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Physiological Responses of C_4 Perennial Bioenergy Grasses to Climate Change: Causes, Consequences, and Constraints

Robert W. Heckman,[1] Caio Guilherme Pereira,[2,3] Michael J. Aspinwall,[4] and Thomas E. Juenger[2]

[1]Rocky Mountain Research Station, US Department of Agriculture Forest Service, Cedar City, Utah, USA; email: robert.heckman@usda.gov

[2]Department of Integrative Biology, University of Texas at Austin, Austin, Texas, USA; email: tjuenger@austin.utexas.edu

[3]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

[4]Formation Environmental, Sacramento, California, USA

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Abstract

C_4 perennial bioenergy grasses are an economically and ecologically important group whose responses to climate change will be important to the future bioeconomy. These grasses are highly productive and frequently possess large geographic ranges and broad environmental tolerances, which may contribute to the evolution of ecotypes that differ in physiological acclimation capacity and the evolution of distinct functional strategies. C_4 perennial bioenergy grasses are predicted to thrive under climate change—C_4 photosynthesis likely evolved to enhance photosynthetic efficiency under stressful conditions of low [CO_2], high temperature, and drought—although few studies have examined how these species will respond to combined stresses or to extremes of temperature and precipitation. Important targets for C_4 perennial bioenergy production in a changing world, such as sustainability and resilience, can benefit from combining knowledge of C_4 physiology with recent advances in crop improvement, especially genomic selection.

Contents

1. INTRODUCTION

C_4 grasses are dominant members of many ecosystems worldwide, especially in tropical and subtropical regions (60, 182). Despite making up only 3% of all plant species, plants using the C_4 photosynthetic pathway contribute nearly 25% of global net primary production and include many of the most productive species on earth (60, 161, 182). C_4 species tend to be particularly successful under stressful conditions caused by high temperature, high salinity, and low water availability (166, 167), which may help them maintain high performance as the climate changes. The C_4 grasses include several important crops (e.g., maize, sugarcane, and sorghum), which are characterized by high productivity, water-use efficiency, and nutrient-use efficiency (166). In recent decades, some C_4 grasses have also been targeted for bioenergy production (101, 183, 195). Candidate bioenergy grasses have been identified by their high productivity and broad environmental tolerances (101, 106, 225). Because of their potential utility as a renewable energy source, candidate bioenergy grasses are well studied relative to most undomesticated plants, but they have undergone limited artificial selection—they are largely unimproved (157). As such, they occupy an interesting biological space: They possess traits that distinguish them from most undomesticated species, but they have not undergone the extensive breeding that differentiates most crops from other plants. Thus, researchers can take advantage of the prominence of bioenergy grasses to advance our understanding of fundamental biological processes, including responses to climate change, while also helping advance the future bioeconomy.

Water-use efficiency: ratio of carbon gain to water loss during photosynthesis

In this review, we leverage the considerable suite of ecophysiological, genetic, and biogeographic research performed on C_4 perennial bioenergy grasses and their relatives to assess their responses to current and future climate change. We first outline the major physiological and ecological attributes of C_4 perennial grasses. We then discuss several characteristics that distinguish C_4 perennial bioenergy grasses from other species and the opportunities and challenges that these attributes pose to the improvement of bioenergy crops (see the sidebar titled What Makes a Good Bioenergy Crop?). Next, we assess the predicted and observed physiological responses of C_4 perennial bioenergy grasses to current and future climate change. Finally, we examine

WHAT MAKES A GOOD BIOENERGY CROP?

Lignocellulosic bioenergy production involves the conversion of plant biomass (typically leaves and woody or herbaceous stems) into an energy source, often ethanol or another liquid fuel (195). For bioenergy crops to be a viable energy source, plants should produce substantial biomass with limited inputs of water or nutrients and remain productive in marginal habitats that are unsuitable for conventional agriculture (101). Given this, an ideal bioenergy species would possess high productivity and water- and nutrient-use efficiency; extended periods of vegetative growth and delayed senescence; limited input requirements; and low establishment costs relative to returns (106). In the United States, most candidate bioenergy species were chosen because they exhibited high productivity in common garden trials across broad environmental conditions (e.g., 225). An alternative to carbohydrate-derived bioethanol is lipid-derived biodiesel, which often comes from seed oils such as canola or soybean and may be produced in the future from the stems of perennial C_4 grasses, such as sugarcane, energycane, and *Miscanthus* as well (165).

whether incorporating knowledge of plant physiology into modern crop improvement methods could promote greater bioenergy crop production in a changing world.

2. THE ECOPHYSIOLOGY AND NATURAL HISTORY OF C_4 GRASSES

The C_4 photosynthetic pathway arises from anatomical and biochemical changes that concentrate CO_2 in the presence of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the enzyme responsible for carbon fixation (181). This pathway helps overcome an important limitation of the C_3 photosynthetic pathway: photorespiration. Photorespiration occurs when RuBisCO reacts with O_2 instead of CO_2, which can reduce C fixation by upwards of 20% (132). Photorespiration generally occurs when the CO_2 supply is limited and the concentration of CO_2 decreases relative to O_2 inside leaves (e.g., when drought promotes stomatal closure). Increasing temperatures also increase photorespiration in C_3 plants for two reasons. First, as temperature increases, the CO_2:O_2 specificity of RuBisCO decreases such that oxygenation becomes more likely (181). Second, as temperature increases, there is more O_2 available to RuBisCO, given that the solubility of CO_2 decreases more rapidly than that of O_2 (114).

The efficiency of RuBisCO carboxylation in C_4 plants is increased by an energy-intensive mechanism that concentrates CO_2 at the site of CO_2 fixation. Specifically, CO_2 (in the form of bicarbonate, HCO_3^-) is linked with phosphoenolpyruvate (PEP) by the enzyme PEP carboxylase (PEPC) in the mesophyll cells to form a four-carbon (4-C) sugar [oxaloacetate (OAA)]. OAA is then reduced to malate and shuttled to the bundle sheath cells, where it is decarboxylated, then reduced by RuBisCO as in C_3 photosynthesis (181). Because of the energy required for these additional steps, C_4 photosynthesis should only be advantageous under conditions that increase the likelihood of photorespiration in C_3 plants (133). Early research on the efficiency of photosynthesis in different environments found that the temperature at which C_4 photosynthesis began to outperform C_3 photosynthesis increased with increasing [CO_2] (62). Research examining the environmental determinants of C_4 photosynthesis is ongoing, and our understanding of these processes is still incomplete (see the sidebar titled Key Knowledge Gaps in C_4 Photosynthesis).

Photorespiration: process in which RuBisCO reacts with O_2 instead of CO_2, resulting in reduced photosynthetic efficiency

PACMAD clade: a major monophyletic group of grasses that includes all C_4 grass species

The C_4 pathway has evolved at least 62 times across 19 angiosperm families, primarily within the last 30 million years (182). Research suggests that, over this time, water availability has played an important role in the evolution of C_4 photosynthesis in grasses. Among grasses, the C_4 pathway has evolved multiple times within one major group, the PACMAD clade (subfamilies Panicoideae, Aristidoideae, Chloridoideae, Micrairoideae, Arundinoideae, and Danthonioideae), but not within

KEY KNOWLEDGE GAPS IN C_4 PHOTOSYNTHESIS

Despite decades of research examining the physiology and biochemistry of C_4 photosynthesis, key knowledge gaps still hinder our ability to predict C_4 photosynthetic responses to climate change. Because of its biochemical complexity, our understanding of C_4 photosynthesis relies on a semimechanistic model that has not been tested widely across species and growth environments (196, 214). This model makes several simplifying assumptions, which, if inaccurate, could limit our understanding of C_4 photosynthesis and photosynthetic temperature acclimation. The C_4 photosynthetic model frequently assumes that carbonic anhydrase is nonlimiting; however, this assumption may be invalid at higher temperatures (29). Another common assumption is that the Michaelis-Menten constant for PEPC activity (K_P) is not temperature responsive (22, 214). Complications also arise when calculating the maximum rate of PEPC carboxylation (V_{pmax}) as this requires knowing mesophyll conductance (g_m). Because g_m has multiple anatomical, biochemical, and environmental determinants (135), it is highly plastic and often difficult to measure effectively in C_4 plants (29, 135). To estimate V_{pmax}, then, requires making assumptions about g_m to which current models are very sensitive. When information about specific biochemical process rates is not necessary for making inferences about C_4 plant physiology, fitting empirical functions to photosynthetic–CO_2 response (A-C_i) curves rather than theoretical functions may be appropriate. Clarifying mechanistic details of C_4 physiochemical processes and their temperature sensitivities will improve our ability to model C_4 photosynthesis over space and time.

another, the BOP clade (subfamilies Bambusoideae, Oryzoideae, and Pooideae), which includes important cereal crops such as wheat and rice (161). This probably occurred because prior to the origin of the C_4 pathway, PACMAD grasses had evolved traits that facilitated the subsequent evolution of C_4 photosynthesis when conditions began to favor it (160). These traits, such as increased bundle sheath tissue and more narrowly spaced veins, are thought to be adaptations to drier, more open habitats (160). Other evidence also supports the critical role of water availability in C_4 evolution. In Hawaii, C_3 and C_4 PACMAD grasses occupy habitats with similar temperatures, but C_4 species occur in drier habitats (61). Moreover, work on C_3–C_4 intermediate lineages suggests that high temperatures also contributed to the evolution of C_4 species (134).

The conditions under which the C_4 pathway evolved and the dominance of C_4 grasses in tropical and subtropical environments have implications for responses to climate change: Although rising [CO_2] will reduce photorespiration in C_3 plants at a given temperature or level of water stress, C_4 photosynthesis will still be advantageous under warmer and drier conditions (167, 200), which are predicted to increase in many areas in coming decades (92).

A key challenge for improving the yield of bioenergy crops is maximizing production without increasing inputs (i.e., fertilization or irrigation; 106), which can be accomplished in several ways. First, the vegetative growth period of bioenergy grasses can be extended by moving low-latitude genotypes and species to higher latitudes where flowering will be delayed (45, 99). These low-latitude plants are often highly susceptible to freezing or fail to senesce before freezing kills their leaves and causes loss of valuable nutrients (31, 129, 204). Thus, delayed flowering time may need to be combined with increased freezing tolerance (36). Second, nutrient-use efficiency can be increased through agronomic practices, such as temporally optimizing biomass harvest, which may require not harvesting biomass in some years, or harvesting only after nutrient remobilization has occurred (55, 83, 174). Recently, researchers have also examined the possibility of manipulating rhizosphere nitrogen-fixing bacteria to increase soil nitrogen availability (180). Third, increasing the water-use efficiency of plants usually takes one of two approaches: consuming less water to gain the same amount of carbon, thereby leaving some soil water unused, or efficiently using most

or all of the available water to gain more carbon (118). Of the two approaches, the latter would be more appropriate for bioenergy crops, where high production is critical.

3. IMPORTANT CHARACTERISTICS OF C_4 PERENNIAL BIOENERGY GRASSES

In bioenergy systems, perennial species have several advantages over annual species, including higher soil carbon sequestration and increased sustainability, especially in marginal habitats (65, 67). Perennial species also often have lower input requirements than annual species, which may be offset to some degree because perennials often fail to produce a first-year harvest. These advantages are associated with characteristics that could influence how perennial bioenergy grasses respond to management and climate change. Here, we highlight four interrelated consequences of C_4 perennial grass biology—optimization of growth, reproduction, and survival; internal carbon and nutrient dynamics; bud banks; and polyploidy.

Annual and perennial species exhibit substantial life history differences, which are often related to the habitats they occupy. In natural systems, perennial grass abundance is often determined by the frequency of disturbance (e.g., fire, herbivory, and harvests). When disturbance frequency is intermediate, perennial grasses can thrive, whereas frequently disturbed areas are dominated by annual species and infrequently disturbed areas are dominated by woody species (77, 110). Within and between habitats, selection for differences in life span has resulted in different strategies for allocating resources. In annuals, resource allocation should evolve to optimize reproductive output; in perennial grasses, resource allocation is more complex—some resources must be allocated toward storage for survival and future reproduction, while others are allocated toward current growth and reproduction (93, 213).

In seasonal environments, the balance of resource allocation to survival, growth, and reproduction impacts plant carbon and nutrient dynamics (26) (**Figure 1**). Overwinter survival and subsequent early-season growth require perennial herbaceous species, including perennial C_4 grasses, to store a portion of assimilated carbohydrates and acquired nutrients in perennial tissues like rhizomes (55, 174). Leaves, then, act as an early-season carbohydrate sink and later become a net carbohydrate source (140). Carbohydrate source–sink dynamics can impact the timing and duration of vegetative growth in a manner that is suboptimal for bioenergy production. In switchgrass (*Panicum virgatum*), photosynthesis sharply declined once rhizome starch content peaked (~10% dry weight), even though favorable conditions for growth continued for several weeks afterwards (201). Similarly, nutrient dynamics in undomesticated bioenergy grasses may not be optimized for bioenergy production. Remobilization of nitrogen, phosphorus, and other nutrients from senescing leaves to belowground tissue is critical for plant survival and the sustainability of bioenergy production (83, 229). It is also subject to a trade-off—remobilizing nutrients too early leads to a lost opportunity for additional growth, while remobilizing nutrients too late could lead to loss of nutrients from frost-killed leaves (204). Because vegetative growth largely stops once flowering begins in bioenergy grasses such as switchgrass, sugarcane, and *Miscanthus*, and flowering time is strongly associated with nutrient remobilization (186, 192), delayed flowering can increase biomass yield (36, 99, 191). But later-flowering plants risk nutrient loss from frost-damaged tissue (204).

Overwinter survival and early-season and postdisturbance growth depend on the continued viability of rhizomes and meristems, which form belowground bud banks (81, 162, 186). A key to understanding bud banks and how they impact the growth and ecology of C_4 perennial bioenergy grasses is demography—the birth, dormancy, activation, and death of meristems—although the biological processes underlying transitions between these stages are not well known (186). When

Marginal habitat: land that is economically untenable for conventional agriculture, often due to stressful environmental conditions, which may be well-suited for bioenergy production

Bud banks: dormant belowground meristems that form the basis of vegetative reproduction in many species, including perennial bioenergy grasses

Trade-off: condition in which a phenotype that provides an advantage in one environment results in a disadvantage in that, or another, environment

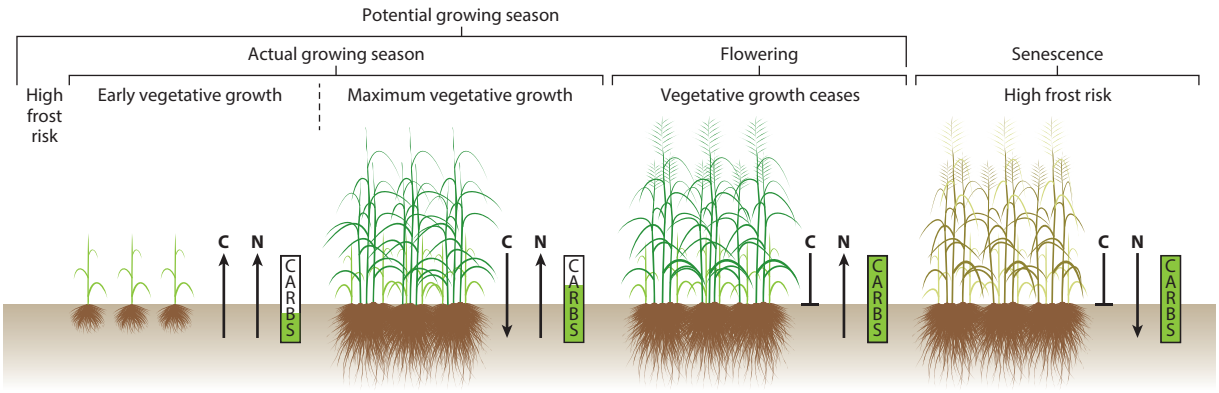

Figure 1

Aspects of perennial grass biology that should be considered in bioenergy production systems. Because C_4 perennial bioenergy grasses have been subject primarily to natural selection for optimized growth and survival under natural conditions, their phenology and carbon (C) and nutrient (N) dynamics may differ from those of domesticated relatives. First, to survive dormancy and initiate growth the following spring, perennial grasses may prioritize carbohydrate (CARBS) storage and end-of-season foliar nutrient remobilization over vegetative growth. To that end, vegetative growth may cease when plants begin flowering and/or acquire sufficient carbohydrate reserves for future growth. Second, the possibility of early- or late-season freezing can further constrain the active growing season in C_4 perennial grasses: Early-season freezing may kill actively growing tissue, while late-season freezing could prevent nutrient remobilization to belowground storage. Thus, increased freezing tolerance, enhanced carbohydrate storage capacity, and delayed flowering could lengthen the active growing season in a manner that could enhance biomass production. Figure prepared by Mark Seniw.

bud bank density is high, such as in early spring or among older plants in temperate grass species, plants may be more responsive to environmental conditions (162). Moreover, because bud banks act as points of new vegetative growth, they can buffer plant populations through poor conditions, such as drought, or promote rapid responses to favorable conditions, such as abundant rainfall (85, 178). There are several advantages of vegetative reproduction from bud banks over sexual reproduction from seeds. First, plants are able to continuously provide carbohydrates and nutrients to their bud banks in a manner that is not possible for seeds (162). Second, buds are genetically identical to the parent plant, which may promote greater local adaptation than can outcrossing seeds (81, 162). Bud banks are also critical for bioenergy production: Biomass yield is largely a function of tiller density and tiller mass (13, 27), both of which are proximally driven by the number and size of active meristems (21, 162).

Local adaptation: condition in which fitness is higher for the local population within an environment than for populations originating elsewhere

Polyploidy: condition in which organisms possess more than two complete sets of homologous chromosomes in their somatic cells; the symbol *x* indicates the number of chromosomes in a set (tetraploids have four sets: 4*x*)

One possible explanation for the high productivity and broad environmental tolerances of many C_4 perennial bioenergy grasses is polyploidy, an increase in the number of copies of homologous chromosomes. Polyploidy provides many ecological and evolutionary advantages. For instance, gene duplication can improve performance in stressful environments (207) and acts as a potential source of evolutionary innovation, where one pair of homologous genes maintains its current function while another pair can evolve a new function (neofunctionalization) or provide an additional dosage for the original function (164). Polyploidy can also mitigate the impact of deleterious mutations through genetic redundancy—the masking of deleterious alleles by extra copies of wild-type alleles—or as a general result of elevated heterozygosity (187). Interspecific hybridization is an important route for the generation of polyploids, which often exhibit hybrid vigor through the masking of deleterious mutations, nonadditive gene action, or the overdominance of gene expression (42). Within C_4 perennial bioenergy species, cytotypes (i.e., plants with different ploidy levels) can differ in environmental tolerance. In switchgrass, octoploids (8*x*) exhibit a generalist strategy—the ability to perform stably and well in a variety of environments

across space and time—while tetraploids (4*x*) exhibit a specialist strategy—performing extremely well in a narrower range of conditions and poorly outside those conditions (149). In big bluestem (*Andropogon gerardii*), the abundance of octoploids increased relative to hexaploids (6*x*) with rising aridity (109), suggesting that higher ploidy can improve stress tolerance. In *Miscanthus* × *giganteus*, tetraploids developed leaves with higher tissue construction costs than triploids (3*x*), indicating a more conservative resource allocation strategy (120).

Together, these aspects of perennial grass biology present opportunities and challenges for developing C_4 bioenergy systems across diverse habitats. In particular, the seeming optimization of many bioenergy grasses toward success in natural environments may constrain their production in a bioenergy context, yet more work is needed to understand the consequences of these key characteristics.

4. FUNCTIONAL AND PHYSIOLOGICAL STRATEGIES OF BROADLY DISTRIBUTED BIOENERGY GRASSES

Several C_4 perennial bioenergy grasses have large ranges covering varied climatic conditions (44, 129). For example, switchgrass and big bluestem occur throughout much of eastern and central North America from Mexico to Canada (129, 232), while *Miscanthus sacchariflorus* and *Miscanthus sinensis* span much of East Asia, from far eastern Russia and northeastern China through Southeast Asia (44). We examine two consequences of this variation in climatic conditions, which may both result from differences in leaf and phytomer longevity: the evolution of distinct resource allocation strategies and differences in acclimation to climatic extremes (158, 193).

In widespread species such as switchgrass and *Miscanthus* spp., selection to occupy diverse habitats may have driven the evolution of locally adapted ecotypes that exhibit distinct leaf economics strategies (e.g., 13, 120). The leaf economics spectrum is thought to arise from a trade-off between rapid resource acquisition in high-resource environments and resource conservation in low-resource environments, which manifests as an acquisitive strategy of short-lived, high-nutrient tissue with high metabolic rates and a conservative strategy of long-lived, low-nutrient tissue with low metabolic rates (176, 224). The leaf economics spectrum within species may also result from differences in growing season length—long growing seasons often promote long-lived leaves that are a key component of the conservative leaf economics strategy (111, 158, 224). Thus, short growing seasons at higher latitudes may promote an acquisitive strategy, while longer growing seasons at lower latitudes may promote a conservative strategy (**Figure 2**). Similar results have been observed in other groups: Acquisitive strategies were associated with cooler environments in *Helianthus* spp. and *Populus fremontii* (76, 141) and with higher elevation sites in four deciduous alpine species (115). Economics strategies can also be important determinants of bioenergy production. Plants with a conservative strategy of long-lived, low-nutrient tissue often have higher tissue construction costs, including higher levels of structural compounds such as lignin (158), which can reduce bioenergy conversion efficiency (231).

Species occupying broad ranges may also exhibit differences in physiological acclimation capacity. Perhaps the best-studied and most important form of physiological acclimation is temperature acclimation of leaf physiology (e.g., 87, 228). Definitions of photosynthetic temperature acclimation have varied among studies and over time (222). Here, we consider temperature acclimation of leaf physiology to be a reversible (occurring within a few days to a month) change in the instantaneous (short-term) temperature response of leaf photosynthesis (*A*) and/or respiration (*R*) in response to a change in growth temperature (28, 177) (**Figure 3*a***), regardless of whether the adjustment improves plant performance. Hikosaka et al. (87) identified several of the likeliest processes explaining temperature acclimation of *A* in C_3 species, which include changes

Ecotype: a genetically distinct group within a species adapted to a particular environment

Leaf economics spectrum: correlated suite of functional traits hypothesized to result from a trade-off between resource acquisition and conservation

Physiological acclimation: reversible change in the instantaneous physiological response to a change in growth conditions, often resulting from physiological or biochemical modifications

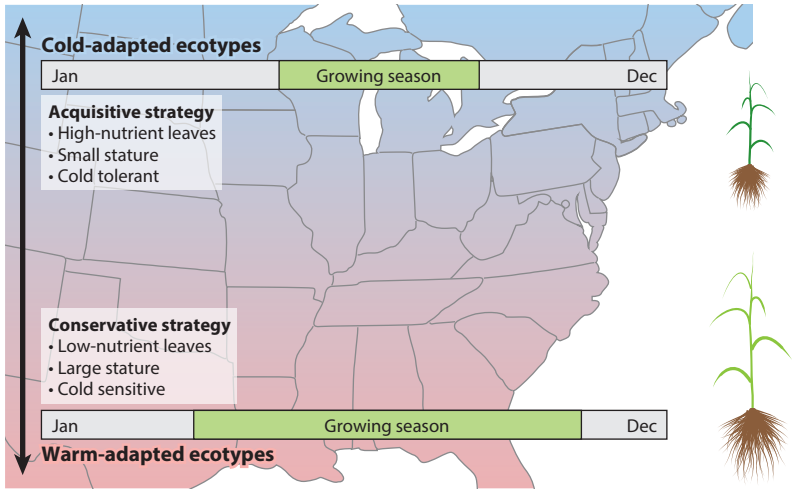

Figure 2

Hypothesized relationship between growing season length and the evolution of plant functional strategies. Ecotypes adapted to warm habitats with long growing seasons are often larger in stature and less cold tolerant than ecotypes adapted to cold habitats with short growing seasons. Growing season length, and the associated change in leaf longevity, may promote the evolution of distinct leaf economics strategies: Longer growing seasons may promote a conservative strategy of longer-lived, low-nutrient tissue with high construction costs (e.g., high leaf mass per area); shorter growing seasons may promote an acquisitive strategy of shorter-lived, high-nutrient tissue with low construction costs. Figure prepared by Mark Seniw.

in intercellular [CO_2] (C_i), changes in the activation energy of RuBisCO carboxylation (V_{cmax}), the temperature sensitivity of electron transport for ribulose-1,5-bisphosphate (RuBP) regeneration (J_{max}), and the ratio of RuBP regeneration to RuBisCO carboxylation (J_{max}/V_{cmax}). Similar processes could be important in C_4 species, as could changes in the activation energy of PEPC. Among these candidates, changes in the activation energy of RuBisCO and the J_{max}/V_{cmax} ratio have been observed most often in C_3 species (87). This suggests that plants adjust the activation state of RuBisCO or the activity of RuBisCO activase as growth temperature changes and may shift nitrogen partitioning to the most limiting process (e.g., RuBP regeneration and RuBisCO carboxylation) to maximize A at the new temperature. More recent studies have highlighted the importance of stomatal sensitivity to increasing atmospheric vapor pressure deficit (D) and the temperature response of R in explaining variation in the temperature response of A (124), as well as the importance and seasonal changes in growth and carbohydrate production and use (11). Therefore, temperature acclimation processes may be best understood when both biochemical and whole-plant responses to growth temperature are considered.

Studies on physiological temperature acclimation in C_4 species are limited. One synthesis study found evidence for reduced acclimation capacity of C_4 species relative to C_3 species: C_4 species exhibited smaller changes in the optimum temperature (T_{opt}) of A with increasing growth temperature than C_3 species, which was likely due to the greater complexity of the C_4 photosynthetic machinery (228). However, individual studies have found that some C_4 species show similar or even greater acclimation capacity than some C_3 species (194, 197).

Physiological temperature acclimation can vary within and among species. A long-standing hypothesis posits that plant populations experiencing variable growing conditions are better able to acclimate than populations experiencing uniform growing conditions (23). Some evidence supports this hypothesis in trees (e.g., 56, 205), but it remains largely untested in C_4 grasses. In broadly

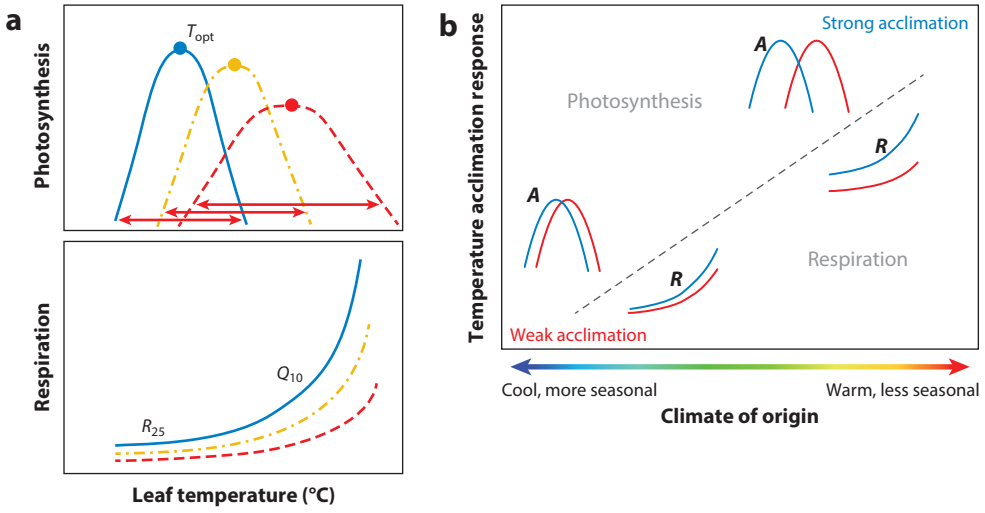

Figure 3

Predicted effects of physiological temperature acclimation in C_4 perennial grasses. (*a*) Conceptual representation of the influence of growth temperature on the instantaneous temperature response of leaf photosynthesis and respiration. As growth temperature changes from cool (*solid blue line*) to moderate (*dotted/dashed yellow line*) to warm (*dashed red line*), the instantaneous temperature response of each parameter changes, reflecting temperature acclimation. T_{opt} is the temperature optimum of photosynthesis (A), Q_{10} is the temperature sensitivity of leaf dark respiration (R), and R_{25} is leaf dark respiration at 25°C. (*b*) Hypothesized relationship between the thermal origin and growing season length of genotypes and thermal acclimation capacity of physiology (ΔT_{opt} of A; ΔR_{25}). Genotypes adapted to warmer environments—where growing seasons are longer and growing season temperature is more variable—are expected to exhibit stronger temperature acclimation of both photosynthesis and dark respiration. Figure prepared by Mark Seniw.

distributed C_4 perennial bioenergy grasses, genotypes from warmer (temperate) sites might exhibit greater acclimation capacity because warmer sites tend to experience more variation in conditions during the growing season—longer frost-free growing seasons and a wider growing season temperature range—and longer-lived leaves than cooler sites (23) (**Figure 3*b***). Thus, genotypic differences in acclimation capacity may be associated with variation in growing season length, flowering time, leaf area retention, and investment in leaf structure (e.g., leaf thickness and leaf carbon concentration) and resource use (e.g., leaf nitrogen concentration), attributes related to temperature adaptation.

Growing season length may be an important link between leaf economics strategies and physiological acclimation. From the perspective of leaf investment, genotypes producing longer-lived, more expensive leaves (i.e., a conservative strategy) may have evolved a greater ability to adjust photosynthetic and respiratory capacity in response to changing temperatures than genotypes that produce shorter-lived, cheaper leaves (i.e., an acquisitive strategy). In switchgrass, for example, adaptation to a longer, more variable growing season in warm-origin genotypes could in turn enable a greater capacity for temperature acclimation of leaf physiology. Moreover, warm-origin switchgrass genotypes tend to flower later and produce longer-lived, thicker leaves with lower nitrogen concentration (i.e., a conservative strategy) than genotypes from cooler climates with shorter, less variable growing seasons (13, 129). Consequently, high physiological acclimation capacity may be correlated with late flowering and a conservative resource allocation strategy. While

evidence supporting parts of these hypotheses exists, the impact of climatic origin and economics strategies on acclimation capacity remains untested in C_4 grasses.

Legacy effect: historical contingency that alters plant responses to current conditions due to physiological and morphological responses to prior conditions; time-lagged effects

Physiological acclimation is an important form of a reversible plastic response to environmental change, but other plastic responses, such as legacy effects, are also common (6, 178). These varied responses to changes in temperature and water availability have traditionally been studied by different researchers at different scales using different terminology (but see 105). At the leaf scale, physiologists often examine physiological acclimation as a short-term biochemical process (222), while at the whole-plant and population scales, ecologists often study legacy effects as a longer-term anatomical or demographic process (85, 178). Despite these differences in approach and scale, legacies and physiological acclimation may simply be on opposite ends of a continuum of responses that result from both molecular (e.g., change in gene expression, DNA methylation, open chromatin, and production of stress hormones) and developmental responses (30, 139). For instance, short-term and longer-term plastic responses may result from rapidly implemented and easily reversible processes, such as osmotic adjustment (16, 18), as well as slower and less reversible processes, such as constructing cavitation-resistant xylem vessels (70).

5. PHYSIOLOGICAL RESPONSES OF C_4 PERENNIAL BIOENERGY GRASSES TO CLIMATE CHANGE

In the last several decades, a growing literature on the physiology of bioenergy grasses has emerged, including in the context of climate change. To understand how C_4 perennial bioenergy grasses will respond to current and future climate change, we surveyed the literature on C_4 perennial bioenergy grasses and their physiological responses to climate change drivers (elevated [CO_2] and changes in temperature and precipitation). Specifically, we performed a keyword search in Google Scholar focused on nine bioenergy grasses (**Table 1**), which included the common

Table 1 Notable C_4 perennial bioenergy grasses

Species	Scientific name(s)	Native range	Ploidy	Status
Aleman grass	*Echinochloa polystachya*	Tropical Americas	6*x*, 12*x*	Under-studied bioenergy candidate; forage species; wetland dominant
Big bluestem	*Andropogon gerardii*	Temperate North America	6*x*, 8*x*, 9*x*	Minor bioenergy candidate; forage species; tallgrass prairie dominant
Foxtail millets	*Setaria viridis*, *Setaria italica*	Eurasia, China	2*x*	Annual model for C_4 bioenergy species; major crop
Guinea grass	*Megathyrsus maximus*	Tropical Africa	Primarily 4*x*; seldom 7*x*, 11*x*	Under-studied bioenergy candidate; invasive in many (sub)tropical regions
Miscanthus	*Miscanthus sinensis*, *Miscanthus sacchariflorus*, *Miscanthus* × *giganteus*	Temperate East Asia	3*x*, 4*x*, 6*x*	Primary bioenergy candidate; *M.* × *giganteus* is frequently an infertile hybrid
Napier grass	*Pennisetum purpureum*	Tropical Africa	4*x*	Under-studied bioenergy candidate; forage species
Prairie cordgrass	*Spartina pectinata*	Temperate North America	4*x*, 6*x*, 8*x*	Minor bioenergy candidate
Sugarcane/ energycane	*Saccharum officinarum*, *Saccharum spontaneum*, and hybrids	New Guinea; cultivated globally	Highly variable	Primary bioenergy candidate; major crop
Switchgrass	*Panicum virgatum*	Temperate North America	4*x*, 8*x*	Primary bioenergy candidate; forage species

or scientific name of each species plus climate change drivers (e.g., "elevated CO_2," "drought," "flooding," "warming," and "cold") plus "physiological responses." We identified 60 studies that described the response to a climate change driver of one or more major physiological parameters (e.g., photosynthetic rate, stomatal conductance, and leaf water potential; see **Supplemental File 1** for parameters described in each study).

Supplemental Material >

5.1. Main Effects of CO_2, Water, and Temperature

Many studies have examined the independent effects of elevated [CO_2] as well as changes in water availability and temperature on C_4 species. Considering these effects in isolation may be misleading for predicting long-term responses to climate change. But understanding the independent effects of water and temperature fluctuations, especially extreme events such as drought, flooding, heat waves, and cold snaps, is still important for managing C_4 bioenergy production under current field conditions. In general, C_4 photosynthesis is thought to be saturated at current [CO_2], so unlike C_3 plants, the benefits for C_4 grasses of increasing [CO_2] tend to be indirect and more limited (184). C_4 grasses also tend to perform better than C_3 grasses at lower water availability, partly due to greater photosynthetic capacity and intrinsic water-use efficiency (166, 200). However, severe drought can still drastically reduce the performance of C_4 grasses (127). Responses to flooding vary among species; many genotypes of lowland switchgrass do well under flooding (15), as does Aleman grass (*Echinochloa polystachya*), a species from the Amazon floodplain which has exhibited some of the highest productivity values ever observed (171). Finally, C_4 perennial grasses typically perform well at high temperatures, likely because PEPC has a high temperature optimum (228). C_4 grasses, especially the major bioenergy grasses, are relatively freezing intolerant, although *Miscanthus*, upland switchgrass, and prairie cordgrass (*Spartina pectinata*) exhibit some cold tolerance (101, 129, 183).

In **Table 2** and **Supplemental File 1**, we summarize the independent effects of these climate change drivers on C_4 perennial bioenergy grass physiology. Below, we describe the predicted and observed interactive effects of climate change drivers on C_4 perennial bioenergy grass physiology.

5.2. CO_2–Water Interactions

In mesic environments, increased [CO_2] will likely have little effect on photosynthesis or productivity because C_4 photosynthesis is already maximized at current atmospheric [CO_2] (117, 184). However, in drier areas, elevated [CO_2] could promote increased productivity of C_4 grasses by further increasing water-use efficiency (144). Increases in water-use efficiency under elevated [CO_2] result from reduced stomatal conductance (g_s), such that more carbon is fixed per unit water loss (4, 5). Likewise, increased water-use efficiency could help C_4 grasses expand into drier and marginal areas or increase their tolerance of episodic droughts.

Among C_4 perennial bioenergy grasses, empirical evidence generally aligns with prediction. In big bluestem and sugarcane, drought-induced declines in A were largely alleviated under elevated [CO_2] even though g_s declined or did not change (1, 107, 217). This indicates that intrinsic water-use efficiency (iWUE) either increased or remained the same under combined drought and elevated [CO_2]. This CO_2-mediated alleviation of drought stress is likely the result of reduced g_s (and increased iWUE) that slowed soil water depletion but provided sufficient CO_2 supply to maintain relatively constant rates of A under dry conditions (217).

5.3. CO_2–Temperature Interactions

Because C_4 photosynthesis is already maximized at current [CO_2] (117), at least under well-watered conditions, elevated [CO_2] is unlikely to interact with warming to alter the responses

Table 2 Summary of environmental effects associated with climate change, their hypothesized impacts on C_4 perennial grass physiology, and the empirical evidence collected for the bioenergy crops included in this study

Stress	Hypothesis	Studied species	Empirical support
Elevated [CO_2]	Increases in atmospheric [CO_2] are not expected to affect A, but should lead to a decrease in g_s, thus increasing iWUE	Big bluestem	No; most studies showed increases in A (1, 104, 107), even if it was conditional to dry years only (1); g_s decreased in all cases (1, 107, 163).
		Foxtail millet	No; open-top experiments have shown increases of up to 123% on A, depending on sampling year and developmental stage (74, 119), as well as consistent increases in iWUE and, occasionally, g_s (119).
		Guinea grass	Mixed; elevated [CO_2] either led to an increase of 25% in A and 71% in iWUE with a 30% decrease in g_s (80) or did not affect A and g_s but increased iWUE (50).
		Miscanthus	Yes; elevated [CO_2] had no effect on A, but significantly reduced g_s and E, thus improving iWUE (123).
		Napier grass	Yes; elevated [CO_2] did not affect A under either cool or warm conditions; it increased iWUE by up to 49% (146).
		Sugarcane	Mixed; several experiments have shown increases of up to 30% in A and 62% in iWUE with a decrease of up to 51% in g_s (52, 218); others have shown no effect of elevated [CO_2] on A but decreases in g_s and increases in iWUE (51, 217).
Drought	Drought is expected to inhibit A of C_4 plants through a decrease in g_s and a reduction in total plant biomass	Aleman grass	Yes; drought led to a decrease in A, g_s, and Ψ_{leaf} (17).
		Big bluestem	Yes; drought led to a decrease in A, g_s, iWUE, F_v/F_m, and Ψ_{leaf} (14, 47, 88, 89, 107, 137, 138, 151).*
		Foxtail millet	Yes; drought led to a decrease in A, g_s, E, F_v/F_m, Φ_{PSII}, and Ψ_{leaf} and an increase in iWUE (58, 185, 199, 206).*
		Miscanthus	Yes; drought led to a decrease in A, g_s, E, F_v/F_m, and Ψ_{leaf}; iWUE was either the same or higher (91, 116, 189, 223).*
		Napier grass	Yes; drought led to significant reduction in total plant growth, as well as a decrease in A, g_s, and E (33, 146, 147).*
		Sugarcane	Yes; drought led to reductions in A, g_s, E, and F_v/F_m; iWUE also increased in most studies (54, 63, 190, 217, 234, 235).*
		Switchgrass	Yes; drought led to reductions in A, g_s, E, and Ψ_{leaf}; iWUE also increased in most studies (82, 125, 130, 142).*
Flooding	Flooding is expected to affect A indirectly through a reduction in growth due to oxygen deprivation	Aleman grass	No; flooding either did not affect A, g_s, and alcohol dehydrogenase activity (17) or led to an increase of 82% in A and 100% in g_s when compared to the dry phase of plant growth (172).
		Big bluestem	No; flooding did not affect A and g_s but decreased iWUE (47, 137).

(*Continued*)

Table 2 (*Continued*)

Stress	Hypothesis	Studied species	Empirical support
		Sugarcane	No; periodic flooding either did not affect or moderately increased A, g_s, and E (73, 96).
		Switchgrass	Mixed; a 50% increase in precipitation led to a 15.9% higher A, as well as a higher E and plant growth in one study (90), but no effect on A despite the increase in g_s and E in another study (53). In a different study, a 25% increase in precipitation did not alter gas-exchange rates but decreased iWUE and Ψ_{leaf} (82). Another study also showed that infrequent, large precipitation events reduced A, g_s, and E compared to frequent, small events (155).
Heat	Very high temperatures (typically >35°C) are expected to inhibit A due to a lower activation of RuBisCO	Big bluestem	Mixed; heat stress reduced A and g_s in some studies (151, 220). It increased A in one (104); in others, either it had no effect on these physiological parameters (136) or the response was dependent on water availability (88, 89).
		Foxtail millet	Yes; heat stress led to a reduction in A, g_s, E, and Ψ_{leaf} (3, 185).
		Guinea grass	No; elevated temperatures led to increased g_s (50) and E (50, 80), thus decreasing iWUE (50), but no change in A.
		Napier grass	Yes; high temperatures led to a decrease in A and plant growth (97).
		Sugarcane	Mixed; two studies showed a strong genotypic effect, with most varieties showing no photosynthetic response to heat, while some showed increases of up to 17% and one showed a 29% decrease in A (51, 126).
Cold	Low temperatures should limit A of C_4 species due to the sensitivity of certain enzymes, especially PPDK and RuBisCO, and due to the lower Φ_{PSII}	*Miscanthus*	Mixed; low temperatures led to reductions in A, g_s, E, and Φ_{PSII} in a cold-sensitive line of *M.* × *giganteus*, while these parameters were unchanged in a cold-tolerant one (24). In two studies, A and Φ_{PSII} decreased with cold exposure, with increases in [PPDK] (69, 221), and, in another, the low temperature had no effect on A but [PPDK] increased (148).
		Napier grass	Yes; low temperatures led to reductions in A_{max}, V_{cmax}, and J_{max} (98).
		Sugarcane	Yes; low temperatures led to reductions in A and g_s (39, 57, 121).

*Not all of the studies listed have data for every physiological parameter presented; for a detailed description of the analyzed parameters in each study, as well as a summary of the results, see **Supplemental File 1**.

Abbreviations: A, leaf photosynthesis, broadly defined; E, leaf transpiration rate; F_v/F_m, maximum quantum yield of photosystem II (PSII) photochemistry; g_s, stomatal conductance; iWUE, intrinsic water-use efficiency; J_{max}, maximum rate of photosynthetic electron transport; PPDK, Pyruvate orthophosphate (P_i) dikinase; V_{cmax}, maximum rate of RuBisCO carboxylase activity; Φ_{PSII}, effective quantum yield of PSII photochemistry at incident photosynthetically active radiation; Ψ_{leaf}, leaf water potential.

Supplemental Material >

of C_4 grasses. Instead, regardless of $[CO_2]$, warming should promote higher rates of photosynthesis, to a point. This is likely to occur through faster enzymatic rates—especially of PEPC, which has a very high temperature optimum—without increases in photorespiration (228). Among C_4 perennial bioenergy grasses, our predictions held in Guinea grass (*Megathyrsus maximus*), where interactive effects of elevated $[CO_2]$ and warming on A or g_s were absent or muted (50, 80), but not in sugarcane, where elevated $[CO_2]$ and warming interactively reduced A and g_s (51), or in big bluestem, where elevated $[CO_2]$ and warming interactively promoted A (104). In these interaction studies, the physiological main effects of warming and elevated $[CO_2]$ differed slightly, even among studies on the same species. In one study in Guinea grass, elevated $[CO_2]$ increased iWUE and had no effect on A or g_s, while warming increased A and g_s and reduced iWUE (50). In the other Guinea grass study, elevated $[CO_2]$ increased A and iWUE and slightly reduced g_s, but warming did not impact these physiological parameters (80). In the sugarcane study, most physiological parameters exhibited significant genotype-by-environment (G×E) interactions. The presence of considerable G×E indicates that sugarcane has sufficient genetic potential to perform well under current and future climate change but complicates efforts to summarize the overall impact of warming and elevated $[CO_2]$ on sugarcane physiology (51).

Genotype-by-environment (G×E) interaction: condition in which different genotypes produce different phenotypes in response to different environments

Some of the observed physiological responses to climate change may be explained by anatomical and biochemical modifications. For instance, elevated $[CO_2]$ reduced stomatal density (stomates mm^{-2}) and stomatal index [percentage derived from the number of stomates divided by the number of stomates and epidermal cells] in Guinea grass (80). In the same study, warming reduced leaf thickness, which may explain the lack of temperature effect on gas exchange physiology. In big bluestem, the increases in A in response to both warming and elevated $[CO_2]$ independently may have resulted from increased PEPC efficiency and electron transport rate (104). Nonstructural carbohydrates may play an important role in physiological temperature acclimation, but they responded differently to warming in the two Guinea grass studies: In one study, sucrose, glucose, and fructose all declined with increasing temperature, but there was no difference in starch content, while in the other study, starch content declined with increasing temperature (50, 80). Together, these studies suggest a complicated relationship between the effects of elevated $[CO_2]$ and warming on plant physiology.

5.4. Water–Temperature Interactions

Unlike interactions between elevated $[CO_2]$ and other drivers, interactions between water and temperature will likely be more complex in a changing climate (184). When warming coincides with adequate water supply, photosynthesis and productivity could increase (10, 222). But the timing, length, and magnitude of changes in water availability could all modify the effects of warming. For example, early-season warming with adequate water supply should considerably increase photosynthesis and productivity, but this rapid early growth could leave plants more vulnerable to later drought (233).

Despite their high drought and heat tolerances, C_4 perennial grasses are still vulnerable to extremes, especially the combination of persistent drought and high temperatures (88, 89). To mitigate this combined stress, plants can implement several stress-alleviation mechanisms, which may depend on the severity, speed of onset, and duration of water limitation. In the short term, plants may transpire to facilitate leaf cooling, even when high g_s may not appear optimal for short-term carbon economics (25). However, rapid-onset droughts may not allow drought acclimation to occur, resulting in greater vulnerability to heat. Longer-term, slow-onset droughts may promote hydraulic or structural adjustments (i.e., drought acclimation) that maintain plant function during heat stress (40). Complex and sometimes conflicting results from previous drought × heat wave studies may be partly explained by plant growth stage, drought features [length,

severity, and timing (34)], and differences in drought acclimation among species or populations (20, 71).

Interactive drought × warming effects on C_4 perennial bioenergy grass physiology were largely absent in the studies we examined. For instance, in big bluestem, warming reduced A and g_s under moderate precipitation but not under drought (88, 89). Additionally, A, g_s, and midday leaf water potential were more responsive to soil water availability than to leaf temperature, while iWUE was more responsive to leaf temperature (151). Moreover, in Guinea grass, drought and warming did not interact to impact stomatal density, length, or index (79). Only in Napier grass (*Pennisetum purpureum*) were drought × warming effects common: g_s declined with drought under low temperatures, but not at high temperatures, while A declined in response to drought slightly more at low temperatures than at high temperatures (147).

Experimental design may have contributed to the results in some of these studies. The drought-induced decline in A at low temperatures in Napier grass may have been driven by temperature effects on plant water status; at high temperatures, midday leaf water potentials of the more mesic treatments were lower (i.e., more negative) and more similar to the severe drought treatment than at low temperatures (147). Similarly, interactive warming × drought effects on A and g_s may be lacking in big bluestem because the drought treatment elicited such severe water stress that warming had no additional negative effects (88, 89). Notably, air warming also tends to increase D, which can impact leaf function independent of air temperature (78). Yet few warming studies are designed to separate the effect of temperature and D, mainly because doing so requires humidity control, which can be logistically challenging. Thus, temperature effects in most studies are likely a combination of temperature and D effects (78). More studies are needed to understand the independent and interactive effects of D and temperature on C_4 grass physiology. Some results also shed light on possible biological mechanisms underlying responses to warming and drought. In Guinea grass, drought alone enlarged bulliform cells, which can promote improved leaf rolling, a key mechanism of water conservation in grasses (103), while warming alone increased epidermal thickness, which may also increase drought tolerance (79). In the annual C_4 species *Setaria viridis* (wild foxtail millet), associations between molecular and physiological responses are informative (185). Specifically, among stress-tolerant accessions, drought and heat (2 h per day at 42°C) induced high expression of aquaporins, which correlated well with high leaf water potentials, A, and g_s, and altered the expression of several genes associated with the abscisic acid signaling pathway.

5.5. CO_2–Water–Temperature Interactions

Elevated [CO_2] may alter the interaction between drought and warming in important ways. First, elevated [CO_2] could alleviate drought stress when heat waves are short or not severe. Specifically, warming could trigger rapid evapotranspiration that promotes intense drought when adequate water supply is not sustained. In this scenario, elevated [CO_2] could provide some benefits by reducing g_s and thereby limiting the loss of soil water. However, elevated [CO_2] could also exacerbate plant stress under drought and prolonged heat waves. Rising [CO_2] and drought both frequently reduce g_s, and without this transpiration-induced cooling, sustained high temperatures could push C_4 leaves close to their thermal limits, potentially resulting in tissue damage or death (94, 117).

Only one study in C_4 perennial bioenergy grasses has examined this three-way interaction. The combined effects of elevated [CO_2], drought stress, and warming increased stomatal number, reduced transpiration, and promoted strong osmotic adjustment in Napier grass (146). Osmotic adjustment is a key mechanism of physiological acclimation to water stress, which contributes strongly to turgor loss point (16). Thus, osmotic adjustment and other acclimation responses may have helped to maintain relatively high A and leaf water potential in this study.

5.6. Impacts of Experimental Design on Study Outcomes

Differences in the length and severity of temperature and precipitation treatments can impact the results that we see across studies. For instance, experimental drought often elicits weaker plant responses than natural drought, which may result because natural droughts often co-occur with heat waves, exacerbating water stress (113, 202). Another potential limitation of experimental droughts is the inability to control *D*, a key determinant of stomatal behavior (78). In fact, high *D* and warming can elicit strong physiological responses even when soil water is not limiting (188); future work examining drought responses of C_4 bioenergy grasses should consider the interaction between *D* and temperature. The rate of drought onset can also be important: Lovell et al. (130) found that the same genotype of *P. virgatum* experiencing the rapid onset of drought in small greenhouse pots had 10× more differentially expressed genes than plants experiencing a longer-term experimental drought in the field. While slow increases in water stress may be common, as in the Lovell et al. (130) field study, the rapid onset of flash drought, where high temperatures and extremely low humidity rapidly elicit severe water stress in plants, is being acknowledged as an important phenomenon (43).

Likewise, the speed with which warming treatments are implemented can impact plant responses. Natural heat waves often build over several days before reaching their peak intensity, potentially giving plants time to physiologically acclimate to higher temperatures (28, 177), while experimental heat waves often increase in temperature more abruptly. Moreover, the difference between pulse (i.e., heat wave) and press (i.e., long-term warming) studies can be large (94). Longer-term press studies often warm plots by a relatively small amount above ambient conditions (e.g., 1–3°C), which is valuable for understanding long-term physiological acclimation or responses at the population, community, or ecosystem levels. In many habitats, this level of consistent warming may have positive impacts on C_4 physiology because C_4 grasses often have high T_{opt} of *A* (228). On the other hand, short-term heat wave experiments often seek to induce heat stress by increasing temperature more (e.g., ≥5°C above ambient), which could push plants past the T_{opt} of *A*, thereby reducing their performance and increasing the chance of mortality (94).

Going forward, climate change studies in bioenergy grasses and other species could be improved through several minor changes. First, drought or warming studies could vary the length, frequency, or timing of stress to more closely mimic natural events (112, 202). Second, studies could incorporate climatic extremes, which are important drivers of changes in plant populations and communities (66, 153). Third, performing more studies that include combinations of stresses that could exacerbate one another will be informative. For instance, Novick et al. (154) examined the soil water conditions where surface conductance (which incorporates canopy/stomatal conductance) shifts from control by *D* to control by soil water availability. A similar approach could be implemented for warming × drought studies to identify the soil water threshold where heat effects on physiology become severe.

It is also critical to quantify treatment levels so they can be compared across studies. One way to improve comparisons across studies, especially across regions, is to report indices that describe the severity of a treatment relative to the local climate [e.g., the standardized precipitation evapotranspiration index (SPEI) (19)]. Another way to improve comparisons across precipitation studies is to quantify plant water status. This can be done by measuring predawn and midday leaf water potential, or another indicator of plant water status, when destructive sampling is acceptable, or soil water content or soil water potential when integrated measures of water status are desirable and repeated destructive sampling would be challenging (211, 213).

An important assumption that we make about C_4 physiology—based on theoretical considerations and substantial empirical evidence—is that C_4 photosynthesis is already maximized at

current [CO_2] and thus is unlikely to respond strongly to elevated [CO_2] (117, 184). However, ongoing research is attempting to improve photosynthesis via a combination of approaches from biochemistry and synthetic biology—such as improved RuBisCO functioning or light energy conversion efficiency (159, 215)—which might enable future C_4 bioenergy grasses to better exploit elevated [CO_2]. While the predicted responses of improved C_4 perennial bioenergy grasses to specific climate change drivers are beyond the scope of this review, we describe some of these promising approaches in Section 7.

6. IMPACTS OF PHYSIOLOGY ON BIOENERGY PRODUCTION

For physiological responses to climate change to impact bioenergy production, these responses must be linked to biomass yield or conversion. Yet relatively few studies examine these responses across scales (e.g., P.A. Fay, L.G. Reichmann, M.J. Aspinwall, C.V. Hawkes, T.E. Juenger, unpublished data) (**Figure 4**). Instead, many climate change studies focus on a single scale of response (e.g., molecular, physiological, whole plant, and seeded plots) or link a few closely related scales, such as multiple omic responses or omic and physiological responses (213). These smaller-scale studies can be highly mechanistic but may not accurately scale up to responses at the whole-plant or population level. Larger-scale studies often better reflect natural conditions but lack a strong mechanistic explanation and thus may not be as successful at predicting responses to different climate change drivers. Moreover, an important unknown in physiological temperature acclimation is whether high temperature acclimation of photosynthesis corresponds to broader thermal niches for productivity. In switchgrass, warm-origin lowland genotypes have broader biomass distributions and potentially a broader temperature response of biomass production than cool-origin upland genotypes (95, 226), but this assumption has not been widely tested. Even scaling from leaf-level to canopy-level physiology can be challenging due to canopy architecture and physiological differences due to leaf age (168). Often, leaf-level photosynthetic measurements target young leaves and expose them to saturating light conditions (170). Thus, photosynthetic rate is measured under optimal conditions, which may not exist on shaded lower canopy leaves or among older leaves with lower assimilation rates (46, 173). Scaling is also an important aspect of breeding. Many studies have examined biomass yield in spaced plantings because taking measurements on individual plants in spaced plantings is often less time-, money-, and labor-intensive than measuring closed-canopy swards. But this individual-level measure of performance may fail to predict sward-level production because spaced plantings cannot account for intraspecific competition (37; but see 203).

A lack of strong links across scales could also limit modeling efforts to forecast bioenergy grass productivity. Process-based models determine plant productivity, including A and R, from first principles of plant function, which can produce forecasts of biomass production at fine temporal and spatial scales [e.g., Windows intuitive model of vegetation response to atmosphere and climate change (WIMOVAC) (143, 198)]. In bioenergy grasses such as switchgrass and *Miscanthus*, these models are limited, often relying on scant photosynthetic and respiratory measurements collected on a limited number of genotypes and under a narrow range of environmental conditions. New studies and additional photosynthetic and respiratory data collected across broader temporal and spatial scales, and on a broader range of germplasm, will improve the parameterization and predictive capacity of process-based bioenergy models.

7. IMPROVING BIOENERGY CROPS FOR A CHANGING WORLD

Future climates will pose a number of challenges to the implementation of successful bioenergy production. First, C_4 perennial bioenergy grasses are likely to experience more climatic extremes

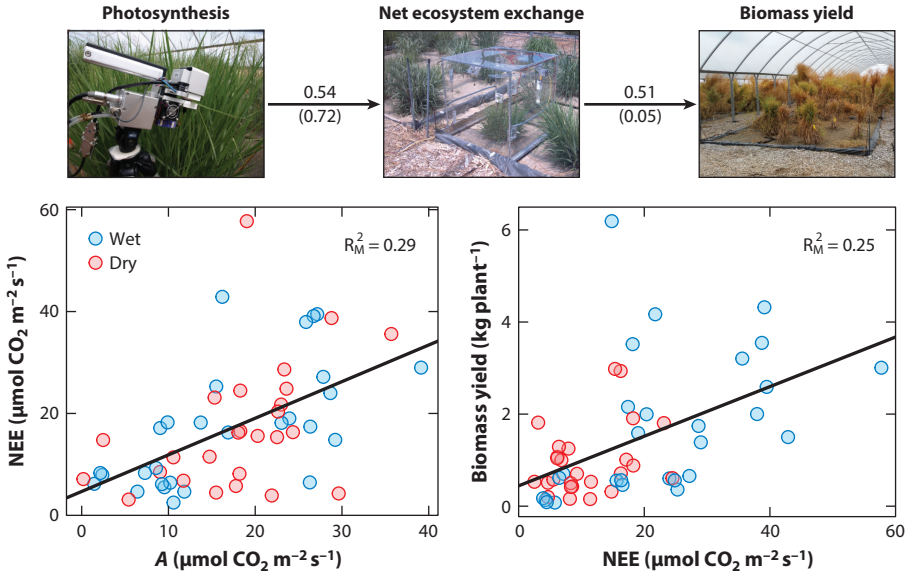

Figure 4

The challenge of scaling from leaf-level to canopy-level processes in bioenergy grasses. Physiological theory and measurements often focus on leaf-level processes, such as photosynthesis and respiration, while bioenergy production is most concerned with end-of-season biomass yield, potentially leading to a disconnect between data and agronomic targets. To demonstrate the importance of understanding physiological function at multiple scales, we present results from an experiment in which eight diverse genotypes of switchgrass were grown at two levels of experimental precipitation that represented an extremely dry year (530 mm annually) and an extremely wet year (1,541 mm annually) at a site in central Texas (Temple, Texas, USA). Across both precipitation levels in a confirmatory path analysis, *A* was a significant predictor of canopy-level NEE ($P < 0.001$), and NEE was a significant predictor of harvested aboveground biomass (biomass yield; $P < 0.001$), but *A* was not significantly related to biomass yield ($P = 0.11$). This highlights the challenge of identifying the scale at which physiological measurements best predict biomass yield, which will be critical for crop improvement and modeling predictions. The path diagram in the top panel shows standardized coefficients with unstandardized coefficients in parentheses; in this confirmatory path analysis, the model adequately fit the data (goodness of fit test, Fisher's $C = 4.42$; degrees of freedom $= 2$; $P = 0.11$). Data on NEE provided by P.A. Fay, L.G. Reichmann, M.J. Aspinwall, C.V. Hawkes, and T.E. Juenger; data on biomass yield from Reference 12. Photos provided by Philip Fay. Abbreviations: *A*, leaf photosynthesis; NEE, net ecosystem exchange; R^2_M, marginal R^2, the amount of variance in a mixed effects model explained by fixed effects only.

than in their recent evolutionary history (157). As such, these grasses will need to acclimate to harsher environments or be replaced in production by better-adapted varieties. Second, to avoid competition with food and feed production, bioenergy production will likely be pushed into habitats unsuitable for conventional agriculture where limited inputs will be available, which will exacerbate the impacts of climatic extremes (106). Improving the responses of C_4 perennial bioenergy grasses, which are largely undomesticated, to climate change could be an important step toward meeting these bioenergy production goals (106). Based on our understanding of future climates, bioenergy grass genetics, and C_4 grass physiology, there are several promising avenues for improvement. However, there are also challenges in working with species that often exhibit complex genetics and life histories.

To date, breeding in bioenergy grasses such as switchgrass and *Miscanthus* has primarily focused on direct phenotypic selection for large size (e.g., height and biomass yield) and freezing

tolerance (45). To accomplish this, breeders grow and phenotype large diversity panels in common environments. Plants exhibiting high values of the trait of interest are identified and crossed to produce synthetic populations, which are then grown and phenotyped for multiple generations (35). Due to the long generation times in perennial bioenergy grasses, cultivar development using phenotypic selection is a slow process. For instance, the development of several new switchgrass cultivars took 25–30 years (45). Newer molecular approaches, such as marker-based selection and genomic selection, which rely on genetic mapping to link genotypes to phenotypes, could drastically increase the speed of breeding programs (48, 216). Because breeders will not need to wait until the plant is mature to measure its phenotype, genomic selection could double or triple the rate of improvement in quantitative traits such as biomass yield in switchgrass (38, 175). Of the two molecular approaches, genomic selection appears more promising, especially for polygenic traits with small-effect loci (48), such as performance-related traits possessing complex genetic architecture in bioenergy grasses. Moreover, marker-assisted breeding may lack the statistical power necessary to detect a sufficient proportion of loci associated with these traits in the small mapping populations typical of bioenergy grass breeding (156, 191). Although genomic selection is a promising approach, there are many unknowns in predicting genomic responses under climate change (32).

Bioenergy grasses also frequently exhibit strong G×E interactions (45, 129, 179), which result from variation in genotype performance across environments (150). This can lead to local adaptation and the formation of ecotypes (108). For instance, switchgrass has three major ecotypes that occupy distinct habitats within their respective regions and possess large phenotypic differences (129). Dealing with diverse, locally adapted populations can be challenging for crop improvement, so breeders often split species' ranges into adaptive zones (212). Instead of targeting the same phenotypes using the same genetic material across the entire range, breeders focus on the attributes that produce high-performing plants in each adaptive zone. The size of these adaptive zones may also be driven by the spatial scale of G×E interactions.

Another challenge with frequent G×E interactions is that they can reduce the efficacy of genomic selection if statistical models assume that all genotypes will respond similarly to environmental changes (32). This challenge is partly due to our lack of information on plant performance in many of the environments that future bioenergy crops will need to occupy, including more extreme climates in their current range and newly cultivated marginal habitats. For instance, climatic extremes may expose hidden beneficial phenotypic plasticity that allows populations to thrive outside their natural habitat or maladaptive phenotypic plasticity that limits their ability to persist in new habitats (72). To that end, genomic prediction models can forecast phenotypes of tested genotypes in tested environments with reasonably high accuracy, but when models are asked to forecast phenotypes of untested genotypes or environments, especially environments outside the range of tested environments, prediction accuracy can decline substantially (122). Thus, developing techniques to improve genomic prediction of untested environments will be critical in the short term for the improved viability of bioenergy grasses. In the longer term, this issue could be alleviated by establishing common gardens in these more extreme environments to provide a solid training set for subsequent genomic selection (209). To date, common garden networks have been established in some C_4 perennial bioenergy grasses, but the density and extent of gardens may be smaller than what will be required for genomic selection into marginal habitats. For instance, Lovell et al. (129) lacked switchgrass common gardens from the range of the Atlantic coastal ecotype and the more xeric regions that may be used for future bioenergy production.

Going forward, genomic selection could be complemented and greatly sped up by combining it with approaches such as high-throughput phenotyping and envirotyping (86, 216, 227). High-throughput phenotyping uses remote sensing techniques to identify spectral proxies for traits of

Genomic selection: use of molecular markers to estimate relatedness among individuals and predict their breeding value for traits of interest in order to identify individuals to select for subsequent crosses

Quantitative trait: a trait arising from the contribution of many genes, the environment, and their interaction, which results in a continuous distribution of phenotypes

Genetic architecture: the genetic basis of phenotypic traits, including the number of loci, their effect sizes and patterns of interaction (gene–gene and gene–environment), and pleiotropy

Phenotypic plasticity: the ability of a single genotype to produce different phenotypes in response to different environments

Envirotyping: the use of detailed environmental information, including soils and microclimate, to understand the environmental responses of plants, especially genotype-by-environment interactions

interest on which selection can be applied (216). This can be performed in specialized greenhouse facilities or in the field using drones more rapidly and extensively than traditional phenotyping (9, 230). Envirotyping utilizes detailed information about the environments in which plants grow—including multiple facets of climate, soil, biotic factors, and management methods—to predict plant phenotypes (227). For example, Mu et al. (145) found that combining an environmental index of diurnal temperature range during the rapid growth window in sorghum with genomic prediction could improve the prediction of plant performance. Because envirotyping uses fine-scale environmental data, it may outperform coarse-scale site-based approaches, which would be particularly valuable for predicting responses to novel future climates (68).

Ideotype: in plant breeding, a plant possessing the attributes necessary to maximize desired functions or breeding goals in a particular environment

Genomic prediction will also benefit from being combined with gene editing. In particular, gene editing will be especially valuable for traits with genes of major effect, such as flowering time, disease resistance, and freezing tolerance (75, 100, 208). Given the positive effect of delayed flowering on biomass production in C_4 perennial bioenergy grasses (36, 45, 99, 106), a genetic manipulation that alters expression of the major flowering time genes could substantially increase biomass production (152). Genetic engineering could also be valuable for improving C_4 photosynthetic efficiency (159). For instance, increasing RuBisCO concentrations or enzymatic activity could improve C_4 photosynthesis at low temperatures, and increasing PEPC activity may be beneficial under water stress (215). In any case, the benefits of more successful bioenergy crops should be weighed against concerns about genetic modifications escaping into natural populations—especially in outcrossing species.

Some locations of future bioenergy production are likely to be drier and have shorter growing seasons than current sites. The strategies of plants that typically occupy these stressful habitats may seem promising for bioenergy crops given their success in these environments, but these plants are often poorly suited to bioenergy production. First, the plants that are adapted to marginal habitats are often slow growing (77)—a poor characteristic in a bioenergy crop. Similarly, strategies that evolved to increase plant survival under stress often result in reduced growth rates, leaf abscission, or early flowering (64). This may make stress-tolerant genotypes poor targets for crop improvement. Instead, strategies that circumvent these stress responses, including delayed leaf senescence in response to water limitation, as in stay-green sorghum and maize (102, 236), may be advantageous for bioenergy production. Likewise, high water-use efficiency is a common response to drought that enables plants to consume less water while acquiring the same amount of carbon, which leaves water in the soil and limits potential growth (118). Instead, this response could be targeted so that plants efficiently use as much of the available water as possible to maximize growth (118).

Genetic architecture can speed up or slow down evolution in response to selection. When selection and genetic architecture are at odds—for instance, when artificial selection occurs for a particular combination of traits, but an opposing combination is genetically linked—the response to selection will be constrained; when selection and genetic architecture act in the same direction, the response to selection will likely be strong (219). When more traits are subject to selection, the strength of constraints is expected to increase (169). To date, most traits related to yield in bioenergy grasses and their relatives have been polygenic and possess relatively complex genetic architecture. For instance, quantitative trait locus (QTL) mapping in switchgrass shows strong overlap in QTLs associated with aboveground and belowground plant size (41, 131). Selection to break trade-offs between these functions would be slow but is likely to succeed when selection is consistently applied orthogonally to the trade-off (2, 210). Moreover, genetic architecture can be leveraged to break trade-offs, as when trade-offs result from conditional neutrality. Because conditionally neutral alleles have a fitness benefit in one environment and no fitness cost in another environment (7), generalist ideotypes could be generated through breeding that combines many

conditionally neutral alleles in the same genome (210). Finally, multivariate genetic architecture may provide opportunities for improved genomic selection. Genomic selection on biomass yield in *Miscanthus* can be improved by considering genetic correlations with phenological traits such as green up and flowering time (49, 191).

To date, most breeding in C_4 perennial bioenergy crops has focused on improving biomass yield (35, 45). Future breeding efforts could also use genomic selection on trait-based ideotypes to promote sustainability. For instance, bioenergy crops deployed at scale could sequester ample carbon in soils, offsetting some of the anthropogenic increase in atmospheric [CO_2] (8, 67). Breeding plants that produce large amounts of recalcitrant root tissue—perhaps by selecting for conservative root economics strategies, large and deep root systems, or a combination of the two—could drastically increase the carbon storage potential of these soils relative to annual crops (59), but this type of selection may hinder increases in aboveground biomass yield. Specifically, root and leaf economics strategies are often positively correlated (41, 84, 176), yet a combination of recalcitrant root tissue that increases soil carbon stores and labile aboveground tissue that enables efficient biofuel conversion is desirable. Another approach is to breed plants whose roots produce large amounts of suberin, a highly recalcitrant compound that can contribute to soil carbon accumulation (128). Promising research on this approach is already underway in some annual crops (59). Similarly, targeting higher resource-use efficiency could reduce or eliminate the need for inputs to bioenergy cropping systems (106). This could be done by directly selecting plant traits that increase resource-use efficiency or by promoting microbial symbioses (e.g., mycorrhizal fungi and nitrogen-fixing bacteria) that increase the plant's ability to acquire resources such as water, nitrogen, and phosphorus (106, 180). In sum, developing bioenergy systems that provide ecosystem services beyond energy production may improve their economic viability and contribute to abating the worst impacts of climate change.

SUMMARY POINTS

1. C_4 perennial grasses, whose biology has been well-studied in recent decades, are important candidates for bioenergy production. In addition to their economic potential in the future bioeconomy, their close relationship to important crops and ecologically dominant species makes them valuable for understanding basic biological processes, such as responses to climate change.
2. Perennial wild species often exhibit strategies that are optimal in environments that differ drastically from typical agronomic settings. As such, bioenergy fields will need to be managed differently from crop fields or perennial bioenergy grasses will need to be bred to possess the attributes of domesticated species.
3. The wide geographical ranges of many C_4 perennial bioenergy grasses likely lead to local adaptation and ecotype formation. Differences in growing season length may promote different leaf economics strategies and thermal acclimation ability with implications for their resilience to climate change.
4. While the interactive effects of climate change drivers (e.g., elevated [CO_2] and changes in temperature and precipitation) on C_4 perennial bioenergy grasses have been understudied, predictions from other systems suggest that these species will maintain productivity under future climates in nonmarginal habitats. Insights concerning responses to climate extremes, especially drought and heat waves, are needed

for a more robust understanding of climate risks for bioenergy crops. Long-term multi-environment field trials are a valuable way to fill these research gaps.

5. A longstanding challenge is linking physiological responses measured at the leaf or canopy level with agronomic-scale responses. We encourage researchers to work across scales as these efforts will provide much needed insight into the mechanisms underlying varied responses to climate change and potential strategies for bioenergy crop improvement.
6. Genomic selection addresses a critical limitation of perennial bioenergy crop improvement: reducing the selection cycle, sometimes by several years per generation. It may be a promising approach for breeding bioenergy crops for changing climates and growth in more marginal habitats if genotype-by-environment interactions can be accounted for.

FUTURE ISSUES

1. Perennial grasses depend on finely tuned resource allocation and carbohydrate sink and source balance to maintain belowground rhizomes and meristems. How do these belowground traits impact the sustainability and success of C_4 perennial bioenergy grasses?
2. What causes physiological temperature acclimation? Is physiological temperature acclimation an adaptive process of optimizing the balance of carbon fixation and use across changing environments?
3. How general is the evolution of leaf economics spectra within widespread bioenergy grasses? How strong of an effect does growing season length exert on leaf economics evolution?
4. Can we implement more effective climate change studies that incorporate interacting climate change drivers and mimic the speed and duration of climatic extremes?
5. Will C_4 perennial bioenergy grasses respond differently to short-term versus long-term changes in temperature and precipitation? Do acclimation and adaptation promote different physiological responses to climate change in C_4 bioenergy grasses?
6. Is it possible to breed bioenergy crops for productivity in unknown future climates or for other targets, such as carbon sequestration potential or climate resilience?
7. What role does polyploidy play in adaptation to climate change and performance/resilience of these crops?
8. How will the economic drivers of a biomass-based bioenergy industry develop?

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