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Review

Crops, Nitrogen, Water: Are Legumes Friend, Foe, or Misunderstood Ally?

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Biological nitrogen fixation (BNF) by crop legumes reduces demand for industrial nitrogen fixation (INF). Nonetheless, rates of BNF in agriculture remain low, with strong negative feedback to BNF from reactive soil nitrogen (N) and drought. We show that breeding for yield has resulted in strong relationships between photosynthesis and leaf N in non-leguminous crops, whereas grain legumes show strong relations between leaf N and water use efficiency (WUE). We contrast these understandings with other studies that draw attention to the water costs of grain legume crops, and their potential for polluting the biosphere with N. We propose that breeding grain legumes for reduced stomatal conductance can increase WUE without compromising production or BNF. Legume crops remain a better bet than relying on INF.

The Nitrogen and Water Problems of Global Agriculture

Crops fulfil the vast majority of global food requirements for both humans and livestock. However, projected increases in population will require the production of more nitrogen (N) fertilizer to support crops, which will consume an even greater proportion of currently available fresh water and energy [1], a difficult problem known as the energy–water–food nexus [2].

The production [industrial nitrogen fixation (INF) via the Bosch-Haber process] and use of fertilizer will generate even more greenhouse gas emissions [3,4]. According to current estimates only ~50% of applied fertilizer N is captured by crops [4], with much of the remaining 50% driving pollution of the biosphere, with sometimes disastrous results [5]. The economic cost of this pollution has been estimated to be in the hundreds of billions of US dollars annually in the USA [6] and Europe [7].

Warnings have also been raised [8] that the world is approaching the limit in terms of water availability for agriculture. This has been emphasized by reports of alarming and unsustainable drawdown of groundwater to support irrigated agriculture in developing countries, as well as in the USA and elsewhere [9]. Changes in seasonal patterns and amounts of rainfall in many cropping areas are reinforced by model predictions of future distributions of rainfall that are not well matched to the current spatial distribution of crops globally. These mismatches pose particular problems for developing countries [10–13]. Already, crop production comprises the great majority of freshwater use by humans worldwide. For example, the annual US soybean [*Glycine max* (L.) Merr.] crop alone consumes more water than evaporates from the entire Great Lakes (MI, USA), the largest group of freshwater lakes on earth [14].

In this context, discussions about the roles of leguminous crops in N and water budgets at a range of scales assume global significance. Legume crops, with their distinctive ability for biological nitrogen fixation (BNF), are grown worldwide in broad-acre, as well as small-holder agriculture. Grain legume seeds (e.g., pulses, beans) and other foods (e.g., those derived from

Highlights

Rates of biological nitrogen fixation (BNF) via crop legumes remain low by comparison with amounts of nitrogen (N) added in fertilizer produced using industrial nitrogen fixation (INF). This remains a key sustainability issue on a global scale.

Lumping BNF and INF together as sources of N pollutants ignores the strong end-product inhibition that regulates BNF, but not INF. Mitigating the pollutant costs of overuse of INF is presently impractical.

The intrinsic water use efficiency (WUE) of crop legumes is generally greater than that of non-legume crops, but we make poor use of this knowledge in agriculture. Breeding programs for legumes, with well-defined goals and trait metrics, could greatly enhance the WUE of current global agriculture.

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soybeans) have been staples for hundreds of years and longer, in regions ranging from the middle-east and northern Africa, through significant parts of Asia, to South America [15]. So important are grain legumes to global food supplies that the 68th United Nations General Assembly declared 2016 the International Year of Pulses.

For readers interested in other aspects of legumes and BNF, there are many recent reviews. Newcomers to the field, are referred to [16] for an overview of legume crops and their environmental and production significance. Other reviews focus on the bacterial and actino-rhizal aspects of BNF under global change [17], BNF in non-legumes [18], and optimizing BNF via coordinated development and metabolism of legume host and rhizobial symbiont [19]. A comprehensive overview of the evolution of BNF and efforts to 'engineer' symbiotic relation-ships between organisms capable of BNF and host plants is given in [20–23]. The authors of [24] explored links between plant N and phosphorous (P) status and the persistence of BNF by legumes. Broader perspectives are offered by reviews of limited-transpiration trait(s) and their potential use in agriculture (including non-legumes and legumes) [25], and of the biogeography of agricultural and nonagricultural legume species and that of their N-fixing symbionts [26]. Finally, a major compilation of a global data for grain legume production includes a range of attributes that can be used to help identify many of the key features of legume crops [27].

Given this breadth of other studies, we focus here on just two key issues for legumes: (i) BNF and nitrogen use efficiency (NUE) in annual and perennial cropping systems, and (ii) water use efficiency (WUE) of grain legumes and non-legume annual crops.

Biological Nitrogen Fixation and Nitrogen Use Efficiency of Crops: A Discussion

NUE of cropping systems is generally a large subject, and well beyond the scope of this review. Nonetheless, it is worth noting that 'mass balance' approaches to quantifying N inputs, pools, and losses at the ecosystem scale have made valuable contributions to our understanding of why some practices produce negative environmental outcomes via 'surplus' N (e.g., [28,29]). A recent synthesis [30] highlighted that such surpluses are especially evident within the early phases of economic growth, and tend to decline as growth proceeds.

Profoundly negative environmental effects of increased concentrations of reactive N in the biosphere have led some researchers to call into question the often assumed benefits of replacing INF with legumes and BNF. Rosenstock *et al.* [31] posed the 'friend or foe' question of tree legumes within agroforestry systems, but concluded that concerns about exacerbation of fertilizer additions of reactive N to the environment lacked support. For cropping systems, however, Battye *et al.* [32] lumped BNF in with INF in as being harmful to the environment. The key example used by Battye *et al.* [32] (and by others) is that of soybean production in North America, and to a lesser extent in Brazil.

At a global scale, rates of BNF by crop legumes are notoriously difficult to quantify. Nearly all large-scale estimates of crop fixation rely on converting the N content of either grain yield or above-ground biomass (very few include estimates of N in belowground biomass) to an amount of N fixed, via an estimated proportion of the N derived from the atmosphere (%N_{dfa}). Jensen *et al.* [33] cited a figure of 33–46 Tg of N due to BNF by crop legumes. Fowler *et al.* [34] cited a range {as previously estimated by Herridge *et al.* [35] and Peoples *et al.* [36], based on the Food and Agriculture Organization (FAO) data for crops} of 50–70 Tg of N with a median value of 60 Tg of N. Lassaletta *et al.* [37] calculated a much lower figure (around 25 Tg of N), despite adjusting for belowground N.

As noted [34], none of these estimates is more than that estimated for preindustrial BNF, even though the preindustrial figure is also seriously questionable.

Battye *et al*. [32] drew upon previous data [35,36] and the same FAO database as used by others (e.g., [34]), reaching an estimate of 43 Tg of N, with a range of 30–51 Tg. They then suggested that BNF is now increasing much more quickly than in the past 100 years, largely on the basis of rapidly increasing areas of soybean. However, many studies (e.g., [37]) have shown that NUE increases rapidly (and possibly exponentially) with the proportion (%) of BNF's contribution to total N inputs to soil (reaching a plateau at ~60%) and, conversely, falls linearly with increasing use of mineral N fertilizer. The differences between Battye *et al*. [32] and Lassaletta *et al*. [37] can be better understood by closer consideration of other recent studies [38]. Gelfand and Robertson [38] used isotopic data to assign tissue N to either a BNF or soil N origin. In support of many previous studies (e.g., [37]), they found a linear decrease in contributions of BNF to plant N with increasing rates of mineral N fertilizer. Other key results were that annual contributions of BNF to soybean crops in the USA were around 3.5 Tg of N, and globally around 10 Tg of N (Battye *et al*. [32] estimated 25 Tg N). Moreover, the contribution of BNF to whole plant N was reported to fall from around 85% without fertilizer, to around 35% at rates of fertilizer application $> \sim 80$ kg N ha^{-1}. Differences in estimated rates of BNF among studies (e.g., [32,37,38]) are at least partly due to methodological difficulties and the often variable quality of crop-based data. In this methodological context, it is worth noting that for natural ecosystems, a major study [39] has recently and substantially revised down estimated rates of BNF in some of the world's most N-rich ecosystems: wet tropical forests (c.f., [40]).

What is clear, however, from large-scale studies of agriculture [37,38] and from decades of detailed physiological and biochemical studies, is that BNF is usually (albeit not always) sensitive to feedback from soil N (Figure 1). In a study of a range of herbaceous legumes that grow in the Bay area of California, BNF was strongly downregulated by moderate additions of N fertilizer [41]. In a study using manures as a source of organic N, BNF was downregulated relative to no N treatments [42]. The precise mechanism of regulation within root nodules remains an active area of research. There is good evidence that accumulation of ureides in nodules is a corollary to slowing rates of BNF (see [43]), but accumulation of other amino acids may also be involved [44]. The source(s) of the ureides and amino acids (leaf tissues or nodules) is an open question and may be species specific. Irrespective of exact process(es), end-product regulation of BNF offers tremendous advantages as a low-cost mechanism for regulating farm and ecosystem N budgets and preventing pollution.

'Lumping' of BNF with INF as a cause of environmental pollution (*sensu* [32]) seems unwarranted, given the evidence that accumulation of end-products of BNF (largely amino-N) acts as a potent regulator of the process. Furthermore, we lack hard evidence that BNF itself produces 'excess N'. Instead, and provided crop legumes are not augmented with INF, there is every prospect of maintaining balanced N budgets when crop legumes are used as both a source of grain and N. Recent detailed analysis of the N balance of legume-based cropping systems in Europe [45] showed that for a typical legume–cereal rotation, there was little N (a few kg ha^{-1}) that could not be accounted for in the crop or soil (i.e., no surplus N). As noted [38]: 'Results underscore the unnecessary expense and environmental burden of adding N fertilizer to modern soybean varieties'. We acknowledge that there are other problems associated with monoculture cropping over large areas, and that some (e.g., tillage) can compound N pollution risks.

Recent studies of interactions of legumes with drought further emphasize the significance of feedback from environmental conditions to rates of BNF. Legumes are frequently noted as being

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'drought sensitive', to the extent that farmers can be wary of growing them under dry seasonal conditions. A strong, N-based response to drought (accumulation of amino acids) was interpreted [45] as being a potential mechanism to account for slowing rates of N fixation for the perennial legume *Medicago*. For grain legumes, a very similar response was noted for *Cicer* [46], while *Phaseolus* accumulated ureides in foliage (but not nodules) under drought conditions that also inhibited BNF [47]. The clear responses of N metabolism to drought (enhanced concentrations of the same metabolites involved in the inhibition of BNF under conditions of excessive soil N) support an argument that BNF is under strong control by external availability of N and water.

Water Use Efficiency of Grain Legume versus Non-Legume Crops: A Meta-analysis

Trait analysis has become a popular tool in ecology and evolution of native plants [48,49], while traits such as WUE have guided plant breeding in agriculture for decades [50–52]. A recent trait study compared wild and cultivated species for photosynthesis and leaf N [53] and concluded that cultivated species have not yet surpassed the biological limit on N use (for photosynthesis) established by wild species.
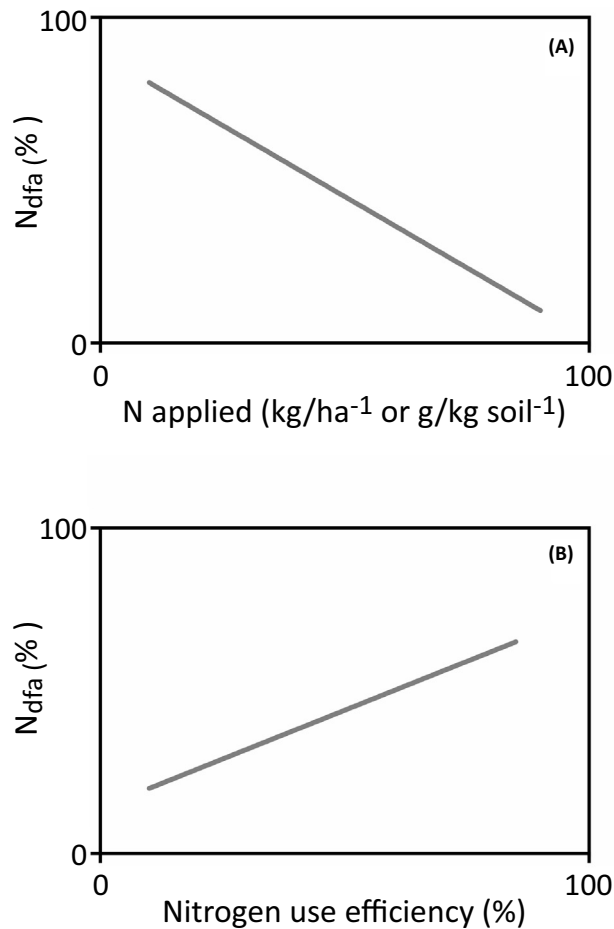
Figure 1. Rates of BNF are Related to Rates of Applied Fertilizer N and NUE. Generalized relationships (e.g., [33–35,42,90]) between biological nitrogen (N) fixation [depicted as % of plant N derived from the atmosphere (N_{dfa})] and (A) N application rate, and (B) N use efficiency for legumes. Abbreviations: BNF, biological nitrogen fixation; NUE, nitrogen use efficiency.

In physiological terms, WUE can be defined as the ratio of the net rate of carbon fixation (A), to the stomatal conductance to water vapor (g_s; a function of stomatal aperture and density [54]). This is usually described as an intrinsic measure (WUE_i) of the efficiency of exchange of CO_2 for water at the leaf surface. Whereas crop transpiration efficiency (yield/water inputs) is influenced by soil and landscape hydrology, WUE_i is a purely plant-based trait and has long been regarded as an important target for plant breeding [50–52]. Theoretically, WUE_i should improve with the availability of N, provided that N results in enhanced production or activity of the major photosynthetic enzymes and pigments. For example, Rubisco, the major enzyme responsible for carbon fixation, may account for up to 25% of crop leaf N [55] and is a major target for genetic improvement [56]. Enhanced photosynthetic capacity could allow plants to acquire more carbon per unit of water transpired, either by enhancing A without increasing g_s, or by maintaining A whilst diminishing g_s (e.g., by modifying stomatal density [57] or selecting for stronger reduction of g_s at high evaporative demand [58]). A controlled-environment study of WUE_i in soybean noted that g_s, much more than A, dictates WUE_i [14]. Another recent study showed that the expected benefits of rising atmospheric CO_2 concentration did not eventuate, owing to modified stomatal function and canopy energy balance [59]. One of very few direct comparisons of C_3 grasses (including cereals) with legumes, revealed that A was related to N per unit leaf area (N_{area}) for both groups, but that A was consistently greater in C_3 grasses than in legumes [60]. A meta-analysis of legumes and non-legumes in natural ecosystems [61] showed that WUE_i in legumes is more tightly linked to leaf N than is the case for non-legumes.

Using an approach similar to those used in other meta-analyses (e.g., [53]), we synthesized leaf trait data for annual grain legume and non-legume crops using meta-analysis of the existing literature [62–93]. We used a parsimonious approach, only accepting data that met key criteria (Box 1). On the basis of the general literature on leaf traits (incorporating natural ecosystems), we hypothesized that leaf N (N_{area}) would be a strong predictor of light-saturated A (A_{sat}) and g_s for all crop species. Given evidence [61] that legumes differ from non-legumes in their relationships of N_{area} to WUE_i, we also hypothesized that grain legume crops would show stronger relations between these variables than non-legumes.

Box 1. Leaf Nitrogen and Water Use Efficiency for Agricultural Legumes

We completed a global meta-analysis of published literature that contains both a measure of leaf nitrogen content and a measure of leaf water use efficiency for agricultural legumes: *Cajanus cajan* (pigeon pea), *Cicer arietinum* (chick pea), *Glycine max* (soybean), *Lens culinaris* (lentil), *Lupinus* spp. (lupin), *Medicago* spp. (alfalfa/lucerne), *Phaseolus vulgaris* (common bean), *Vicia faba* (broad bean/faba bean), and *Vigna unguiculata* (cowpea); and agricultural non-legumes with C_3 metabolism: *Avena sativa* (oat), *Brassica* spp. (canola), *Gossypium* spp. (cotton), *Helianthus annuus* (sunflower), *Hordeum vulgare* and *Hordeum jubatum* (barley), *Oryza* spp. (rice), and *Triticum* spp. (wheat).

We identified relevant literature for each species of interest by screening the Web of Science and Google Scholar search engines for 'nitrogen' combined with any of the following keywords: WUE, water use efficiency, photosynth*, gas exchange, and stomatal conductance. We also included relevant citations documented in these articles. We constructed a dataset for 24 legumes and 28 non-legumes from field, pot, and controlled-environment studies, where leaf nitrogen content per unit area (N_{area} g m^{-2}, or nitrogen per unit mass together with a measure of leaf mass per unit area) was reported concurrently with intrinsic water use efficiency (WUE_i μmol CO_2 mol^{-1} air) or both light-saturated photosynthesis (A_{sat} μmol CO_2 m^{-2} s^{-1}) and stomatal conductance to water vapor (g_s mol air m^{-2} s^{-1}) so we could calculate WUE_i. We included only well-fertilized controls, owing to paucity of studies that include all variables of interest whilst manipulating exogenous (N).

Shapiro-Wilk tests showed the data from the literature [62–93] for A_{sat}, g_s, and WUE_i were significantly non-normal (skewed to the right); log$_{10}$ transformations improved normality distributions of these data. We used Pearson correlations to determine the nature of bivariate relationships between measures of water use efficiency and leaf nitrogen content.

Bivariate analyses of this data set [62–93] show that increased leaf N confers increased WUE_i in grain legumes (Figure 2A). Non-legume crops show no change in WUE_i over a wide range of N_{area} (Figure 2D). Instead, leaf N drives A for non-legumes (Figure 2E) to the extent that N_{area} explains almost a quarter of the variation in A_{sat} for these crops [in contrast to grain legumes in which A in unrelated to N_{area} (Figure 2B)]. Neither grain legumes nor non-legumes adjust g_s with varying N_{area} (Figure 2C,F). These patterns among N_{area}, WUE_i, A, and g_s for grain legumes all mirror those of legumes found in native ecosystems [61].

In a recent controlled-environment study [93], WUE_i was largely invariant to N supply in both grain legume and non-legume annual agricultural crops. However, multivariate analyses revealed a clear differentiation between grain legumes and non-legumes in how A_{sat} and g_s contribute to WUE_i. In non-legumes, g_s dominated variation in WUE, irrespective of externally
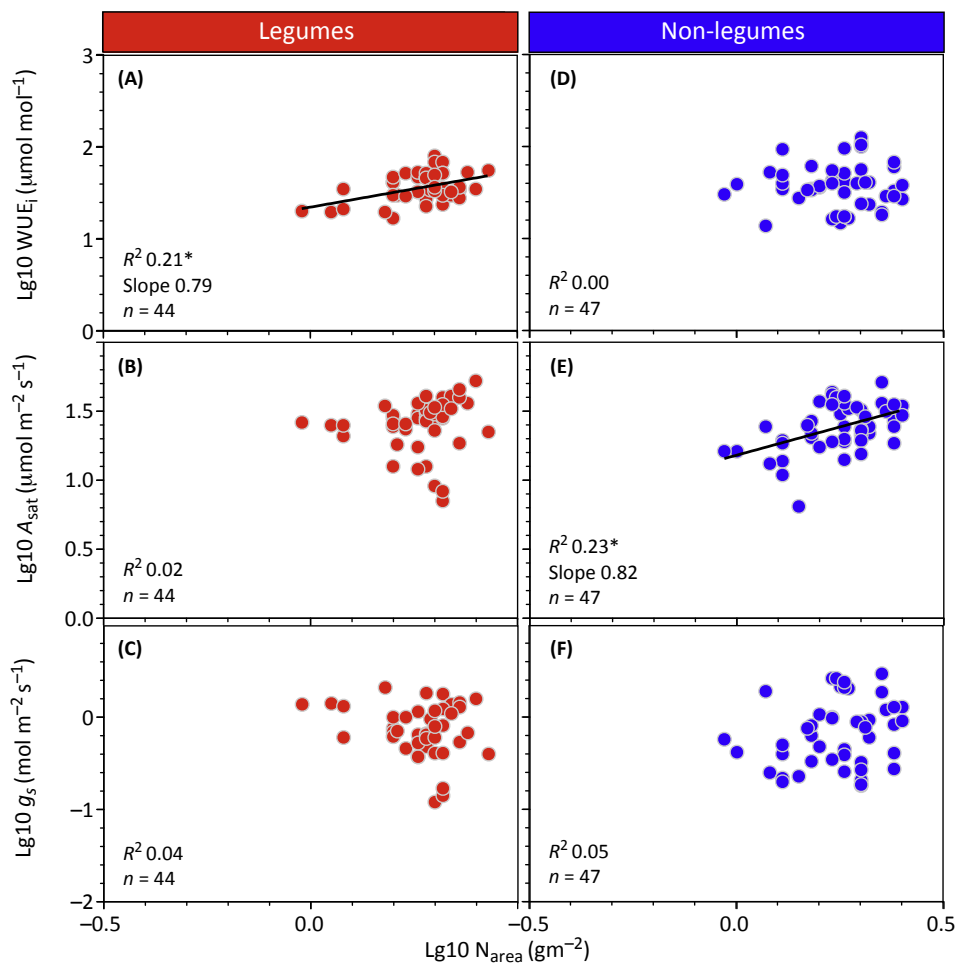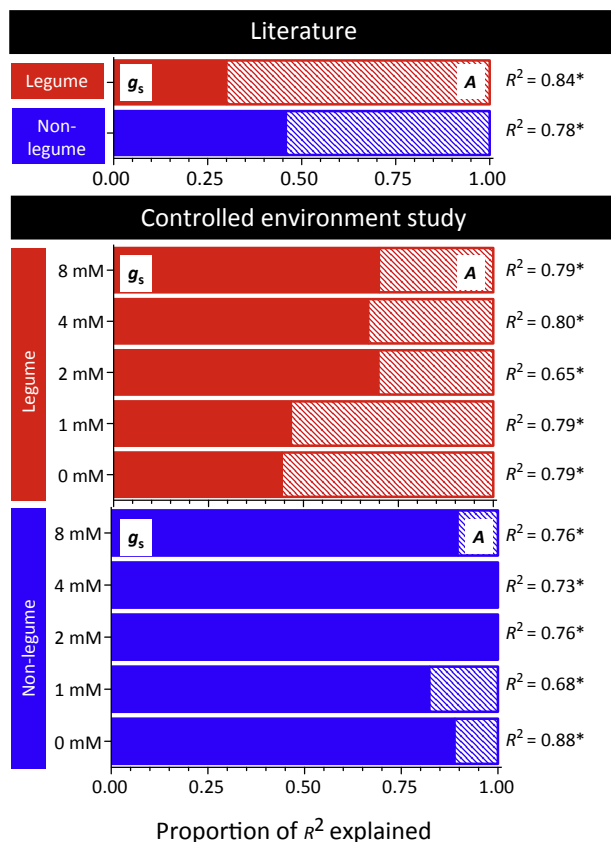

Figure 2. Leaf N, Carbon Fixation, Stomatal Conductance, and WUE. Relationships between N_{area} (g m^{-2}) and WUE_i (μmol CO_2 mol^{-1} H_2O), light-saturated photosynthesis (A_{sat} μmol m^{-2} s^{-1}), and stomatal conductance (g_s mol m^{-2} s^{-1}) for agricultural crops from the literature [55–86] for both legumes (A–C) and non-legumes (D–F). Pearson correlations for log-transformed data, slopes are shown for significant relationships only (*$P < 0.01$). Abbreviations: A_{sat}, Light-saturated carbon fixation; g_s, stomatal conductance; N_{area}, nitrogen per unit leaf area; WUE_i, intrinsic measure of water use efficiency.

supplied N concentration. In grain legumes, and in contrast to annual non-legumes, A_{sat} remained a significant source of variation in WUE_i. The significance of A_{sat} declined as the external supply of N increased (Figure 3). For grain legumes, enhanced Rubisco amount or activity cannot be eliminated as a cause of enhanced WUE. This is a point of clear distinction from non-legume crop species, where variation in N_{area} explains almost 25% of the variance in *A* (e.g., Figure 2E). Selecting physiological traits will allow breeding programs to produce lines of grain legumes that will support more sustainable agricultural practices: practices with reduced requirements for water and N.

Past syntheses [94] and meta-analyses [48,49] support predictions that enhanced leaf N can improve WUE, but those analyses have not focused on crop species, nor have they separated grain legumes from other crop species. In agreement with meta-analysis of legumes in native ecosystems [61], the synthesis here suggests that grain legumes grown as crops have consistently better WUE if they have greater N per unit leaf area (N_{area}). Extrapolating the relationship between WUE_i and N_{area} for grain legumes, to match the upper limit in N_{area} for native legumes, would result in a 20% gain in WUE_i (see Figure I in Box 2).

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Figure 3. Is WUE Better Related to Carbon Fixation or Stomatal Conductance? Proportional contributions by quotients g_s (filled) and A_{sat} (hatched) in explaining variance in WUE_i for legume and non-legume agricultural crops from the literature [59–90] and a controlled environment comparison of the response of physiological traits to nitrogen application [90]. Stepwise multiple regressions were performed on data; *$P < 0.001$. Abbreviations: *A*, Carbon fixation; g_s, stomatal conductance; WUE_i, intrinsic measure of water use efficiency.

Box 2. Opportunities for Plant Breeding

Legumes' greater demand for CO_2 via photosynthesis could provide large (2.5-fold) increases in WUE_i if legumes were instead bred for reduced g_s, (i.e., conservative water use, rather than for maximum A) (Figure I). Breeding programs for legumes, especially for seasonally dry climates, have traditionally focused on rapid development to avoid 'terminal drought' [102]. Such an approach may or may not incorporate properties of WUE_i. In temperate environments, it has been proposed for soybeans [14] that selecting for reduced stomatal conductance and then breeding in enhanced photosynthetic capacity could enhance WUE. The data presented here support extension of this to drier regions, insofar as it is matched by enhanced nitrogen filling of seeds (sink strength). Furthermore, the data suggest that breeding for conservative water use will be robust to external N supply. The legacy of decades of traditional breeding of non-legume crop species for growth, via a focus on photosynthetic carbon fixation, is evidenced by conservation across native and crop species of the relationship between A and N_{area}. That legumes are fundamentally different in this respect (e.g., [61]), as well as showing an equally strongly conserved relationship between WUE_i and N_{area}, is a guide to both further research and to current and future breeding programs.
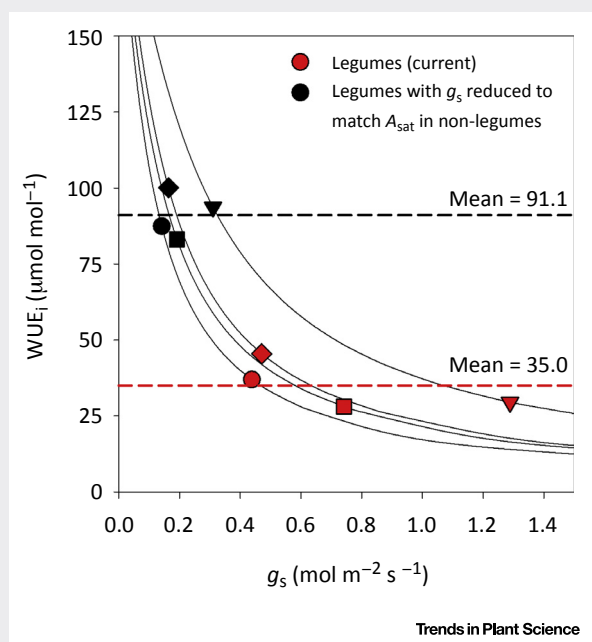

Figure I. Potential Gain in WUE From Different Approach to Breeding. Simulated gains in intrinsic water use efficiency (WUE_i) that would result from breeding legumes with reduced stomatal conductance (g_s). Lines represent g_s versus WUE_i relationships observed for four legume species. Key: circles, *Vicia faba*; diamonds, *Glycine max*; squares, *Lupinus alba*; triangles, *Cicer arietinum*. (See [90] for further detail.) Abbreviation: WUEi, intrinsic water use efficiency.

The meta-analysis presented here shows agricultural (grain) legumes accumulate N in foliage to concentrations well above those needed for photosynthesis, a result which is also similar to that for woody legumes in natural ecosystems [61]. Much of this leaf N is eventually remobilized to fill seeds. As an example, between 30% and 70% of seed N originated in leaf N for *Pisum sativum* [95]. Manipulating membrane-related transport mechanisms to enhance the sink for N in grain legume seeds has been shown to upregulate growth [96,97] in addition to a range of metabolic processes. These points fit well the 20-year-old observation by Hardwick [98], that rates of photosynthesis tend to follow seed development in grain legumes, rather than the reverse. Coupled with evidence that enhanced N transport to seeds promotes growth, and that the carbon:N ratio of phloem sap of legumes is generally conserved, it is reasonable to propose that engineering an enhanced ability to store N in seeds could well enhance rates of carbon fixation by legumes, a variant of the well-known, and increasingly well-elucidated, sink limitation of photosynthesis (Box 2) [99–101].

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Concluding Remarks and Future Perspectives

The availability of water and N will regulate agricultural yields across the globe in the coming decades. The intricate coupling of water and N use in legume grain crops commands further research: we need to fully elucidate the causes of stronger relationships of WUE_i to N_{area} for grain legumes than for non-legumes and then exploit these relationships to help sustainably feed the world (see Outstanding Questions).

In a review of resource use efficiency and climate resilience, Carmo-Silva *et al*. [103] noted that a negative relationship between Rubisco activase and Rubisco content could cancel out potential benefits for WUE of additional allocation of N to the latter. At a molecular level, and in the absence of considerations of N, drought downregulates many photosynthesis-related genes, possibly enhancing WUE_i and aiding survival via a slowing of growth [104]. Further possible contributors to enhanced WUE in legumes arise from clear links between leaf N and enhanced rates of respiration and the energy required for a range of processes, including carbon and N metabolism and storage (in seeds) and N acquisition.

The lowest hanging fruit is perhaps the flexibility in N allocation afforded legumes by BNF. For any plant, ability to up- or downregulate the supply of N to leaves, according to the light environment and to soil supplies of water and N, can confer extraordinary advantages for both WUE and NUE. The special case of legumes, with their distinctive ability to acquire atmospheric N, is deserving of much more detailed analysis. As just one example, we lack a clear understanding of the drivers of temporal variability in N allocation in different tissues, including crossmembrane N transport. Yet these understandings are easily improved.

It seems obvious that research needs to be directed towards understanding the combined WUE and NUE of major legume crops, and that current stop-gap measures are insufficient to prevent further degradation of global biogeochemical cycles. For example, N pollution following excessive additions of fertilizer to non-legume agricultural crops will remain a risk associated with food production, regardless of the energy source for INF. Similarly, the challenge of meeting the water needs for future crop production will remain, even if further groundwater reserves are tapped. Analyses of the 'water footprint' of legumes and other crops emphasizes that there are significant gains to be made (increasing production per unit of water consumed) through better understanding of fundamental aspects of plant biology and its management (e. g., [105]). While there is little evidence of a global increase in drought over the past 60 years [106], there is good evidence of changing amounts and seasonal distribution of rainfall in many of the world's most important grain cropping areas (e.g., in USA [107]), and that this is already reducing crop yields in drier areas (e.g., in Australia [108]). Furthermore, projections of future climates strongly suggest an increase in drought in many regions [109]. Such changes may be partially offset by increased productivity per unit water transpired (e.g., [110]) due to the beneficial physiological effects of rising atmospheric CO_2 concentration. A greatly increased global area of legume crops, grown for their grain and for their N, and with further substitution of legume grains for cereal grains in human and animal diets, would reduce the water and energy demands of agriculture, while maintaining availability of protein. Such an increase would also help alleviate needs for alternative forms of protein, such as could be produced by insects [111].

Lumping BNF with INF as potential sources of polluting reactive N (*sensu* [32]) does help to highlight risks associated with excess additions of N to the biosphere, and, more specifically, to cropping systems (see also [30]). However, while BNF has strong regulatory feedbacks from both too much N and too little water in soils, human use of INF in non-legume cropping systems requires extraordinarily expensive and presently impractical monitoring and remediation. It

Outstanding Questions

The stronger relationships of WUE_i to N_{area} for legumes than for non-legumes need elucidation. The processes of breeding for yield (including selection) under conditions of ample N may at least partially account for the leaf N–A_{sat} relationships of non-legumes, cereals especially. The clear differences displayed by legumes offer the opportunity to enhance yield and WUE.

Further possible contributions to enhanced WUE in legumes arise from clear links between leaf N and enhanced rates of respiration and the energy required for a range of processes, including carbon and N metabolism and storage (in seeds) and N acquisition. Better understanding of the flexibility of N allocation in legumes, and the drivers thereof, seems a straightforward research issue.

Temporal variability in N allocation in different tissues, and the roles of crossmembrane N transport therein, may help unlock further opportunities for enhancing yield and legume protein.

While there are reports that BNF in some crop legumes is not as well-regulated via end-product inhibition as in other legumes, the evidence is sketchy. Further research here is warranted, especially if the areas planted to legumes are to be greatly increased.

seems to us counterproductive to use such a collective description, as it distracts from the core problem of overuse of fertilizer N, and overreliance globally on INF. As noted by Zhang *et al.* [30], we do not yet know 'how close to 100% efficiency the use of N inputs could become', given continued economic development and current temporal patterns in NUE. However, the overwhelming weight of evidence suggests that NUE is strongly enhanced by replacing INF with BNF. The further WUE advantages conferred on legumes (relative to non-legumes) by the ability to both and up- and downregulate BNF according to changing availability of soil N, water, light, and even atmospheric CO_2, concentration, are only just beginning to be recognized. As a gift of evolution, BNF is of inestimable value. Ensuring a sustainable supply of food on earth requires that we not misunderstand or misuse one of our strongest allies.

References

1. Elliot, J. *et al.* (2014) Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3239–3244

2. Leck, H. *et al.* (2015) Tracing the energy-water-food nexus: description theory and practice. *Geogr. Comp.* 9/8, 445–460

3. Gruber, N. and Galloway, J.N. (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296

4. Erisman, J.W. *et al.* (2011) Reactive nitrogen in the environment and its effect on climate change. *Curr. Opin. Environ. Sustain.* 3, 281–290

5. Diaz, R. and Rosenberg, R. (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321, 926–929

6. Sobota, D.J. *et al.* (2015) Cost of reactive nitrogen release from human activities to the environment in the United States. *Environ. Res. Lett.* 10, 025006

7. van Grinsven, H.J.M. *et al.* (2013) Costs and benefits of nitrogen for Europe and implications for mitigation. *Environ. Sci. Technol.* 47, 3571–3579

8. Rost, S. *et al.* (2009) Global potential to increase crop production through water management in rainfed agriculture. *Environ. Res. Lett.* 4, 044002

9. Dalin, C. *et al.* (2017) Groundwater depletion embedded in international food trade. *Nature* 453, 700–707

10. Lesk, C. *et al.* (2016) Influence of extreme weather disasters on global crop production. *Nature* 529, 84–87

11. Wheeler, T.R. and von Braun, J. (2013) Climate change impacts on global food security. *Science* 341, 508–513

12. Schewe, J. *et al.* (2014) Multimodel assessment of water scarcity under climate change. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3245–3273

13. Wang, G. (2005) Agricultural drought in a future climate: results from 15 global climate models participating in the IPCC 4th assessment. *Clim. Dynam.* 25, 739–753

14. Gilbert, M.E. *et al.* (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *J. Exp. Bot.* 62, 2875–2887

15. Foyer, C.H. *et al.* (2016) Neglecting legumes has compromised human health and sustainable food production. *Nat. Plants* 2, 16112

16. Stagnari, F. *et al.* (2017) Multiple benefits of legumes for agricultural sustainability: an overview. *Chem. Biol. Technol. Agric.* 4, 1–13

17. Olivares, J. (2013) Biological nitrogen fixation in the context of global change. *Mol. Plant Microbe Interact.* 26, 486–494

18. Santi, C. *et al.* (2013) Biological nitrogen fixation in non-legume plants. *Ann. Bot.* 111, 743–767

19. Terpolilli, J.J. *et al.* (2012) What determines the efficiency of N_2-fixing *Rhizobium*-legume symbioses? *Adv. Microb. Physiol.* 60, 325–389

20. Boyd, E.S. and Peters, J.W. (2013) New insights into the evolutionary history of biological nitrogen fixation. *Front. Microb.* 4, 201–209

21. Oldroyd, G.E. and Dixon, R. (2014) Biotechnological solutions to the nitrogen problem. *Curr. Opin. Biotechnol.* 26, 19–24

22. Delaux, P.-M. *et al.* (2015) Tracing the evolutionary path to nitrogen-fixing crops. *Curr. Opin. Plant Biol.* 26, 95–99

23. Mus, F. *et al.* (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl. Environ. Microb.* 82, 3698–3710

24. Werner, G.D.A. *et al.* (2015) Evolutionary signals of symbiotic persistence in the legume-rhizobia mutualism. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10262–10269

25. Sinclair, T.R. *et al.* (2017) Limited-transpiration response to high vapor pressure deficit in crop species. *Plant Sci.* 260, 109–118

26. Sprent, J.I. *et al.* (2017) Biogeography of nodulated legumes and their nitrogen fixing symbionts. *New Phytol.* 215, 40–56

27. Cernay, C. *et al.* (2016) A global experimental dataset for assessing grain legume production. *Sci. Data* 3, 160084

28. Gardner, J.B. and Drinkwater, L.E. (2009) The fate of nitrogen in grain cropping systems: a meta-analysis of ^{15}N field experiments. *Ecol. Appl.* 19, 2167–2184

29. Blesh, J. and Drinkwater, L.E. (2013) The impact of nitrogen source and crop rotation on nitrogen mass balances in the Mississippi River Basin. *Ecol. Appl.* 23, 1017–1035

30. Zhang, X. *et al.* (2015) Managing nitrogen for sustainable development. *Nature* 528, 51–59

31. Rosenstock, T.S. *et al.* (2014) Agroforestry with N_2-fixing trees: sustainable developments friend or foe? *Curr. Opin. Environ. Sustain.* 6, 15–21

32. Battye, W. *et al.* (2017) Is nitrogen the next carbon? *Earth's Future* 5, 894–904

33. Jensen, E.S. *et al.* (2011) Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agron. Sustain. Dev.* 32, 329–364

34. Fowler, D. *et al.* (2013) The global nitrogen cycle in the twenty-first century. *Philos. Trans. R. Soc. B Biol. Sci.* 368, 20130164

35. Herridge, D.F. *et al.* (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311, 1–18

36. Peoples, M.B. *et al.* (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48, 1–17

37. Lassaletta, L. *et al.* (2014) 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environ. Res. Lett.* 9, 105011

38. Gelfand, I. and Robertson, G.P. (2015) A reassessment of the contribution of soybean biological nitrogen fixation to reactive N in the environment. *Biogeochemistry* 123, 175–184

39. Cleveland, C.C. *et al.* (1999) Global patterns of terrestrial nitrogen (N2) fixation in natural ecosystems. *Glob. Biogeochem. Cycles* 13, 623–664

40. Sullivan, B.W. *et al.* (2014) Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical N cycle. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8101–8106

41. Menge, D.N.L. *et al.* (2015) Diversity of nitrogen fixation strategies in Mediterranean legumes. *Nat. Plants* 1, 15064

42. Snap, S. *et al.* (2017) Compost legacy down-regulates biological nitrogen fixation in a long-term field experiment. *Crop Ecol. Physiol.* 109, 1–8

43. Carter, A.M. and Tegeder, M. (2016) Increasing nitrogen fixation and seed development in soybean requires complex adjustments of nodule nitrogen metabolism and partitioning processes. *Curr. Biol.* 26, 2044–2051

44. Gil-Quintana, E. *et al.* (2012) Is N-feedback involved in the inhibition of nitrogen fixation in drought-stressed *Medicago truncata*? *J. Exp. Bot.* 64, 281–292

45. Anglade, J. *et al.* (2015) Relationships for estimating N$_2$ fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. *Ecosphere* 6, 1–24

46. Esfahani, M.N. *et al.* (2014) Mechanisms of physiological adjustment of N$_2$ fixation in *Cicer arietinum* L. (chickpea) during early stages of water deficit: single or multi-factor controls. *Plant J.* 79, 964–980

47. Coleto, I. *et al.* (2014) Comparison of inhibition of N$_2$ fixation and ureide accumulation under water deficit in four common bean genotypes of contrasting drought tolerance. *Ann. Bot.* 113, 1071–1082

48. Wright, I.J. *et al.* (2004) The worldwide leaf economics spectrum. *Nature* 428, 821–827

49. Osnas, J.L.D. *et al.* (2013) Global leaf trait relationships: mass, area and the leaf economics spectrum. *Science* 340, 741–744

50. Condon, A.G. *et al.* (1990) Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat leaf gas-exchange and whole plant studies. *Aust. J. Plant Physiol.* 17, 9–22

51. Condon, A.G. (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Sci.* 42, 122–131

52. Morrison, J.I.L. *et al.* (2008) Improving water use in crop production. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 639–658

53. Rotundo, J.L. and Cipriotti, P.A. (2017) Biological limits on nitrogen use for plant photosynthesis: a quantitative revision comparing cultivated and wild species. *New Phytol.* 214, 120–131

54. de Boer, H.J. *et al.* (2016) Optimal allocation of leaf epidermal area for gas exchange. *New Phytol.* 210, 1149–1151

55. Parry, M.A.J. *et al.* (2013) Rubisco activity and regulation as targets for crop improvement. *J. Exp. Bot.* 64, 717–730

56. Lin, M.T. *et al.* (2014) A faster Rubisco with potential to increase photosynthesis in crops. *Nature* 513, 547–550

57. Gilbert, M.E. *et al.* (2011) Field confirmation of genetic variation in soybean transpiration response to vapor pressure deficit and photosynthetic compensation. *Field Crop. Res.* 124, 85–92

58. Franks, P.J. (2015) Increasing water-use efficiency directly through genetic manipulation of stomatal density. *New Phytol.* 207, 188–195

59. Gray, S.B. *et al.* (2016) Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. *Nat. Plant.* 2, 16132

60. Del Pozo, A. *et al.* (2000) Contrasted nitrogen utilization in annual C$_3$ grass and legume crops: physiological explorations and ecological considerations. *Acta Oecol.* 21, 79–89

61. Adams, M.A. *et al.* (2016) Legumes are different: leaf nitrogen, photosynthesis, and water use efficiency. *Proc. Natl. Acad. Sci. U. S. A.* 113, 4098–4103

62. Bolger, T.P. and Turner, N.C. (1998) Transpiration efficiency of three Mediterranean annual pasture species and wheat. *Oecologia* 115, 32–38

63. Brugnoli, E. and Björkman, O. (1992) Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. *Planta* 187, 335–347

64. Comstock, J. and Ehleringer, J. (1993) Stomatal response to humidity in common bean (*Phaseolus vulgaris*): implications for maximum transpiration rate, water-use efficiency and productivity. *Aust. J. Plant Physiol.* 20, 669–691

65. da Silva, E.S. *et al.* (2015) Nitrogen components, growth and gas exchange in spring wheat plants grown under interaction of silicon (Si) and nitrogen (N). *Aust. J. Crop Sci.* 9, 790–798

66. Del Pozo, A. *et al.* (2007) Gas exchange acclimation to elevated CO$_2$ in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. *Environ. Exp. Bot.* 59, 371–380

67. Detmann, K.C. *et al.* (2012) Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice. *New Phytol.* 196, 752–762

68. Edwards, C.E. *et al.* (2011) The genetic architecture of ecophysiological and circadian traits in *Brassica rapa*. *Genetics* 189, 375–390

69. Fredeen, A.L. *et al.* (1991) Responses of photosynthesis and carbohydrate-partitioning to limitation in nitrogen and water availability in field-grown sunflower. *Plant Cell Environ.* 14, 963–970

70. Guo, S. *et al.* (2007) Relationship between water and nitrogen uptake in nitrate- and ammonium-supplied *Phaseolus vulgaris* L. plants. *J. Plant Nutr. Soil Sci.* 170, 73–80

71. Hall, N.M. *et al.* (2005) Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. *Plant Breed.* 124, 557–564

72. Issarakraisila, M. *et al.* (2007) Photosynthetic and growth responses of juvenile Chinese kale (*Brassica oleracea* var. *alboglabra*) and Caisin (*Brassica rapa* subsp. *parachinensis*) to waterlogging and water deficit. *Sci. Hortic.* 111, 107–113

73. Jensen, C.R. *et al.* (1996) Leaf photosynthesis and drought adaptation in field-grown oilseed rape (*Brassica napus* L.). *Aust. J. Plant Physiol.* 23, 631–644

74. Jifon, J.L. and Wolfe, D.W. (2002) Photosynthetic acclimation to elevated CO$_2$ in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. *Glob. Change Biol.* 8, 1018–1027

75. Li, Y. *et al.* (2012) Chloroplast downsizing under nitrate nutrition restrained mesophyll conductance and photosynthesis in rice (*Oryza sativa* L.) under drought conditions. *Plant Cell Physiol.* 53, 892–900

76. Li, Y. *et al.* (2013) Does chloroplast size influence photosynthetic nitrogen use efficiency? *PLoS One* 8, 1–10

77. Lopes, M.S. and Araus, J.L. (2006) Nitrogen source and water regime effects on durum wheat photosynthesis and stable carbon and nitrogen isotope composition. *Physiol. Plant.* 126, 435–445

Trends in Plant Science

78. Lopes, M.S. *et al.* (2004) Nitrogen source and water regime effects on barley photosynthesis and isotope signature. *Funct. Plant Biol.* 31, 995–1003

79. Madhu, M. and Hatfield, J.L. (2014) Interaction of carbon dioxide enrichment and soil moisture on photosynthesis, transpiration, and water use efficiency of soybean. *Agric. Sci.* 5, 410–429

80. Mythili, J.B. and Nair, T.V.R. (1996) Relationship between photosynthetic carbon exchange rate, specific leaf mass and other leaf characteristics in chickpea genotypes. *Aust. J. Plant Physiol.* 23, 617–622

81. Raeini-Sarjaz, M. *et al.* (1998) Water stress, water use efficiency, carbon isotope discrimination and leaf gas exchange relationships of the bush bean. *J. Agron. Crop Sci.* 180, 173–179

82. Rosenthal, D.M. *et al.* (2014) Biochemical acclimation, stomatal limitation and precipitation patterns underlie decreases in photosynthetic stimulation of soybean (*Glycine max*) at elevated CO_2 and temperatures under fully open air field conditions. *Plant Sci.* 226, 136–146

83. Roumet, C. (2000) Short and long-term responses of whole-plant gas exchange to elevated CO_2 in four herbaceous species. *Environ. Exp. Bot.* 43, 155–169

84. Schmid, I. *et al.* (2016) Effects of CO_2 enrichment and drought on photosynthesis, growth and yield of an old and modern barley cultivar. *J. Agron. Crop Sci.* 202, 81–95

85. Seemann, J.R. and Critchley, C. (1985) Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta* 164, 151–162

86. Sreeharsha, R.V. *et al.* (2015) Delayed flowering is associated with lack of photosynthetic acclimation in pigeon pea (*Cajanus cajan* L.) grown under elevated CO_2. *Plant Sci.* 231, 82–93

87. Tambussi, E.A. *et al.* (2005) Does higher yield potential improve barley performance in Mediterranean conditions? A case study. *Field Crop. Res.* 91, 149–160

88. van den Boogaard, R. *et al.* (1995) Association of water use efficiency and nitrogen use efficiency with photosynthetic characteristics of two wheat cultivars. *Plant Cell Environ.* 20, 200–210

89. Wong, S.H. *et al.* (1985) Leaf conductance in relation to rate of CO_2 assimilation. *Plant Physiol.* 78, 821–825

90. Xue, W. *et al.* (2016) Soil water availability and capacity of nitrogen accumulation influence variations of intrinsic water use efficiency in rice. *J. Plant Physiol.* 193, 26–36

91. Yong, J.H.W. *et al.* (2010) Effects of root restriction on growth and associated cytokinin levels in cotton (*Gossypium hirsutum*). *Funct. Plant Biol.* 37, 974–984

92. Zhu, C. *et al.* (2012) The temporal and species dynamics of photosynthetic acclimation in flag leaves of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) under elevated carbon dioxide. *Physiol. Plant.* 145, 395–405

93. Adams, M.A. *et al.* (2018) Contrasting responses of crop legumes and cereals to nitrogen availability. *New Phytol.* 217, 1475–1483

94. Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78, 9–19

95. Schlitz, S. *et al.* (2005) Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N *in vivo* labeling throughout seed filling. *Plant Physiol.* 137, 1463–1473

96. Götz, K.P. *et al.* (2007) Uptake and allocation of carbon and nitrogen in *Vicia narbonensis* plants with increased seed sink strength achieved by seed-specific expression of an amino acid permease. *J. Exp. Bot.* 58, 3183–3195

97. Zhang, L. *et al.* (2015) Improvement of pea biomass and seed productivity by simultaneous increase of phloem and embryo loading with amino acids. *Plant J.* 81, 134–146

98. Hardwick, R.C. (1988) Critical physiological traits in pulse crops. In *World Crops: Cool Season Food Legumes* (Summerfield, R. J., ed.), pp. 885–896, Kluwer Academic Publishers

99. Paul, M.J. and Foyer, C.H. (2001) Sink limitation of photosynthesis. *J. Exp. Bot.* 52, 1383–1400

100. Burnett, A.C. *et al.* (2016) Carbon source-sink limitations differ between two species with contrasting growth strategies. *Plant Cell Environ.* 39, 2460–2472

101. Ruiz-Vera, U.M. *et al.* (2017) The role of sink strength and nitrogen availability in the down-regulation of photosynthetic capacity in field-grown *Nicotiana tabacum* L. at elevated CO_2 concentration. *Front. Plant Sci.* 8, 998

102. Siddique, K.H.M. *et al.* (2015) Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. *Eur. J. Agron.* 15, 267–280

103. Carmo-Silva, E. *et al.* (2015) Optimizing Rubisco and its regulation for greater resource use efficiency. *Plant Cell Environ.* 38, 1817–1832

104. Tien Le, D. *et al.* (2012) Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. *PLoS One* 7, e49522

105. Mekonnen, M.M. and Hoekstra, A.Y. (2014) Water footprint benchmarks for crop production: a first global assessment. *Ecol. Indic.* 46, 214–223

106. Sheffield, J. (2012) Little change in global drought over the past 60 years. *Nature* 491, 435–438

107. Wuebbles, D.J. *et al.* (2017) *Climate Science Special Report: Fourth National Climate Assessment, Volume I*, p. 470, U.S. Global Change Research Program

108. Hochman, Z. *et al.* (2017) Climate trends account for stalled wheat yields in Australia since 1990. *Glob. Change Biol.* 23, 2071–2081

109. Field, C.B. *et al.* (2012) *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change,* Cambridge University Press, p. 582

110. Deryng, D. *et al.* (2016) Regional disparities in the beneficial effects of rising CO_2 concentrations on crop water productivity. *Nat. Clim. Chang.* 6, 786–790

111. van Huis, A. *et al.* (2013) *Edible Insects: Future Prospects for Food and Feed Security*. FAO Forestry Paper 171, p. 187, Food and Agriculture Organization of the United Nations