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REVIEW PAPER

Senescence and nitrogen use efficiency in perennial grasses for forage and biofuel production

Jiading Yang[1,2] and Michael Udvardi[1,2,*]

[1] Noble Research Institute, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA
[2] Bioenergy Sciences Center (BESC), Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

* Correspondence: mudvardi@noble.org

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Abstract

Organ senescence is an important developmental process in plants that enables recycling of nutrients, such as nitrogen, to maximize reproductive success. Nitrogen is the mineral nutrient required in greatest amount by plants, although soil-N limits plant productivity in many natural and agricultural systems, especially systems that receive little or no fertilizer-N. Use of industrial N-fertilizers in agriculture increased crop yields several fold over the past century, although at substantial cost to fossil energy reserves and the environment. Therefore, it is important to optimize nitrogen use efficiency (NUE) in agricultural systems. Organ senescence contributes to NUE in plants and manipulation of senescence in plant breeding programs is a promising approach to improve NUE in agriculture. Much of what we know about plant senescence comes from research on annual plants, which provide most of the food for humans. Relatively little work has been done on senescence in perennial plants, especially perennial grasses, which provide much of the forage for grazing animals and promise to supply much of the biomass required by the future biofuel industry. Here, we review briefly what is known about senescence from studies of annual plants, before presenting current knowledge about senescence in perennial grasses and its relationship to yield, quality, and NUE. While higher yield is a common target, desired N-content diverges between forage and biofuel crops. We discuss how senescence programs might be altered to produce high-yielding, stress-tolerant perennial grasses with high-N (protein) for forage or low-N for biofuels in systems optimized for NUE.

Key words: Bioenergy, biomass, NAC transcription factor, nitrogen remobilization, perennial grass, senescence, sustainability, switchgrass.

Nitrogen, NUE, and plant senescence

Nitrogen (N) is an essential element in many biomolecules, including nucleic acids, which encode genetic information, and proteins, which perform the chemistry of life and fulfil other functional as well as structural roles. Low concentrations of available N limit primary production of plants and other autotrophs in natural and agricultural systems (Marschner, 1995; O'Neill *et al.*, 2004; Elser *et al.*, 2007). Massive use of industrial N-fertilizers relieved the N-limitation in many agricultural systems and contributed to the Green Revolution during the second half of the 20th century. Global fertilizer-N use increased ten-fold, to over one hundred million tonnes, while production of cereal grains increased three-fold and the population more than doubled between 1961 and 2009 (Godfray *et al.*, 2010; FAO, 2012). Fertilizer use has doubled the flux of N through the terrestrial N-cycle (Canfield *et al.*, 2010) and loss of reactive-N from agricultural systems damages ecosystems and human health, and impacts climate (Michael Beman *et al.*, 2005; Galloway *et al.*, 2008; Sutton *et al.*, 2011). Current

rates of fertilizer-N use are probably unsustainable (Rockström *et al.*, 2009). Despite this, the use of fertilizer-N is projected to increase to meet the demands of the growing global population (Ladha and Chakraborty, 2016). There is room for optimism, however, as several strategies have been identified that could reduce the amount of fertilizer-N needed by agriculture globally for food, feed, fiber, and fuel production. These include: greater use of legumes that add N into agricultural systems via symbiotic nitrogen fixation in a sustainable manner (Bohlool *et al.*, 1992; Herridge *et al.*, 2008; Biswas and Gresshoff, 2014); increased N use efficiency (NUE) at the field scale through better management of the type, amount, and timing of fertilizer application (Frink *et al.*, 1999; Cassman *et al.*, 2002); improved management of planting density, planting and harvesting times, water, other nutrients, and pest and pathogen control to ensure optimal yields and NUE of crops (Alam *et al.*, 2013; Chu *et al.*, 2016); selection and breeding of plant cultivars that capture fertilizer-N and convert it to food and feed protein more efficiently (Saengwilai *et al.*, 2014*a*, 2014*b*; Lynch and Wojciechowski, 2015); genetic engineering for greater NUE in crop species (Xu *et al.*, 2012); development of chemical or biological strategies to interfere with microbial turnover and loss of fertilizer-N from agricultural systems (Subbarao *et al.*, 2009); development of associative nitrogen fixing symbioses in non-legumes (Chen *et al.*, 2011, 2014; Santi *et al.*, 2013); and synthetic biology approaches to biological and symbiotic nitrogen fixation in plants (Rogers and Oldroyd, 2014; Mus *et al.*, 2016). Off the farm, the demand for food and its associated requirement for fertilizer-N could be reduced by minimizing food spoilage before it reaches consumers and waste afterwards. It is estimated that spoilage and waste account for approximately one-third of food production, so there is great scope here for reducing the need for fertilizer-N (Gustavsson *et al.*, 2011). Dietary choice is another means of reducing the amount of fertilizer needed to produce a unit of food protein, with vegetarian diets requiring fewer fertilizer-N inputs than meat-based diets, although much of the land that is used for animal production is not suitable for food-crop production (Pimentel and Pimentel, 2003; van Zanten *et al.*, 2016). On the other hand, substantial cropland is devoted to producing grain for animal production rather than direct human consumption. Finally, there is huge variation in the rates of N-fertilizer application in different regions of the world (Mueller *et al.*, 2012), and scope to increase yields dramatically in the poorest regions with relatively modest N-inputs. In other words, global food production could be increased substantially at current rates of N-fertilization simply by using fertilizer where it is most needed and where the protein produced from each additional unit of N is greatest. Clearly, there are many opportunities to improve NUE in agriculture. One of these, related to improving plant NUE, is the optimization of plant senescence to conserve N in perennial systems or to maximize protein production and overall yield in seed and vegetative organs of annual and perennial systems for food or forage.

NUE is a measure of how efficiently input-N is converted into harvested product, either total biomass or harvested-N (Masclaux-Daubresse *et al.*, 2010). Measuring N-inputs and N-outputs in harvested material along with soil-N prior to fertilization and after harvest enables calculation of true NUE and estimates of losses to the surrounding environment, which is important in the context of agricultural sustainability. Measuring losses of N from agricultural systems directly, as gaseous-N, leaching, and erosion losses, is a more challenging enterprise (Sutton *et al.*, 2007).

At the plant level, NUE depends on the efficiencies of various physiological and biochemical processes from transport of soil-N into roots to N- and C-metabolism and accumulation in harvested plant parts. It can be divided into two components: N-uptake efficiency and N-utilization efficiency. The latter encompasses N assimilation and deployment for photosynthesis and growth, and N remobilization efficiency (NRE) for growth of new vegetative and reproductive organs, including seed. NRE from vegetative organs explains much of variation in grain yield in wheat, for instance (Barraclough *et al.*, 2010). Crop NUE is determined not only by plant processes, but also by physical, chemical, and biological processes occurring in the soil, which compete for N and otherwise remove it from the crop rhizosphere. Management practices also impact crop NUE, as mentioned above (Alam *et al.*, 2013; Chu *et al.*, 2016). Thus, systems-based approaches that incorporate the many factors affecting N uptake and utilization by plants are required to understand and improve overall NUE in agriculture.

Recent reviews of NUE and leaf senescence in annual crop species provide a useful starting point for exploring these processes in perennial grasses (Masclaux-Daubresse *et al.*, 2008, 2010; Garnett *et al.*, 2015).

Plant senescence and nutrient remobilization

Senescence is the last developmental stage prior to death of an organ or the whole plant (Lim *et al.*, 2007; Rapp *et al.*, 2015). Macromolecule degradation and nutrient remobilization during senescence allows plants to reuse endogenous nutrients (Himelblau and Amasino, 2001; Buchanan-Wollaston *et al.*, 2003; Gregersen *et al.*, 2008). Developmental senescence is primarily an age-based process, which is controlled by both internal and external seasonal cues and generally mediated by hormones (Jibran *et al.*, 2013). Various external stresses, such as extreme temperatures, high or low irradiation, water/nutrient deficiency, and pathogen infection, may induce precocious senescence that enables plants to reinvest nutrients for growth elsewhere in the plant and, in the case of biological attack, to constrain the invader (Lim *et al.*, 2003; Fischer, 2012; Rapp *et al.*, 2015).

For annual staple crops such as wheat, rice, maize, and soybean, leaf senescence usually coincides with reproductive development, perhaps in response to signals of resource demand in the nascent sink tissues (Noodén and Penney, 2001). Nutrients remobilized from leaves and stems are used primarily for seed (grain) production following flowering, ensuring maximal reproductive success. Greater N remobilization from flag leaves resulted in increased protein and micronutrient contents in wheat and rice grain (Uauy *et al.*, 2006*b*; Liang *et al.*, 2014). Grain yield and quality are closely related to nutrient

remobilization efficiency of crops, although grain yield and protein content generally are negatively related (Bogard *et al.*, 2010). It was estimated that small-grained cereals such as rice, wheat, and barley may mobilize up to a maximum of 90% of the N in vegetative organs to the grain, although this varies with species and genotype (Barbottin *et al.*, 2005; Kichey *et al.*, 2007; Gregersen *et al.*, 2008). In pea, N remobilization from vegetative organs contributed up to 71% of the total N in mature seeds borne on the first two nodes (first stratum; Schiltz *et al.*, 2005). Nonetheless, a substantial proportion of nutrients remains in leaves and stems after senescence. For example, about 7.0 kg N, 1.1 kg P, and 9.7 kg K remained in each tonne of corn stover under field conditions (Johnson *et al.*, 2010). If such stover is removed from the field, matching amounts of fertilizer must be added to maintain soil fertility.

Most perennial grasses, including dedicated bioenergy crops such as switchgrass, miscanthus, and giant reed, cease vegetative growth after entering reproductive development, unlike many dicots (Moore *et al.*, 1991). Following anthesis and pollination, all above-ground organs (leaves, stems, and spikes) undergo senescence annually, at least under natural conditions in the field. In addition to supporting seed production, a portion of the nutrients remobilized from leaves and stems of perennials is allocated to underground organs (roots, rhizomes, and crowns; Lemus *et al.*, 2008; Heaton *et al.*, 2009; Nassi o Di Nasso *et al.*, 2011, 2013; Strullu *et al.*, 2011; Dohleman *et al.*, 2012; Kering *et al.*, 2012) where they serve as nutritional reserves for regrowth in the next season. Nutrient remobilization to underground organs is important for sustainability of biomass production for biofuels. We have shown that some switchgrass accessions can remobilize up to 61% of the N in a mature tiller (Yang *et al.*, 2009). However, substantial amounts of soil nutrients are still removed within the harvested shoot biomass (Propheter and Staggenborg, 2010; Guretzky *et al.*, 2011). For example, the total N removed with switchgrass biomass varied from 31 to 63 kg N ha^{-1} $year^{-1}$ in a one-cut autumn harvest system, and from 90 to 144 kg N ha^{-1} $year^{-1}$ for a two-cut system, over 5 years of measurements (Reynolds *et al.*, 2000). As the N in biomass is not beneficial for biofuel production but necessitates the addition of more fertilizer-N to maintain switchgrass productivity in the next growth season, it is desirable to decrease the N content of biomass for bioenergy production further, by enhancing N-remobilization during shoot senescence for instance.

In contrast to biomass for biofuels, perennial grass biomass used as forage for livestock is best when it contains high levels of protein-N. Therefore, delayed senescence (and delayed N remobilization) would be a desirable trait for forages to keep more protein in leaves prior to consumption, especially if it enables continued photosynthesis and growth until then.

Molecular basis and regulation of senescence

Genetic and genomic approaches using model plant species, especially arabidopsis and rice, have revealed much about the molecular mechanisms of leaf senescence. Transcriptomic analyses and characterization of various mutants have uncovered complex molecular regulatory networks and a number of regulatory factors involved in senescence, including transcription factors, receptors, and signaling components for hormones and stresses, and metabolic regulators (Guo *et al.*, 2004; Buchanan-Wollaston *et al.*, 2005; van der Graaff *et al.*, 2006; Lim *et al.*, 2007; Li *et al.*, 2012). Interestingly, but perhaps not surprisingly, similar biochemical, transcriptional, and cellular processes appear to be engaged during leaf senescence in perennial plants and annual plants (Munné-Bosch, 2008).

Using microarrays to measure global gene expression, thousands of genes have been found that respond to developmental or environmental cues for senescence (Buchanan-Wollaston *et al.*, 2005; van der Graaff *et al.*, 2006; Breeze *et al.*, 2011). Some of the senescence-associated genes (SAGs) are involved in hormone biosynthesis or signaling. Initiation and progression of leaf senescence have long been known to be affected by almost all plant hormones. In general, ABA, ethylene, jasmonic acid, and salicylic acid can promote senescence, whereas cytokinin, auxin, and gibberellic acid may suppress leaf senescence (Nooden *et al.*, 1997; Jibran *et al.*, 2013).

Other SAGs that have potential to alter senescence and increase stress tolerance, yield, and /or NUE are those encoding transcription factors (TFs) that orchestrate transcription of the many other genes encoding proteins required for the organized deconstruction of macromolecules and the export of nutrients to other parts of the plant. Among the largest groups of senescence-associated TFs in arabidopsis are the NAC, WRKY, MYB, C2H2 zinc-finger, bZIP, and AP2/EREBP families (Guo *et al.*, 2004; van der Graaff *et al.*, 2006). In grasses, however, so far only TFs of the NAC family have been implicated directly through genetic studies in the regulation of senescence and N remobilization from leaves.

Wheat *TtNAM-B1* encoding a NAC family TF was identified at a quantitative trait locus, *Gpc-B1*, which was associated with delayed senescence and increased grain protein content (GPC) (Joppa *et al.*, 1997; Uauy *et al.*, 2006*a*). Sequence comparisons between different alleles of *TtNAM-B1* from various wheat cultivars linked differences in GPC between wild wheat and modern wheat varieties to the function of NAM-B1, which is dysfunctional due to a frame-shift in modern varieties. When the transcript levels of all four NAM copies in hexaploid wheat were reduced by RNA interference (RNAi), flag leaves and main-spike peduncles of the transgenic plants turned yellow later than in non-transgenic controls, by 24 d and more than 30 d, respectively. *NAM-B1* RNAi plants exhibited 30% reduction in GPC and lower Zn and Fe concentrations in grain, with higher levels of N, Zn, and Fe in the flag leaves. This suggested that reduction in *NAM* transcript levels delayed whole-plant senescence, which reduced nutrient remobilization from flag leaves to developing grains. This work provided the first evidence that nutrient remobilization is genetically tractable and could be related to functional expression of a single transcription factor.

Rice *OsNAC5* (Locus ID Os11g08210) exhibited higher transcript levels during grain-filling relative to the panicle

exertion stage in two cultivars with distinctive concentrations of seed protein, Zn, and Fe (Sperotto *et al.*, 2009). *OsNAC5* transcript levels increased following dark-incubation, ABA application, salinity, cold, and Fe-deficiency treatments, all of which can induce senescence in rice. Application of nicotinamide, an inhibitor of ABA action, abolished salt-stress induction of *OsNAC5* expression, implicating ABA in *OsNAC5* induction by salt stress. Interestingly, a positive correlation between *OsNAC5* expression in flag leaves (at stages of panicle exertion and anthesis) and concentrations of Fe, Zn, and protein in seeds was found (Sperotto *et al.*, 2009).

A rice gain-of-function mutant, prematurely senile 1 (*ps1-D*), was identified with premature leaf senescence after tillering (Liang *et al.*, 2014). *LOC_Os03g21060* (*OsNAP*) was found to be the only gene within the vicinity of the T-DNA insertion in the mutant, and its expression was elevated 20-fold in *ps1-D* mutant seedlings. Consistent with this, transgenic overexpression (OE) of *OsNAP* in wild-type plants also resulted in premature leaf senescence. *OsNAP* expression increased with developmental age in leaves in the wild-type, as well as in the endosperm over the course of grain-filling. When *OsNAP* was down-regulated through RNAi in wild-type rice, transgenic plants displayed delayed leaf senescence. Compared with wild-type plants, both *ps1-D* mutant and *OsNAP* OE lines exhibited significant increases in total grain protein content and concentrations of seven nutrient elements while RNAi plants showed about 10% decreases. Conversely, flag leaves of *ps1-D* and *OsNAP* OE lines had a lower nutrient concentration, while *OsNAP* RNAi flag leaves retained more nutrient elements than wild-type plants. Thus, OsNAP positively affects nutrient remobilization from leaves to grain during rice reproduction/senescence process, similarly to TtNAM-B1 in wheat (Uauy *et al.*, 2006*b*).

Two full-length cDNAs, *PvNAC1* and *PvNAC2*, were isolated from perennial switchgrass, with both genes showing increased expression in leaves during natural senescence and under prolonged darkness (Yang *et al.*, 2015). Estradiol-inducible expression of *PvNAC1* in arabidopsis triggered yellowing of mature leaves on whole plants and resulted in 53% less chlorophyll and 24% less N after 5 d of treatment, consistent with a role of PvNAC1 in controlling leaf senescence and N remobilization. Overexpression of *PvNAC2* in switchgrass, using the constitutive 35S-promoter, increased total aboveground biomass (sum of leaves, stems, and inflorescences) of greenhouse-grown plants, without affecting the biomass of underground organs (crown and roots) of *35S::PvNAC2* plants relative to controls. Note that under these greenhouse conditions, switchgrass plants did not senesce (Yang *et al.*, 2015).

Recently, we completed a study of N-dynamics of the various organs of the switchgrass cultivar 'Alamo' grown over four years, which captured nicely the annual increase in leaf-N during spring and decrease during autumn senescence, concomitant with storage of N in the root system (Yang *et al.*, 2016). Parallel analysis of transcriptomes of switchgrass organs from spring though autumn revealed several thousand genes that exhibited at least two-fold changes in transcript levels in leaves or stems before and after senescence. A large number of these genes (597 in leaves and 437 in stems) were homologous to known SAGs in arabidopsis, rice, maize, and wheat (Yang *et al.*, 2016). Approximately 15% of the switchgrass SAGs were found to encode putative TFs, mainly of the following families: NAC, WRKY, AP2/EREBP, bZIP, and C3H, bHLH, and C2C2-CO-like. In particular, 19 senescence-associated NAC TF genes were identified in leaves or stems, four of which were common to both. Phylogenetic analysis of 17 complete protein sequences derived from these NAC TF genes was performed, using previously described wheat, rice, and switchgrass sequences (TtNAM-B1, OsNAC5, OsNAP, and PvNAC1, PvNAC2) as references for comparison. Eight of the PvNAC SAGs are in the same clade as the five reference proteins (Fig. 1). Thus, these NACs are most likely to regulate transcription of genes involved in senescence and N remobilization in switchgrass shoots.

Although underground organs of switchgrass appear to play a role in N-storage between growing seasons (Yang *et al.*, 2016), to our knowledge no published studies have explored the mechanisms of senescence and N mobilization in grass root systems. Given the likely importance of N-storage and remobilization in underground organs for multi-year growth and competitiveness of perennial grasses, these are attractive areas for future research.

The stay-green trait

Stay-green is a heritable trait of delayed foliar senescence that can be classified as either cosmetic or functional stay-green (Thomas and Ougham, 2014). Cosmetic stay-green mutants have impaired or delayed chlorophyll catabolism due to defects in chlorophyll degradation (Hörtensteiner and Kräutler, 2011), while other physiological processes, especially photosynthetic decline, are normal (Thomas and Ougham, 2014). *STAY-GREEN* (*SGR*) genes encode chloroplast proteins that are highly conserved in plant species and are thought to destabilize chlorophyll–protein complexes as a prerequisite to degradation of both chlorophyll and apoprotein (Hörtensteiner, 2009). In the perennial legume forage species alfalfa (*Medicago sativa*), down-regulation of a *SGR* gene (*MsSGR*) by RNAi led to the production of stay-green transgenic plants (Zhou *et al.*, 2011). Together with a more greenish appearance, most of the alfalfa RNAi lines retained more than 50% of chlorophyll during senescence and had increased crude protein content compared to the wild-type, indicating that a cosmetic stay-green phenotype can improve forage quality.

The functional stay-green trait couples delayed chlorophyll degradation to extended photosynthetic capacity in leaves (Thomas and Ougham, 2014). N remobilization from the leaves to the grain is slower in stay-green hybrids compared with senescent genotypes of sorghum (Borrell and Hammer, 2000). In durum wheat, four stay-green mutants maintained photosynthetic competence for longer than normal and also had higher seed weights and grain yields per plant than the parental line (Spano *et al.*, 2003). Down-regulation of *TtNAM-B1* in wheat resulted in delayed leaf yellowing

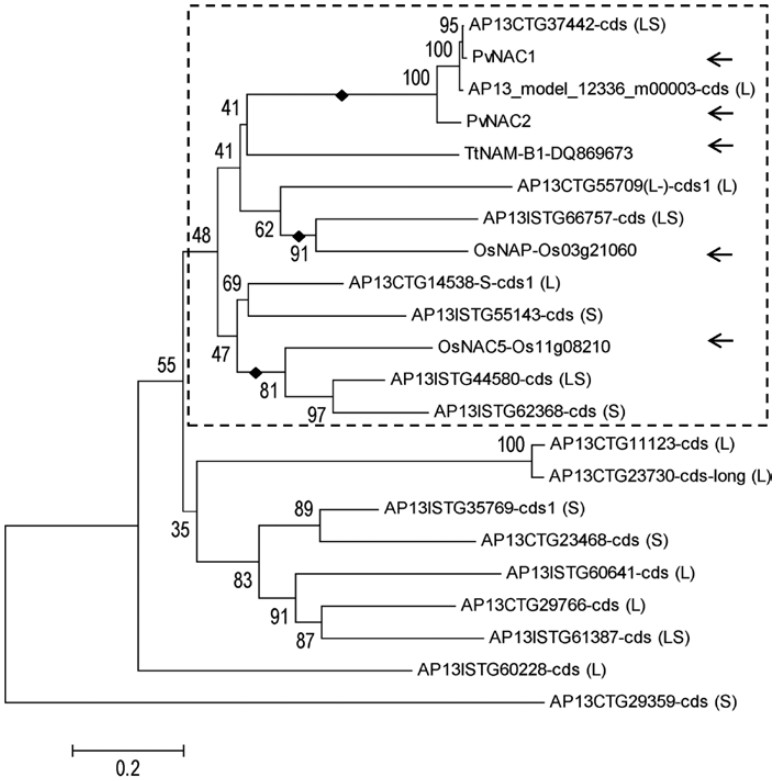

Fig. 1. Phylogenetic analysis of 17 NAC TFs preferentially expressed in switchgrass leaves and stems, and five reference NAC proteins that were previously characterized to be associated with nitrogen remobilization (TtNAM-B1, OsNAC5, OsNAP, PvNAC1, and PvNAC2; indicated by arrows) (Yang *et al.*, 2016). The letters L and S following a transcript ID stand for preferential expression in either leaves or stems, respectively. The clade containing eight PvNACs closely related to the five reference NACs is enclosed within the dashed-line rectangle. Five PvNACs are found in three sub-branches with high bootstrap values (>80, indicated with diamonds) containing the reference NACs.

and greater N retention in flag leaves, with a reduction in grain protein, zinc, and iron content (Uauy *et al.*, 2006*b*). Suppression of *ZmNAP* via RNA silencing resulted in a stay-green phenotype and a 15–30% increase in 1000 grain weight of *ZmNAP* RNAi plants compared to the wild-type (Zhang *et al.*, 2012). So far, no similar study has been performed in a perennial grass.

Delaying leaf senescence by over-producing senescence-inhibiting cytokinin has been achieved in multiple species, as recently reviewed by Guo and Gan (2014). Tissue-specific or inducible promoters are preferred in such approaches to minimize perturbation of normal development. Overexpression of isopentenyl transferase (IPT), required for cytokinin-biosynthesis, driven by a senescence-inducible promoter (P_{SAG}-*IPT*), was used to delay leaf senescence in various plant species, including rice, creeping bentgrass, and *Zoysia sinica* (Guo and Gan, 2014).

Stay-green and stress-tolerance traits can go hand-in-hand, as exemplified in many P_{SAG}-*IPT* transgenic plants that exhibited not only delayed senescence, but also greater tolerance of environmental stresses, such as drought (Merewitz *et al.*, 2011), flooding (Huynh *et al.*, 2005), cold (Khodakovskaya *et al.*, 2005), heat (Xu *et al.*, 2010), darkness (García-Sogo *et al.*, 2012; Zakizadeh *et al.*, 2013), and low N (Sykorová *et al.*, 2008). Genetic studies showed that quantitative trait loci (QTLs) for temperature and drought tolerance coincide with loci for leaf senescence (Ougham *et al.*, 2007; Vijayalakshmi *et al.*, 2010; Emebiri, 2013). In sorghum, hybrids possessing the stay-green trait maintained more photosynthetically active leaves than hybrids not possessing this trait when water was limiting during the grain-filling period, and produced 47% more post-anthesis biomass than their senescent counterparts (920 versus 624 g m^{-2}), while the stay-green trait did not constrain yield in the well-watered plants (Borrell *et al.*, 2000*a*, 2000*b*). Sorghum stay-green lines also showed more charcoal rot tolerance than non-stay-green lines (Burgess *et al.*, 2002). These results highlight the potential of utilizing the stay-green trait in perennial grasses to increase biomass yield and quality, especially under abiotic or biotic stress conditions. Very recently, it was reported that overexpression of the *Medicago WOX* gene *STENOFOLIA* (*STF*), which encodes a transcriptional repressor, in three monocots (switchgrass, rice, and *Brachypodium*) resulted in an increase in active cytokinin, via direct binding of the WOX protein to the promoters of cytokinin oxidases/dehydrogenase genes (CKXs; Wang *et al.*, 2017). STF-overexpressing switchgrass plants produced approximately 2-fold more biomass and released approximately 1.8-fold more sugar without pre-treatment. In addition, leaves of transgenic plants exhibited a stay-green phenotype (Chunxiang Fu, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, personal communication). These results show the potential value of the functional stay-green phenotype for improvement of biofuel grasses.

Effects of flowering time on N remobilization

Development of annual and perennial grasses can be divided into two distinct stages: vegetative and reproductive. Vegetative growth generates most of a plant's biomass and essentially ends with initiation of reproductive growth, while timing of heading (inflorescence initiation and development) is closely associated with grain production and biomass nutritional quality. Flowering time is highly variable among switchgrass varieties and associated with differences in photoperiod along a north–south gradient, i.e. northern ecotypes flower earlier than southern types (Casler *et al.*, 2004). 'Lowland' switchgrass ecotypes from the southern USA usually have heading dates 2–4 weeks later than 'upland' ecotypes from the north (Cortese *et al.*, 2010). Higher biomass production of lowland ecotypes is correlated with a longer vegetative growth period (Casler, 2012). Notably, moving northern ecotypes south prompts early flowering and reduced biomass production, while moving southern ecotypes north delays reproductive development, extends their growth period and increases biomass yields (Casler *et al.*, 2004).

In *Brachypodium* accessions, flowering time influences the final N content in biomass; late-flowering accessions produce more leaves and accumulate more total N (Schwartz and Amasino, 2013). We investigated 31 switchgrass accessions for N remobilization efficiency and found that upland ecotypes generally have lower NRE, despite flowering earlier (Yang *et al.*, 2009). Calendar days to heading, i.e. from January 1, were recorded for 14 lowland and 22 upland ecotypes from 2007 to 2011 at Ardmore, Oklahoma, USA (Fig. 2A), and showed later onset of reproductive development of lowland ecotypes, as expected. Interestingly, N-concentration in tillers harvested in mid-August, prior to senescence (i.e. still green) but after heading in all cases, were similar for lowland and upland ecotypes, despite the greater number of days between heading and harvest (DHH) for most upland ecotypes (Fig. 2B). In other words, there was no correlation between DHH and N concentration in tillers harvested in August. NRE was calculated as the difference between tiller N content at mature and senescent stages (N_m and N_s) harvested in August and December, respectively, divided by tiller N content at the mature stage $[(N_m-N_s)/N_m]$ (Yang *et al.*, 2009). Somewhat surprisingly, a positive correlation was found between DHH and N concentration in senescent tillers harvested in December, with linear correlation coefficient R=0.68, P<0.001 (Fig. 2C). In other words, upland ecotypes were less efficient at remobilizing N from tillers, despite initiating reproductive development earlier than lowland ecotypes. This is reflected in the negative correlation between DHH and N remobilization efficiency for all ecotypes taken together, with R=–0.64, P<0.001 (Fig. 2D).

In horticultural practice, surgical removal of inflorescences in monocarpic plants such as pea and soybean can reverse the fate of senescing leaves to juvenile leaves (Nooden, 1988). Similarly, maintenance of soybean plants under photoperiods that prevent induction of flowering promotes indefinite vegetative growth, indicating potential control of plant senescence by reproductive growth (Nooden *et al.*, 1997). In the model legume, *Medicago truncatula*, the *vernalization-insensitive delayed flowering in long days* (*vdf*) mutant with delayed flowering produced more above-ground biomass than the wild-type (Tadege *et al.*, 2015). In another study, overexpression of a miR156 precursor in switchgrass resulted in differential trait modification depending on the expression level of miR156 (Fu *et al.*, 2012). miR156 targets the SQUAMOSA PROMOTER BINDING PROTEIN LIKE transcription factor (SPL) that promotes apical dominance and floral transition (Fornara and Coupland, 2009). Transgenic plants with relatively low overexpression of miR156 exhibited increased biomass yield with normal flowering time, while moderate levels of miR156 led to improved biomass but no flowering. These two groups of plants produced 58–101% more biomass compared with the control, possibly due to an increase in tiller number. It would be interesting to investigate the senescence phenotype, NUE, and NRE of transgenic switchgrass with moderate miR156 expression under natural/field growth conditions.

Approaches to improve yield, yield stability, and NUE in perennial grasses for forage versus biofuel

For perennial grasses used either for forage or for bioenergy production, such as switchgrass, high biomass yield is desired (Table 1). Therefore, in addition to a fast growth rate, prolonged vegetative growth associated with delayed reproductive development (heading) and extended leaf photosynthetic capacity through functional stay-green traits are attractive breeding targets (Uauy *et al.*, 2006*b*; Guo and Gan, 2014). The functional stay-green trait may also enhance stress tolerance by making plants less prone to senescence under moderate or severe stress (Guo and Gan, 2014). Importantly, delayed flowering and delayed senescence phenotypes arise naturally via genetic mutation, which provides 'grist' for the plant breeding 'mill'. Knowledge of some of the key genes controlling flowering time, such as *SPL*, *LEAFY*, *APETALA1* (Fornara and Coupland, 2009; Yamaguchi *et al.*, 2009), and/or senescence, including *TtNAM-B1*, *OsNAP* and *PvNAC1/2* (Uauy *et al.*, 2006*b*; Liang *et al.*, 2014; Yang *et al.*, 2015) in different plant species makes it possible to design and implement mutational-breeding approaches, using chemically mutagenized seed and targeting induced local lesions in genomes (TILLING; McCallum *et al.*, 2000) or genome editing (Bortesi and Fischer, 2015), to produce desired mutations and phenotypes. Genome editing may be the most effective way to generate homozygous mutations in polyploid genomes, especially in out-crossing species such as switchgrass (Wang *et al.*, 2014). While delaying flowering and senescence could increase biomass in perennial grasses, completely abolishing these developmental processes may be counterproductive. No flowering means no seed, which is a problem for plant breeders, even if producers of perennial forages and biofuel feedstocks care little for seed production in their own fields. No senescence means no wholesale recycling and transfer of

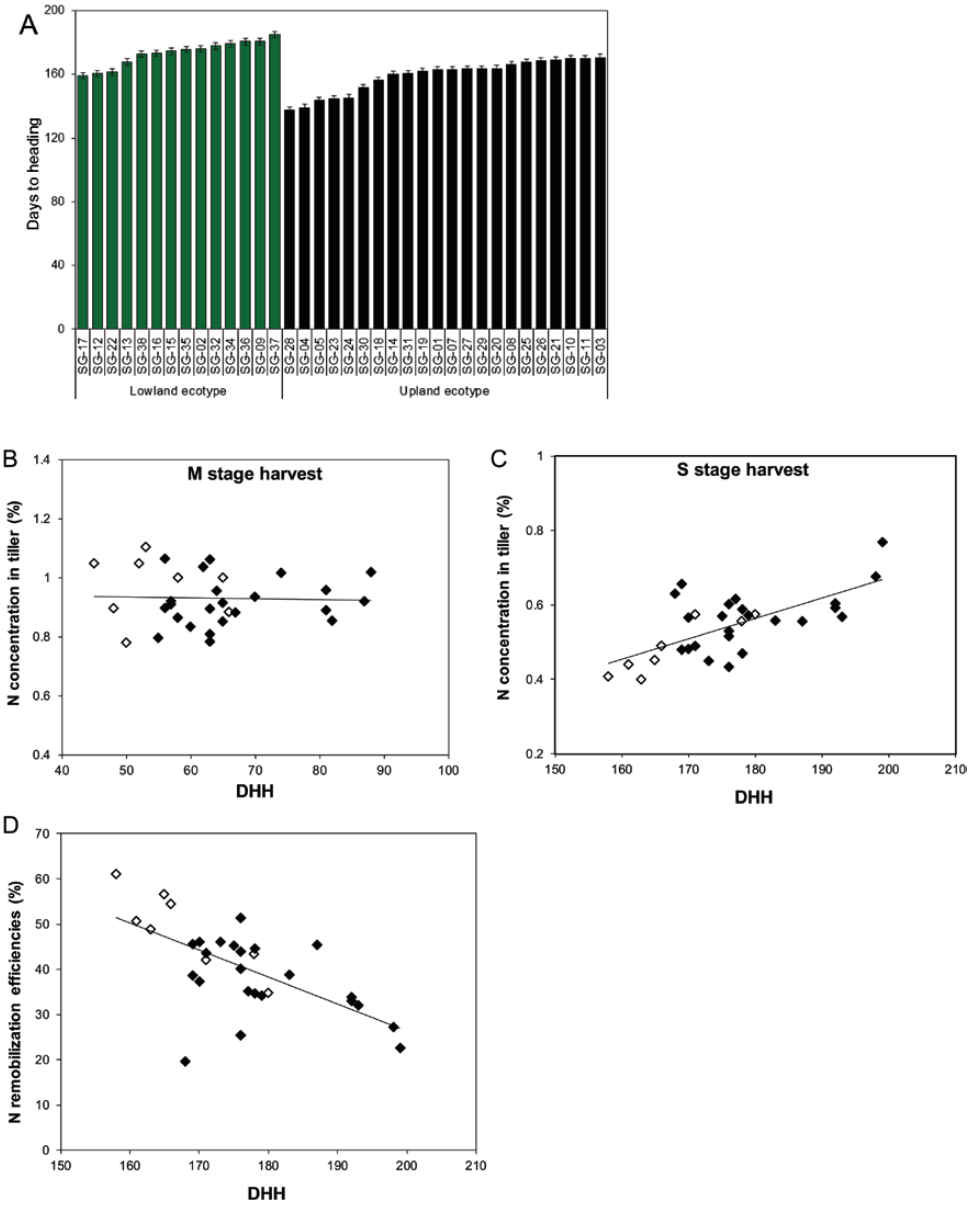

Fig. 2. Relationship between heading time and nitrogen remobilization. (A) Heading time comparison between switchgrass lowland and upland ecotypes. (B) Nitrogen concentration in switchgrass tillers in the pre-senescence stage (in August). (C) Nitrogen concentration in switchgrass tillers in the post-senescence stage (in December). (D) Correlation between nitrogen remobilization efficiency (NRE) and days between heading and harvest (DHH). NRE was calculated from tiller N content at mature (N_m) and senescent (N_s) stages, as follows: NRE=(N_m–N_s)/N_m. Diamonds in (B–D) indicate switchgrass accessions as reported previously (Yang *et al.*, 2009). The open diamonds indicate lowland ecotypes and solid diamonds indicate upland ecotypes.

Table 1. *Ideal ideotypes and approaches to optimize biomass production and nitrogen content of forage and biofuel perennial grasses*

	Forage crop	**Biofuel crop**
Biomass production	**High**	**High**
Growth period	Prolonged vegetative growth / delayed flowering	
Leaf function	Increased leaf area and photosynthetic capacity	
Leaf phenotype	Functional stay-green/delayed senescence	
Physiological adaptability	Increased biotic/abiotic stress tolerance	
Root function	Improved nitrogen transport and assimilation	
Nitrogen accumulation	**High**	**Low**
Biomass harvest time	Before senescence	After senescence
Nitrogen remobilization	Inhibited	Promoted
Chlorophyll degradation	Inhibited	Promoted
Expression of NAC TFs	Inhibited	Promoted

nutrients from old and damaged organs to new ones, which could limit the photosynthetic capacity and growth potential of perennial grasses, especially in nutrient-poor soils. No senescence and remobilization of nutrients into storage organs, such as root crowns, could compromise re-growth of shoots in the next growing seasons, and ultimately persistence of the grass stand. Fortunately, nature has shown us that it is possible to delay flowering and senescence without eliminating these important processes, so there is potential to improve yield and quality in this way.

While increased yield per unit area and time is a common target for producers of forage and biofuel-feedstock grasses, biomass protein and N-content is not (Table 1). While forage and feedstock producers all desire energy-rich carbohydrates in their biomass, forage producers also seek moderate protein levels while producers of biomass for biofuels do not. In fact, to conserve N in fields for the next growing season, it is desirable to harvest protein-poor shoots for biofuel, and store protein-N in underground organs following shoot senescence. Natural variation for NRE in switchgrass (Yang *et al.*, 2009) and other biofuel grasses will enable breeders to improve N-conservation in production systems. As we have noted above, greater NRE is not tied to earlier senescence, or at least not to earlier reproductive development, so it does not necessarily come at the cost of reduced yield (Fig. 2).

Retaining protein in vegetative organs may be achieved while maximizing biomass production by delaying reproductive development and/or delaying senescence, as described above. Obviously, this may come at the cost of seed production or N-storage in roots and crowns, which may compromise growth in the next season. On the other hand, some of the N consumed as protein in leaves by grazing animals is returned in urine to the soil, where it is available again to plants. N removed with animal protein and otherwise lost from the system through leaching or gaseous-N losses to the atmosphere needs to be replaced by addition of fertilizer-N in systems that don't include nitrogen-fixing legumes.

It is an interesting time to be working on senescence in plants, both from the perspective of basic research and plant breeding. Annotated genome sequences for many model and crop species and easy-to-use tools for transcriptomics have uncovered thousands of genes that are potentially involved in senescence, including regulators of this important developmental process (see above). Genetic studies in model and crop species have shown that some of these genes are not only required for normal senescence, but are also attractive targets to increase yield, improve quality, and/or increase NUE of plant production, through delayed and/or enhanced senescence. Recent developments in genome editing (Bortesi and Fischer, 2015) empower technologists and breeders to tailor senescence genes to produce new food, forage, and biofuel crops with these desired traits. On the other hand, there is still much to be learned about the regulation and mechanics of cellular deconstruction and nutrient-remobilization during senescence (Masclaux-Daubresse *et al.*, 2010). Especially for perennial grasses, it would be interesting to know how N remobilized during yearly senescence of shoots is allocated to the two major sinks: seeds and underground organs. Convergence of many experimental approaches, including genomics, genetics, biochemistry, molecular and cellular biology, physiology, bioinformatics, and modeling will lead to a systems-level understanding of senescence that will support further advances in plant breeding and nutrient conservation in agricultural systems.

Summary

N fertilizers are a double-edged sword for agriculture, essential for high yields (per unit area and time) of food, feed, fiber, and fuel, but damaging to the environment and human health at current rates of application. By increasing NUE, plant senescence can help to sharpen one edge of the N-sword while blunting the other via reduction in losses of reactive-N to the environment. Optimizing the timing and/or magnitude of senescence, via conventional breeding and/or genome-editing, may lead to plant cultivars that are not only more efficient in their use of N, but also less sensitive to environmental stress and, therefore, more productive overall. This is an exciting prospect that will be tested in the next few years.

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References

Alam MM, Karim MR, Ladha JK. 2013. Integrating best management practices for rice with farmers' crop management techniques: A potential option for minimizing rice yield gap. Field Crops Research **144,** 62–68.

Barbottin A, Lecomte C, Bouchard C, Jeuffroy M-H. 2005. Nitrogen remobilization during grain filling in wheat. Crop Science **45,** 1141–1150.

Barraclough PB, Howarth JR, Jones J, Lopez-Bellido R, Parmar S, Shepherd CE, Hawkesford MJ. 2010. Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. European Journal of Agronomy **33,** 1–11.

Biswas B, Gresshoff PM. 2014. The role of symbiotic nitrogen fixation in sustainable production of biofuels. International Journal of Molecular Sciences **15,** 7380–7397.

Bogard M, Allard V, Brancourt-Hulmel M, Heumez E, Machet JM, Jeuffroy MH, Gate P, Martre P, Le Gouis J. 2010. Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. Journal of Experimental Botany **61,** 4303–4312.

Bohlool BB, Ladha JK, Garrity DP, George T. 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. Plant and Soil **141,** 1–11.

Borrell AK, Hammer GL. 2000. Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop Science **40,** 1295–1307.

Borrell AK, Hammer GL, Douglas ACL. 2000a. Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. Crop Science **40,** 1026–1037.

Borrell AK, Hammer GL, Henzell RG. 2000b. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Science **40,** 1037–1048.

Bortesi L, Fischer R. 2015. The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnology Advances **33,** 41–52.

Breeze E, Harrison E, McHattie S, *et al*. 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. The Plant Cell **23,** 873–894.

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, Pink D. 2003. The molecular analysis of leaf senescence–a genomics approach. Plant Biotechnology Journal **1,** 3–22.

Buchanan-Wollaston V, Page T, Harrison E, *et al*. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. The Plant Journal **42,** 567–585.

Burgess MG, Rush CM, Piccinni G, Steddom K, Workneh F. 2002. Relationship between charcoal rot, the stay-green trait, and irrigation in grain sorghum. Phytopathology **92,** S10.

Canfield DE, Glazer AN, Falkowski PG. 2010. The evolution and future of Earth's nitrogen cycle. Science **330,** 192–196.

Casler MD. 2012. Switchgrass breeding, genetics, and genomics. In: Monti A, ed. *Switchgrass*. New York: Springer, 29–54.

Casler MD, Vogel KP, Taliaferro CM, Wynia RL. 2004. Latitudinal adaptation of switchgrass populations. Crop Science **44,** 293–303.

Cassman KG, Dobermann A, Walters DT. 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. Ambio**31,** 132–140.

Chen X, Cui Z, Fan M, *et al*. 2014. Producing more grain with lower environmental costs. Nature **514,** 486–489.

Chen X-P, Cui Z-L, Vitousek PM, *et al*. 2011. Integrated soil–crop system management for food security. Proceedings of the National Academy of Sciences, USA **108,** 6399–6404.

Chu G, Wang Z, Zhang H, Yang J, Zhang J. 2016. Agronomic and physiological performance of rice under integrative crop management. Agronomy Journal **108,** 117–128.

Cortese LM, Honig J, Miller C, Bonos SA. 2010. Genetic diversity of twelve switchgrass populations using molecular and morphological markers. BioEnergy Research **3,** 262–271.

Dohleman FG, Heaton EA, Arundale RA, Long SP. 2012. Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus×giganteus* and *Panicum virgatum* across three growing seasons. GCB Bioenergy **4,** 534–544.

Elser JJ, Bracken ME, Cleland EE, *et al*. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10,** 1135–1142.

Emebiri LC. 2013. QTL dissection of the loss of green colour during post-anthesis grain maturation in two-rowed barley. Theoretical and Applied Genetics **126,** 1873–1884.

FAO. 2012. *FAO Statistical Yearbook 2012*. Rome: Economic and Social Development Department, FAO.

Fischer AM. 2012. The complex regulation of senescence. Critical Reviews in Plant Sciences **31,** 124–147.

Fornara F, Coupland G. 2009. Plant phase transitions make a SPLash. Cell **138,** 625–627.

Frink CR, Waggoner PE, Ausubel JH. 1999. Nitrogen fertilizer: Retrospect and prospect. Proceedings of the National Academy of Sciences, USA **96,** 1175–1180.

Fu C, Sunkar R, Zhou C, *et al*. 2012. Overexpression of miR156 in switchgrass (*Panicum virgatum* L.) results in various morphological alterations and leads to improved biomass production. Plant Biotechnology Journal **10,** 443–452.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science **320,** 889–892.

García-Sogo B, Pineda B, Roque E, Antón T, Atarés A, Borja M, Beltrán JP, Moreno V, Cañas LA. 2012. Production of engineered long-life and male sterile *Pelargonium* plants. BMC Plant Biology **12,** 156.

Garnett T, Plett D, Heuer S, Okamoto M. 2015. Genetic approaches to enhancing nitrogen-use efficiency (NUE) in cereals: challenges and future directions. Functional Plant Biology **42,** 921–941.

Godfray HCJ, Beddington JR, Crute IR, *et al*. 2010. Food security: the challenge of feeding 9 billion people. Science **327,** 812–818.

Gregersen PL, Holm PB, Krupinska K. 2008. Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biology **10**(Suppl 1), 37–49.

Guo Y, Cai Z, Gan S. 2004. Transcriptome of Arabidopsis leaf senescence. Plant, Cell & Environment **27,** 521–549.

Guo Y, Gan SS. 2014. Translational researches on leaf senescence for enhancing plant productivity and quality. Journal of Experimental Botany **65,** 3901–3913.

Guretzky J, Biermacher J, Cook B, Kering M, Mosali J. 2011. Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant and Soil **339,** 69–81.

Gustavsson J, Cederberg C, Sonesson U, van Otterdijk R, Meybeck A. 2011. *Global food losses and food waste: extent causes and prevention*. Rome: Food and Agriculture Organization of the United Nations.

Heaton EA, Dohleman FG, Long SP. 2009. Seasonal nitrogen dynamics of *Miscanthus×giganteus* and *Panicum virgatum*. GCB Bioenergy **1,** 297–307.

Herridge DF, Peoples MB, Boddey RM. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil **311,** 1–18.

Himelblau E, Amasino RM. 2001. Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. Journal of Plant Physiology **158,** 1317–1323.

Hörtensteiner S. 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends in Plant Science **14,** 155–162.

Hörtensteiner S, Kräutler B. 2011. Chlorophyll breakdown in higher plants. Biochimica Et Biophysica Acta **1807,** 977–988.

Huynh LN, Vantoai T, Streeter J, Banowetz G. 2005. Regulation of flooding tolerance of *SAG12:ipt* Arabidopsis plants by cytokinin. Journal of Experimental Botany **56,** 1397–1407.

Jibran R, A Hunter D, P Dijkwel P. 2013. Hormonal regulation of leaf senescence through integration of developmental and stress signals. Plant Molecular Biology **82,** 547–561.

Johnson J, Wilhelm W, Karlen D, *et al*. 2010. Nutrient removal as a function of corn stover cutting height and cob harvest. BioEnergy Research **3,** 342–352.

Joppa LR, Du C, Hart GE, Hareland GA. 1997. Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. Crop Science **37,** 1586–1589.

Kering MK, Butler TJ, Biermacher JT, Guretzky JA. 2012. Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. BioEnergy Research **5,** 61–70.

Khodakovskaya M, Li Y, Li J, Vanková R, Malbeck J, McAvoy R. 2005. Effects of *cor15a-IPT* gene expression on leaf senescence in transgenic *Petunia×hybrida* and *Dendranthema×grandiflorum*. Journal of Experimental Botany **56,** 1165–1175.

Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J. 2007. In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Research **102,** 22–32.

Ladha JK, Chakraborty D. 2016. Nitrogen and cereal production: Opportunities for enhanced efficiency and reduced N losses. Proceedings of the 2016 International Nitrogen Initiative Conference, *'Solutions to improve nitrogen use efficiency for the world'*. Melbourne, Australia, http://www.ini2016.com/1715.

Lemus R, Parrish D, Abaye O. 2008. Nitrogen-use dynamics in switchgrass grown for biomass. BioEnergy Research **1,** 153–162.

Li Z, Peng J, Wen X, Guo H. 2012. Gene network analysis and functional studies of senescence-associated genes reveal novel regulators of Arabidopsis leaf senescence. Journal of Integrative Plant Biology **54,** 526–539.

Liang C, Wang Y, Zhu Y, *et al*. 2014. OsNAP connects abscisic acid and leaf senescence by fine-tuning abscisic acid biosynthesis and directly targeting senescence-associated genes in rice. Proceedings of the National Academy of Sciences, USA **111,** 10013–10018.

Lim PO, Kim HJ, Nam HG. 2007. Leaf senescence. Annual Review of Plant Biology **58,** 115–136.

Lim PO, Woo HR, Nam HG. 2003. Molecular genetics of leaf senescence in Arabidopsis. Trends in Plant Science **8,** 272–278.

Lynch JP, Wojciechowski T. 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. Journal of Experimental Botany **66,** 2199–2210.

Marschner H. 1995. *Mineral nutrition of higher plants*. London: Academic Press.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany **105,** 1141–1157.

Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M. 2008. Leaf nitrogen remobilisation for plant development and grain filling. Plant Biology **10**(Suppl 1), 23–36.

McCallum CM, Comai L, Greene EA, Henikoff S. 2000. Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. Plant Physiology **123,** 439–442.

Merewitz EB, Gianfagna T, Huang B. 2011. Photosynthesis, water use, and root viability under water stress as affected by expression of *SAG12-ipt* controlling cytokinin synthesis in *Agrostis stolonifera*. Journal of Experimental Botany **62,** 383–395.

Michael Beman J, Arrigo KR, Matson PA. 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. Nature **434,** 211–214.

Moore KJ, Moser LE, Vogel KP, Waller SS, Johnson BE, Pedersen JF. 1991. Describing and quantifying growth stages of perennial forage grasses. Agronomy Journal **83,** 1073–1077.

Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley JA. 2012. Closing yield gaps through nutrient and water management. Nature **490,** 254–257.

Munné-Bosch S. 2008. Do perennials really senesce? Trends in Plant Science **13,** 216–220.

Mus F, Crook MB, Garcia K, *et al*. 2016. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Applied and Environmental Microbiology **82,** 3698–3710.

Nassi o Di Nasso N, Roncucci N, Bonari E. 2013. Seasonal dynamics of aboveground and belowground biomass and nutrient accumulation and remobilization in giant reed (*Arundo donax* L.): a three-year study on marginal land. BioEnergy Research **6,** 725–736.

Nassi o Di Nasso N, Roncucci N, Triana F, Tozzini C, Bonari E. 2011. Seasonal nutrient dynamics and biomass quality of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus×giganteus* Greef et Deuter) as energy crops. Italian Journal of Agronomy **6,** e24.

Nooden LD. 1988. The phenomena of senescence and aging. In: Nooden L, Leopold AD, ed. *Senescence and aging in plants*. San Diego: Academci Press, Inc., 1–50.

Nooden LD, Guiamet JJ, John I. 1997. Senescence mechanisms. Plant Physiology **101,** 746–753.

Noodén LD, Penney JP. 2001. Correlative controls of senescence and plant death in *Arabidopsis thaliana* (Brassicaceae). Journal of Experimental Botany **52,** 2151–2159.

O'Neill PM, Shanahan JF, Schepers JS, Caldwell B. 2004. Agronomic responses of corn hybrids from different eras to deficit and adequate levels of water and nitrogen. Agronomy Journal **96,** 1660–1667.

Ougham H, Armstead I, Howarth C, Galyuon I, Donnison I, Thomas H. 2007. The genetic control of senescence revealed by mapping quantitative trait loci. In: Gan S, ed. *Senescence processes in plants*, Annual Plant Reviews, vol.26. Blackwell Publishing Ltd, 171–201.

Pimentel D, Pimentel M. 2003. Sustainability of meat-based and plant-based diets and the environment. The American Journal of Clinical Nutrition **78,** 660S–663S.

Propheter JL, Staggenborg S. 2010. Performance of annual and perennial biofuel crops: nutrient removal during the first two years. Agronomy Journal **102,** 798–805.

Rapp YG, Ransbotyn V, Grafi G. 2015. Senescence meets dedifferentiation. Plants **4,** 356–368.

Reynolds JH, Walker CL, Kirchner MJ. 2000. Nitrogen removal in switchgrass biomass under two harvest systems. Biomass and Bioenergy **19,** 281–286.

Rockström J, Steffen W, Noone K, *et al*. 2009. Planetary boundaries: exploring the safe operating space for humanity. Ecology and Society **14,** 32

Rogers C, Oldroyd GE. 2014. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. Journal of Experimental Botany **65,** 1939–1946.

Saengwilai P, Nord EA, Chimungu JG, Brown KM, Lynch JP. 2014a. Root cortical aerenchyma enhances nitrogen acquisition from low-nitrogen soils in maize. Plant Physiology **166,** 726–735.

Saengwilai P, Tian X, Lynch JP. 2014b. Low crown root number enhances nitrogen acquisition from low-nitrogen soils in maize. Plant Physiology **166,** 581–589.

Santi C, Bogusz D, Franche C. 2013. Biological nitrogen fixation in non-legume plants. Annals of Botany **111,** 743–767.

Schiltz S, Munier-Jolain N, Jeudy C, Burstin J, Salon C. 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 Physiology **137,** 1463–1473.

Schwartz C, Amasino R. 2013. Nitrogen recycling and flowering time in perennial bioenergy crops. Frontiers in Plant Science **4,** 76.

Spano G, Di Fonzo N, Perrotta C, Platani C, Ronga G, Lawlor DW, Napier JA, Shewry PR. 2003. Physiological characterization of 'stay green' mutants in durum wheat. Journal of Experimental Botany **54,** 1415–1420.

Sperotto RA, Ricachenevsky FK, Duarte GL, Boff T, Lopes KL, Sperb ER, Grusak MA, Fett JP. 2009. Identification of up-regulated genes in flag leaves during rice grain filling and characterization of *Os*NAC5, a new ABA-dependent transcription factor. Planta **230,** 985–1002.

Strullu L, Cadoux S, Preudhomme M, Jeuffroy MH, Beaudoin N. 2011. Biomass production and nitrogen accumulation and remobilisation by *Miscanthus* × *giganteus* as influenced by nitrogen stocks in belowground organs. Field Crops Research **121,** 381–391.

Subbarao GV, Nakahara K, Hurtado MP, *et al*. 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proceedings of the National Academy of Sciences, USA **106,** 17302–17307.

Sutton MA, Nemitz E, Erisman JW, *et al*. 2007. Challenges in quantifying biosphere–atmosphere exchange of nitrogen species. Environmental Pollution **150,** 125–139.

Sutton MA, Oenema O, Erisman JW, Leip A, van Grinsven H, Winiwarter W. 2011. Too much of a good thing. Nature **472,** 159–161.

Sykorová B, Kuresová G, Daskalova S, *et al*. 2008. Senescence-induced ectopic expression of the *A. tumefaciens ipt* gene in wheat delays leaf senescence, increases cytokinin content, nitrate influx, and nitrate reductase activity, but does not affect grain yield. Journal of Experimental Botany **59,** 377–387.

Tadege M, Chen F, Murray J, Wen J, Ratet P, Udvardi MK, Dixon RA, Mysore KS. 2015. Control of vegetative to reproductive phase transition improves biomass yield and simultaneously reduces lignin content in *Medicago truncatula*. BioEnergy Research **8,** 857–867.

Thomas H, Ougham H. 2014. The stay-green trait. Journal of Experimental Botany **65,** 3889–3900.

Uauy C, Brevis JC, Dubcovsky J. 2006a. The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. Journal of Experimental Botany **57,** 2785–2794.

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. 2006b. A NAC Gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science **314,** 1298–1301.

van der Graaff E, Schwacke R, Schneider A, Desimone M, Flügge UI, Kunze R. 2006. Transcription analysis of arabidopsis membrane transporters and hormone pathways during developmental and induced leaf senescence. Plant Physiology **141,** 776–792.

van Zanten HHE, Mollenhorst H, Klootwijk CW, van Middelaar CE, de Boer IJM. 2016. Global food supply: land use efficiency of livestock systems. The International Journal of Life Cycle Assessment **21,** 747–758.

Vijayalakshmi K, Fritz AK, Paulsen GM, Bai G, Pandravada S, Gill BS. 2010. Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Molecular Breeding **26,** 163–175.

Wang H, Niu L, Fu C, *et al*. 2017. Overexpression of the *WOX* gene *STENOFOLIA* improves biomass yield and sugar release in transgenic grasses and display altered cytokinin homeostasis. PLOS Genetics **13,** e1006649.

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology **32,** 947–951.

Xu G, Fan X, Miller AJ. 2012. Plant nitrogen assimilation and use efficiency. Annual Review of Plant Biology **63,** 153–182.

Xu Y, Gianfagna T, Huang B. 2010. Proteomic changes associated with expression of a gene (*ipt*) controlling cytokinin synthesis for improving heat tolerance in a perennial grass species. Journal of Experimental Botany **61,** 3273–3289.

Yamaguchi A, Wu MF, Yang L, Wu G, Poethig RS, Wagner D. 2009. The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of *LEAFY*, *FRUITFULL*, and *APETALA1*. Developmental Cell **17,** 268–278.

Yang J, Worley E, Ma Q, *et al*. 2016. Nitrogen remobilization and conservation, and underlying senescence-associated gene expression in the perennial switchgrass *Panicum virgatum*. New Phytologist **211,** 75–89.

Yang J, Worley E, Torres-Jerez I, Miller R, Wang M, Fu C,Wang Z-Y,Tang Y, Udvardi M. 2015. *PvNAC1*and *PvNAC2*are associated with leaf senescence and nitrogen use efficiency in switchgrass. BioEnergy Research **8,** 868–880.

Yang J, Worley E, Wang M, Lahner B, Salt D, Saha M, Udvardi M. 2009. Natural variation for nutrient use and remobilization efficiencies in switchgrass. BioEnergy Research **2,** 257–266.

Zakizadeh H, Lütken H, Sriskandarajah S, Serek M, Müller R. 2013. Transformation of miniature potted rose (*Rosa hybrida* cv. Linda) with P*SAG12-ipt* gene delays leaf senescence and enhances resistance to exogenous ethylene. Plant Cell Reports **32,** 195–205.

Zhang Y, Cao Y, Shao Q, Wang L, Wang H, Li J, Li H. 2012. Regulating effect of *ZmNAP* gene on anti-senescence and yield traits of maize. Journal of Henan Agricultural Seicences **41,** 19–24.

Zhou C, Han L, Pislariu C, *et al*. 2011. From model to crop: functional analysis of a *STAY-GREEN* gene in the model legume *Medicago truncatula* and effective use of the gene for alfalfa improvement. Plant Physiology **157,** 1483–1496.