandquist D, Ehleringer J (2003) Population- and family-level variation of brittlebush (*Encelia farinosa*, Asteraceae) pubescence: its relation to drought and implications for selection in variable environments. *Am J Bot* 90:1481–1486
- Schmer M, Vogel K, Mitchell R et al (2008) Net energy of cellulosic ethanol from switchgrass. *Proc Natl Acad Sci USA* 105:464–496
- Sherrard M, Maherali H (2006) The adaptive significance of drought escape in *Avena barbata*, an annual grass. *Evolution* 60:2478–2489
- Sims D, Kelley S (1998) Somatic and genetic factors in sun and shade population differentiation in *Plantago lanceolata* and *Anthoxanthum odoratum*. *New Phytol* 140:75–84
- Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York
- Stinchcombe JR, Rutter MT, Burdick DS et al (2002) Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *Am Nat* 160:511–523
- Tilman D, Wedin D, Knops JMH (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–720
- Tilman D, Reich PB, Knops JMH et al (2001) Diversity and productivity in a long-term grassland experiment. *Science* 294:843–845
- Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade long grassland experiment. *Nature* 441:629–632

- Tran TS, Simard RR (1993) Mehlich III-extractable elements. In: Carter M (ed) Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, pp 43–49
- Verhoeven KJF, Biere A, Nevo E et al (2004) Differential selection of growth rate-related traits in wild barley, *Hordeum spontaneum*, in contrasting greenhouse nutrient environments. *J Evol Biol* 17:184–196
- Wade M, Kalisz S (1990) The causes of natural selection. *Evolution* 44:1947–1955
- Whitney DA (1998) Micronutrients: zinc, iron, manganese and copper. In: Brown JR (ed) Recommended chemical soil test procedures for the North Central region, NCR Publication Number 221 (Revised). Missouri Agricultural Experimental Station, Columbia, pp 41–44
- Wright IJ, Reich PB, Westoby M et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827
- Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc Natl Acad Sci* 96:1463–1468