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Review

Carbon Assimilation, Biomass Partitioning and Productivity in Grasses

Louis J. Irving

Faculty of Life and Environmental Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan; E-Mail: irving.louis.fb@u.tsukuba.ac.jp; Tel.: +81-029-853-4777

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Abstract: Plant growth correlates with net carbon gain on a whole plant basis. Over the last several decades, the driving factors shaping plant morphology and performance have become increasingly clear. This review seeks to explore the importance of these factors for grass performance. Briefly, these fall into factors influencing photosynthetic rates directly, competition between plants in a canopy, and nutrient status and availability.

Keywords: carbon; nitrogen; biomass partitioning; shoot-root allocation

1. Introduction

Plant growth correlates with net carbon (C) gain on a whole plant basis [1]. Photosynthetic rates vary between species, but leaf nitrogen (N) content and light intensity are known to be major determinants [2]. The other main factor determining plant carbon gain is leaf area per plant, which is a function of mean leaf area and leaf number. In grasses, the production of secondary stems by tillering can greatly increase leaf number per plant, with tiller numbers appearing to be mediated by plant nutrient status, light availability and competition for light between plants in a stand.

A recent review [3] looked at the factors controlling the allocation of biomass to leaves, stems, and roots in a wide range of species. The most important factors were determined to be the quantity of light available (daily photon irradiance), nutrient and water availability, temperature, and plant age/size. However, two of these, water availability and temperature, tended to have large effects only at extremes—very low temperatures, and either strong water deficits or waterlogging conditions. Thus, for most plants, light intensity and nutrient availability seem to be the primary factors driving carbon acquisition and biomass allocation. While Poorter’s review covered a huge number of studies,

unfortunately they could not cover physiological mechanisms in great detail. The purpose of this review is to cover some of the mechanisms controlling plant performance, particularly in the areas of photon capture by shoots and resource acquisition by roots, with a focus on grasses.

Grasses (family Poaceae) are a plant group of unique importance in agriculture. Grasses contribute more than half the calories consumed worldwide—both directly, as cereals, and as forage grasses that form the basis for the production of meat and milk worldwide [4]. Thus, a complete understanding of the factors determining the productivity of grass plants is of fundamental importance in meeting the food needs of the growing human population. Cultivated and semi-natural grasslands cover an area estimated at 52 million km² [5], and account for approximately 15% of global primary productivity [4]. Grass architecture is based around a tiller axis comprised of a stack of phytomers with an apical meristem responsible for the production of new phytomers. Each phytomer has the capacity to produce one leaf, one tiller, and one or more roots. The tiller axis of vegetative grasses is generally small, and is often located around ground level, while the leaves grow from the base, as adaptations to prevent grazing damage to the meristems. Perennial ryegrass (*Lolium perenne* L.) has on average three leaves per tiller, with the initiation of new leaves coinciding with the senescence of the fourth leaf [6], while cereal grasses may have a much higher number of leaves.

Under competitive conditions, we expect plants to pursue a strategy where they use resources such as N and water to maximise growth and ultimately reproduction. Photoassimilates represent both the substrate for growth, and also energy storage for biochemical activities. The partitioning of these resources can have significant implications for plant productivity. For example, generally in grasses, around 80%–85% of plant biomass is partitioned to aboveground organs, such as leaves and stems, with 15%–20% allocated to roots. However, root mass allocation can be significantly affected by nutrient or light availability [3], with greater carbon allocation to the root system under low nutrient, high-light conditions. Even within the root system, the distribution of roots is frequently non-uniform, with localized root proliferation common in nutrient rich patches [7]. This proliferation produces root biomass in excess of that required to fully exploit the nutrient-rich patch, but is thought to increase nutrient capture rates in competitive environments, allowing such plants to outcompete those producing fewer roots, and thus come to dominate the ecosystem. It is worth stressing that the optimal biomass allocation will depend heavily on environmental constraints.

Thornley [8] developed an early model of vegetative plant growth and biomass allocation based upon carbon and nitrogen uptake and assimilation (Figure 1). Thornley's model holds that carbon fixed in the shoot is either used in the shoot or transported to the roots. Similarly, nitrogen absorbed by the roots can either be used directly *in situ*, or transported to the shoot. Although over the subsequent decades many more complex models have been developed [9–14], Thornley's model provides a clear, simple starting point from which to develop a more comprehensive understanding of biomass allocation in grasses. Within each compartment, carbon can be used either for maintenance or growth [15]. In the case of roots, carbon from the stem can be respired to provide energy for metabolic processes such as nutrient uptake and assimilation, or used in growth to explore the soil for further nutrients. Meanwhile, nitrogen in excess of that required by roots can be transported to the shoots for use there. Thus, in order to understand the partitioning of carbon within plants, we need to understand the factors which determine the size of the carbon/nitrogen pool and its usage in each organ. While quantitative descriptions of factors driving

carbon allocation are becoming increasingly robust [3], there remains much to be done on understanding the underlying mechanisms in context. This paper aims to outline some of these processes.

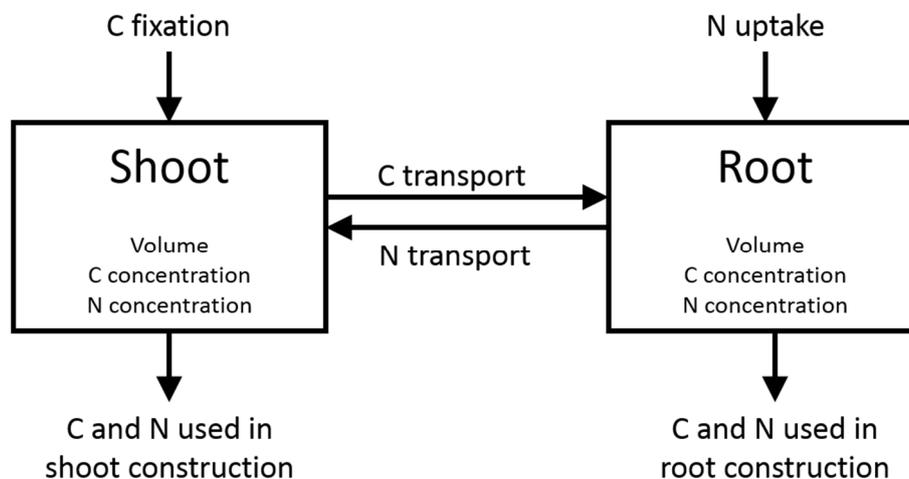


Figure 1. Two pool model of shoot and root growth. C and N status in each pool influences the volume of the pool. Adapted from [8].

2. Carbon Fixation

Given the primacy of photosynthesis in plant growth, it seems logical to first discuss factors influencing photosynthetic rate. However, given the size and complexity of the subject area, this is not intended to be a comprehensive analysis of photosynthesis, or of the factors controlling photosynthetic rates. Rather, the goal is to briefly explore a few of the more important factors, first on an individual plant basis, and then for competing plants in a later section.

At a biochemical level, the inefficiencies of C₃ photosynthesis are well understood, and mainly center on the enzyme Rubisco [16]. The CO₂ fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC4.1.1.39), has a very low catalytic efficiency; thus requiring the investment of 20%–30% of leaf nitrogen in Rubisco [17]. Given its inefficiency, and the relatively high proportion of leaf nitrogen for which Rubisco accounts, a strong positive correlation between photosynthetic rates and leaf nitrogen concentration has been noted [18,19]. As we shall discuss, this requirement for high concentrations of Rubisco in photosynthesizing leaves limits productivity, and has profound implications for plant form and productivity.

Photorespiration is the process by which Rubisco fixes oxygen rather than CO₂, with the resulting production of CO₂ and ammonia, and represents a second major inefficiency of C₃ photosynthesis—and thus plant productivity—with up to one-third of light energy intercepted by the plant used in photorespiration. Photosynthesis and photorespiration are competitive, with an increase in CO₂ or a decrease in O₂ having equivalent effects of increasing carbon fixation. Indeed, classical studies show that growing *Phaseolus vulgaris* L. at reduced oxygen levels led to a 40% increase in photosynthetic CO₂ uptake [20], while a 30% increase in grain yield was noted in rice plants grown at elevated CO₂ [21]. Thus, it is generally assumed that increasing atmospheric CO₂ levels will likewise lead to a decrease in photorespiration, and an increase in plant productivity. However, recent evidence suggests that malate

formed during photorespiration is an important source of energy for the reduction of nitrate to nitrite—the first step in nitrate assimilation. Free air carbon dioxide enrichment (FACE) experiments suggest that future increases in atmospheric CO₂ levels may suppress photorespiration, leading to grain protein levels between 7% and 10% lower than current conditions in C₃ plants—with negative consequences for human nutrition and health globally [22,23]. However, this is strongly species-specific; in loblolly pine (*Pinus taeda* L.) saplings fed nitrate as their sole N source, CO₂ enrichment led to a suppression of growth compared to plants fed ammonium, while in the same study, neither elevated CO₂ levels nor nitrogen form had much of an effect on wheat (*Triticum aestivum* L.) growth [24]. While some studies have demonstrated acclimatory decreases in leaf photosynthesis as a result of elevated CO₂ levels [25], others have noted increases in leaf photosynthetic rates [26], which may be linked to decreased stomatal conductance and improved water use efficiency. Clearly there remain significant questions about the photosynthetic and growth responses in C₃ grasses, and the importance of elevated CO₂ to future productivity.

C₄ plants use a biochemical pump to increase the CO₂ concentration around Rubisco, thus suppressing photorespiration. C₄ Rubisco has carboxylation rates up to double those of C₃ plants [27] with C₄ plants achieving superior photosynthetic nitrogen use efficiencies (CO₂ fixed per unit nitrogen) due to their reduced N requirement for photosynthesis. Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) are agronomically important C₄ grasses, with both species used both for grain and as silages for animal fodder. However, given that approximately 46% of the estimated 10,000 grass species use C₄ photosynthesis [28], there must be many other C₄ grasses which would be suitable targets for improvement for forage, despite their generally low nutritive value. C₄ photosynthesis has evolved at least 24 times in the grasses [29], and there are significant efforts currently to engineer C₄ rice (*Oryza sativa* L.) with the goal of significant increases in grain yield [30]. C₄ plants significantly increase the CO₂ concentration around Rubisco, which in principle would allow the use of more efficient forms of Rubisco, such as those originating in cyanobacteria [31]. Cyanobacterial Rubisco has catalytic rates approximately four-times those of C₃ plants, and might drive up carbon fixation rates and growth, or at least reduce N requirements for photosynthesis. This might be useful in crops destined for biofuel production, but the low tissue N levels would provide poor nutritive ability for animals. A further benefit of developing C₄ agricultural species might be an improvement in stress tolerance. In sorghum, drought induced yield declines were reduced in plants grown under elevated CO₂ conditions, suggesting that future increases in atmospheric CO₂ conditions may alleviate drought stress, even in C₄ plants [32].

Rubisco overexpressing (125% of WT) rice plants exhibited 32% and 15% increased biomass when grown at 280 and 400 ppm CO₂ respectively, but no difference at 1200 ppm. In the same study, Rubisco antisense plants (35% of WT) exhibited significantly lower biomass levels, irrespective of CO₂ level [33]. However, the reductions in relative growth rate in the antisense plants were small compared to the large decrease in leaf Rubisco levels. Clearly Rubisco concentration and growth are inherently linked to the atmospheric CO₂ levels. Increases in CO₂ levels have been shown to cause a decrease in leaf Rubisco levels in *Arabidopsis* [34,35] and rice [36], although Rubisco contents in pea (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.), and parsley (*Petroselinum crispum* Mill.) (all C₃) are relatively unaffected by CO₂ levels [37]. It has been hypothesized that decreased N investment in Rubisco may lead to an increase in photosynthetic nitrogen use efficiency, and greater biomass production [16]. In line with this, wheat (C₃) plants grown under elevated CO₂ were taller, produced more tillers, had lower leaf area ratio (leaf

area per unit shoot mass), and at high N availabilities, had relatively larger root systems than plants grown under control CO₂ levels [38]. CO₂ levels had no effect on maize (C₄) growth in the same experiment, perhaps suggesting that future increases in atmospheric CO₂ levels may have a smaller effect on C₄ plant morphology than for C₃ plants. If these findings can be applied to forage grasses, reduced leaf area and protein content would suggest that increasing CO₂ levels may lead to significant declines in forage quality in C₃ grasses.

3. Plant Morphology as a Partial Consequence of Photosynthetic N Requirement

Plant N concentration decreases allometrically with plant size [39]. The minimum N concentration which allows maximum growth is termed the “critical N concentration”, with plants grown at sub-optimal N levels exhibiting proportionately decreased growth rates. Although there are clear differences in critical N concentrations between C₃ and C₄ plants due to the lower N requirements of C₄ plants, the relationship between plant size and N concentration can be found in both groups, with the primary factor appearing to be a decrease in “leafiness” through plant development [14]. As the plant grows, over time a greater proportion of biomass must be allocated to stems and other support material. Thus, the decrease in N concentration with age may reflect an increase in shaded or senescent leaves over time, or a shift in shoot to root ratio such that C fixation rates are higher than N uptake rates. Hikosaka and Terashima [40] demonstrated that the optimal allocation of N into photosynthetic components varies with light and nitrogen availability. Under high light conditions, high levels of Rubisco are required to maximise photosynthesis. However, as the leaf ages and transitions to a subordinate canopy position shaded by younger leaves, the optimal balance shifts towards the degradation of Rubisco and the remobilization of N to new leaves. These patterns are exacerbated in N-deficient plants.

Given that there is a light gradient down the canopy, with older leaves shaded by younger leaves, plant nitrogen use efficiency can be increased by translocating N in excess of photosynthetic requirements from older, shaded leaves, to younger leaves which have an optimal canopy position [41]. Grasses undergo progressive senescence where the older leaves senesce and are replaced by younger leaves on a continual basis. Photosynthetic rates at ambient CO₂ levels scale with leaf Rubisco content [42], which increases rapidly during leaf emergence, reaches a peak around full expansion, and declines through until senescence [43,44]. However, even under high exogenous supply, approximately half of the nitrogen for new leaf growth comes from the remobilization of leaf proteins including Rubisco from older leaves [45]. Thus, Rubisco represents a key juncture between carbon and nitrogen metabolic pathways. Rubisco retention in older leaves may increase the photosynthetic capacity of that leaf; however, particularly under N limiting conditions, carbon fixation might have been higher had the N been remobilized to younger leaves. In this case, maximising leaf photosynthesis may lead to a decrease in carbon fixation at the plant level. Thus, we expect a balance between the maintenance of leaf photosynthesis, and nitrogen remobilization to new tissues. Nitrogen remobilization is known to be important in supporting the development of new leaves—in *Poa trivialis* L. and *Panicum maximum* (Jacq.), despite removal of the exogenous N supply, new leaves continued to be produced using N remobilized from older organs [46]. Although some loss of N must be expected in senescent biomass, N remobilization is clearly important in determining plant productivity, with a recent paper demonstrating

significantly reduced growth in mutant rice plants with reduced capacity for Rubisco degradation, particularly under N limiting conditions [47]. Thus, we can postulate an optimal leaf protein turnover rate which maximises canopy rather than leaf photosynthesis. Given the importance of CO₂ availability to biomass production in Rubisco overexpressing rice, this optimum is likely environmentally modulated, or contingent on other factors, such as plant size. Development of grasses using high catalytic efficiency Rubisco may reduce the need for internal recycling of nitrogen, leading to a more uniform N allocation between leaves.

A recent paper [48] demonstrated a negative correlation between the maximum leaf Rubisco concentration and plant dry mass in glasshouse grown *Lolium perenne* plants. A second paper, comparing two long-leaved with two short-leaved *L. perenne* genotypes, presented evidence that leaf Rubisco content correlated strongly with leaf elongation duration, tiller weight, and leaf elongation rate, with a weaker correlation noted between leaf Rubisco content and plant dry mass [49]. However, these correlations disappeared when Rubisco levels were considered in terms of concentration rather than content. This suggests that leaf Rubisco concentration is relatively invariant, with plant productivity more strongly controlled by leaf size. If we assume that the maximum Rubisco concentration in leaf cells is essentially a constant, and that leaves grow in proportion to the available carbon and nitrogen resources, we might expect leaf size to be contingent upon photosynthetic rates and nitrogen remobilization within the plant as a whole.

In *Fallopia japonica* (Houtt.), as plant nitrogen content increases a greater proportion of biomass is allocated to leaves, while the specific leaf weight (leaf mass per unit area) decreases [10]. Even assuming that the net assimilation rate is invariant with N availability, this would necessarily lead to an increase in whole plant carbon gain and relative growth rate. Plant biomass has been shown to correlate strongly with intercepted radiation, with the gradient contingent on N availability [50]. In a study using short and long-leaved varieties of tall fescue, MacAdam *et al.* [51] demonstrated that while cell length and the relative leaf elongation rate were apparently genetically controlled, environmental factors such as temperature and light availability, as well as N supply, were important in determining the rate of leaf expansion. Plants receiving high N supply produced a significantly greater number of mesophyll cells, relative to epidermal cells [51]. This may yield thicker leaves, and given the importance of mesophyll cells for photosynthesis, might partially explain higher rates of photosynthesis in plants grown under high N conditions.

Canopy photosynthesis optimization models predict that under low light availabilities, canopies should have a low leaf area index (LAI; leaf area per unit ground area) which minimizes the self-shading of leaves. Under high light conditions, a greater number of leaves, and higher LAI, are predicted. However, as pointed out by Anten [52], optimized canopy models do not take into account the fact that LAIs which are optimal for canopies may not be evolutionarily stable for the individuals within those canopies, since low LAI plants can be outcompeted by plants with higher LAI, which have higher individual photosynthetic carbon gain. Thus, the optimal LAI for the plant is higher than the optimal value for the canopy [53]. However, there must similarly be a maximum LAI for plants, beyond which the carbon cost of increasing LAI is greater than the photosynthetic benefit. At a given LAI, in the absence of N fertilization, canopy photosynthesis in field grown tall fescue (*Festuca arundinacea* Schreb.) was 30% reduced relative to well-fertilized plants. Since the measurements were controlled for LAI, these differences were attributed to a direct N effect on photosynthesis [54]. Grass growth is known

to be strongly seasonal, and even N-deficient plants grown in spring or summer achieved significantly higher LAI than N-replete plants grown in autumn [55]. Presumably this results from a combination of low temperature, which limits leaf elongation rate [56], and reduced day-length and light intensity limiting carbon fixation. Taken together, these results suggest that nitrogen availability directly influences shoot growth via LAI, with photosynthetic declines resulting from N-deficiency important only under otherwise optimal conditions.

Along with absolute leaf area, specific leaf area (SLA; leaf area per unit dry mass) can also vary and is an important factor in determining plant photosynthetic performance. Anten and Hirose [57] demonstrated that in *Xanthium canadense* (Mill.), SLA decreased with increasing light intensity, independent of nutrient availability. Similar results were noted in *Lolium perenne* with comparatively low SLA (160 cm²/g) at high light, and higher values (500 cm²/g) at low light levels (RA Carnevalli, personal communication). Poorter and Evans [58] showed that plants with high SLA had similar photosynthetic rates to low SLA plants on an area basis, but higher rates on a unit mass basis. At lower light intensities, high SLA plants tended to have higher photosynthetic rates per unit nitrogen, due to a greater leaf area production.

4. Nutrient and Water Availability

Nitrogen deficiency is a major factor limiting the growth and yield of agronomically important plants, with inorganic N fertilizers used as a cost-effective method to increase crop yields globally [60]. Nutrient capture by roots is a significant cost for plants, both in terms of soil exploration by root production and the C costs of root activities, which includes both nutrient uptake and assimilation. However, plant N content has a primary role in determining whole plant photosynthetic potential, and thus photosynthesis and productivity, and C investment in roots can thus be seen as a method of maximising future photosynthesis. Given the necessary allometric relationships between leaf area and root biomass, correlations between plant N uptake and shoot growth have been confirmed [60].

Maximum plant growth rates will be achieved when the partitioning of biomass facilitates sufficient nutrient uptake rates to match the rate of carbon supply by photosynthesis [9]. Long-term shifts in canopy photosynthesis (e.g., due to seasonal changes in light availability) have been shown to strongly affect the availability of carbohydrates for the production of leaves, roots, and daughter tillers [61]. Thomas and Davies [62] demonstrated that while the shoot mass of *Lolium perenne* was relatively unaffected by shading, root mass was positively correlated with mean daily insolation. The ability to control biomass partitioning is typically considered to be an adaptive response either as a mechanism to maximise relative growth rates by increasing photosynthetic carbon supply, or as a result of a shift in the site of competition: at low N availabilities, plants compete for nitrogen via the roots, while at higher N levels, light capture becomes more important, and biomass is partitioned towards production of leaves. Conversely, Thomas and Sadras [63] have suggested that rather than being carbon limited, most plants have a carbon surplus, and they posit root growth as a mechanism to balance carbon influx with respiratory usage. However, as well as these evolutionary explanations, we should consider more physiological mechanisms for these phenomena. In nitrogen starved wheat plants, approximately equal proportions of the carbon transported to the roots are incorporated into biomass, respired, and translocated back to the shoot as amino acids [64]. As nitrogen is supplied to roots, we might assume that C demand will increase, as a result of

the respiratory and C-skeleton costs of amino acid synthesis. Assuming C supply to the roots to be relatively invariant over the short term, this might lead to decreased allocation of carbon for root production. In line with this, Scheible *et al.* [65] noted that nitrate accumulation in tobacco shoots was accompanied by a decrease in root sugar content, and that root sugar content correlated with root growth. As exogenous N concentration increases further, despite the ability of plant root cells to store nitrate in the vacuole [66], given the finite size of root storage capacity, we start to see transport of nitrate to the shoots, where it would be assimilated and used directly [67].

Nitrogen uptake in plants scales with root length, and has a strong genetic component [68]. In nitrogen and phosphate deprived plants, roots proliferate in nitrate, ammonium or phosphate rich patches to maximise nutrient capture [69]. A reanalysis of Drew's data suggested that while all the available nitrate could have been captured without proliferation, it increased the rate of nitrate capture, which would be important for plants in competitive environments [7]. However, root proliferation may be more important for ions with low soil mobility, such as ammonium and phosphate. In *Arabidopsis*, nitrate was shown to increase the length but not the number of lateral roots [70]. However, application of other N sources did not stimulate lateral root elongation, suggesting that nitrate was acting as a signal molecule, and its effect on root elongation was not directly as a nutrient. Conversely, very high levels of N supply tended to retard root growth and branching [70]. Ammonium patches also cause root proliferation, although the roots are shorter and much more highly branched when provided ammonium compared with nitrate [71]. This indicates separate mechanisms for root proliferation by nitrate and ammonium, which may have a functional significance, since nitrate has a diffusibility around 10-times higher than ammonium in soil [72].

Nitrogen availability is known to affect the allocation of biomass to the shoots or roots. However, the N form is rarely considered. Zerihun *et al.* [73] noted that there are significantly different C costs associated with assimilating nitrate, ammonium, or glutamine, and that the physical location and time of assimilation could also have effects. For example, malate derived from photorespiration acts as a source of reducing power for nitrate, where assimilation occurs in the leaves during the light period, without significantly affecting carbon fixation rates [23]. N assimilation costs have been postulated to have significant effects on biomass partitioning, and indeed fast-growing grass species have been shown to have lower nutrient assimilation costs than slower growing grass species [74], although the data did not support this hypothesis in *Phaseolus vulgaris* [73]. The factors determining the relative cost of N assimilation remain poorly understood, and further research is required to explore the importance of C-costs of nutrient assimilation in determining plant growth rate and productivity. Work in tobacco has suggested a strong positive relationship between shoot to root ratio and leaf nitrate concentration [65], however that relationship broke down under phosphate deficiency or when plants were supplied ammonium, rather than nitrate. Andrews *et al.* [75] conversely argued that protein concentration, rather than leaf nitrate levels, was primarily important in shoot growth rates. Furthermore, as well as abiotic factors noted here, root mass allocation can also be affected by biotic factors—hemiparasitic plant infection has been shown to cause an increase in host plant root mass allocation, presumably caused by nutrient abstraction [76].

It is generally understood that the majority of grass plant roots occur in the surface layers of the soil [77]. A well-developed root system has the capacity to mitigate local variability in resource variability, providing a continuous supply of water and nutrients to leaves, despite the heterogeneous

nature of the soil environment. Plants require large amounts of water for photosynthesis, since drought suppresses stomatal aperture leading to the depletion of intercellular CO₂ and increased photorespiration. Furthermore, carbohydrate accumulation in tissues during stress [78] presumably leads to a reduction in their availability for growth, and has been shown to cause feedback repression of photosynthesis in *Phaseolus vulgaris* [79]. Thus, plants growing in dry environments may suffer retarded growth as a result of insufficient water availability. Deep roots comprise approximately 30% of the grass root system, and help to maintain access to water even during drought conditions [80]. Huang and Fu [77] noted that in tall fescue, when the surface layer of soil was allowed to dry out but deeper soil had adequate water, carbon allocation to the shoot was decreased by more than 40%, while root production increased in both the upper, drier layer, and the lower layer. Presumably the functional significance of this is to maximise water uptake; however, the physiological mechanism by which it is achieved remains unclear.

5. Competition

The leaf canopy is a dynamic system with a constant turnover of leaves, influenced by environmental conditions. Within a dense stand where plants are competing strongly for light, vertical growth of the plant would be an optimal strategy. However, in more open canopies, vertical growth increases LAI and self-shading. Thus tillering is a preferred strategy, as this increases the number of growing leaves, presumably facilitating a greater increase in whole plant growth rate than could be achieved by an increase in leaf elongation rate alone. Plant C/N ratio is thought to be important in controlling tiller initiation, with nitrogen replete, carbon deficient *Carex rostrata* (Stokes.) plants producing significantly more daughter stems than carbon replete, nitrogen deficient plants [81]. Similarly, Davies [82] presented data showing a strong correlation between tiller number and nitrogen application in *Lolium perenne*, while Gautier *et al.* [83] demonstrated that shading caused a decrease in both leaf appearance rates and tiller formation. Conversely, as discussed previously, wheat plants grown under elevated CO₂ conditions produced more tillers than plants grown under control conditions [38]. The red/far-red ratio (R/FR) of light reaching the base of the canopy is an important shade signal for plants, and mediates a range of plant responses such as increased apical dominance, accelerated flowering and early seed production, and reduced tiller formation. The R/FR of daylight is around 1.2, but this decreases in deep canopies as the red light is absorbed by chlorophyll, making R/FR a signal for canopy closure [84]. A recent paper, which imposed increased R/FR on wheat cultivars demonstrated a 13%–23% increase in the number of tillers produced [85], while reduced tillering was found in wheat plants grown at high densities or under shade conditions where R/FR was decreased to around 0.4 [86]. Similarly, Gautier *et al.* [83] demonstrated a decrease in tiller formation in *Lolium perenne* plants grown under reduced R/FR. R/FR ratio has also been suggested to be a factor in tiller senescence, where decreases in ground-level R/FR coincided with a decline in tiller number [87], although whether this is a causal relationship or simply a result of canopy closure remains unclear. Irrespective of the mechanism, the shift in growth strategy from the production of many stems (tillering) to the concentration of resources in fewer, larger stems as competition for light increases appears common in grasses.

Within grass canopies, we expect a negative relationship between plant density and mass [88]. At low planting densities, the growth of new tillers fills the space in the canopy, while at high densities plant dry weight decreases, presumably due to competitive effects. Using *Lolium perenne* plants sown at a

range of differing densities, Lonsdale and Watkinson [89] reported that plant shoot dry mass followed a $-3/2$ relationship with plant density under high light conditions. However, when the plants were put under heavy shade conditions (83% reduction in light intensity) or when the tiller masses became very large, this relationship shifted from $-3/2$ to approximately -1 . It is thought that at high plant masses, the stand reaches a maximum yield with no further increase in stand mass possible [90]. The -1 slope suggests that at some point resource usage is maximised (e.g., light interception nears 100% for the canopy), leading to a zero-sum game, where an increase in resource use by one plant is necessarily at the expense of a competing plant. This raises the question of whether light attenuation places a maximum limit on the quantity of photosynthetically active biomass produced per unit area. More recent work suggests that rather than $-3/2$, the true relationship between plant mass and density follows a $-4/3$ slope [91].

In Matthew *et al.* [92], plots under low grazing intensity tended to have fewer, larger tillers, and a higher overall biomass. Plots grazed at higher intensities tended to have a greater number of smaller tillers. Grazing led to a more open canopy, presumably prompting tiller initiation and facilitating establishment. Under high light conditions, grazing is thought to have similar implications for plant morphology and yield as growth under low light conditions, due to the reduced leaf area for light interception. Clear seasonal differences in tiller density were apparent, with increased tiller numbers during the summer, suggesting that higher light levels in the summer may act as a substitute for a more open canopy in *Lolium perenne*. Interestingly, there was also seasonality in biomass per unit area for any given tiller density, which was explained by an increase in leaf area index. This suggests environmental factors, other than light, influence the maximum potential biomass. Temperature and light quality both seem to be viable candidates with low temperatures linked to decreased leaf appearance rates [93], although other factors such as water availability and nitrogen availability are also important.

Competition for light between plants promotes an increase in plant height. Taller plants must invest more carbon in support structures and less in leaves. Anten and Hirose [57] demonstrated that *Xanthium canadense* plants grown in stands invested a lower proportion of their mass in leaves than plants grown singly. Increased height allows increased access to light, but comes at the cost of carbon invested in stems. Thus, there must be an optimal plant height at which the C cost of additional stem production is greater than any increases in light interception and C fixation resulting from the increase in plant height. In stands where we have individuals of different species, or unrelated members of the same species, we would expect competitive pressures to drive individuals towards this, presumably environmentally influenced, maximum. However, the situation for clonal plants or tillers of an individual plant remains unclear. As covered earlier, the optimal LAI for an individual plant is higher than that for the canopy. Similarly, we might expect the optimal tiller number per plant to be higher than the optimal tiller density for the canopy. Clearly, excessive competition between multiple stems of a single plant would be disadvantageous for the plant, and we might expect to see a reduction of LAI towards the canopy optimum in clonal stands. On the other hand, limiting competitive effects might be difficult, as it would require the plant to be able to differentiate between tillers and other plants, and to communicate these differences internally. Little work has been conducted on the competitive pressures between tillers in a single plant.

6. Carbon Allocation

Photoassimilate is the major substrate for plant growth, thus it is impossible to understand plant performance without a thorough understanding of the factors determining assimilate transport and partitioning [94]. Phloem is the primary pathway for the transport of carbohydrates from the sites of assimilation (*i.e.*, mature leaves) to the sites of utilization (growth and respiration). However, the direct study of phloem is difficult, requiring the use of radioisotopes with rapid decay rates (e.g., ^{11}C) and expensive equipment, such as positron imaging systems. In the majority of studies, the allocation of carbon to organs is studied using stable or radioisotopes with slower decay rates (e.g., ^{13}C or ^{14}C), and although this is less direct it has allowed us to build models of carbon allocation within plants.

Carbon flux rates are generally assumed to be a function of the difference in concentration between C-sources such as photosynthesizing leaves, and sinks such as growing leaves, roots or seeds [11,13]. While source strength is fairly easily understood as deriving from photosynthetic C fixation, sink strength is a less well-defined concept. One way to think about it may be as the capacity of phloem companion cells in the proximity of a meristem to unload sugars. An upper limit may be established by the ability of cells to use carbon during division, expansion and differentiation [13]. ^{14}C labelling studies show the preferential allocation of carbon to young developing roots, with the oldest roots receiving little from the shoots [95]. Given that the older, established roots have a greater biomass and more apical meristems, we might postulate that they have a greater sink capacity than the younger roots. If C allocation is sink-driven, it would be difficult to understand this allocation. It has been hypothesized that the utilization of photoassimilates by young roots close to the shoot renders the supply insufficient for more distant sinks (C Matthew, personal communication), or it may be that transport resistance between the leaf and the root apical meristem is a key factor determining carbon allocation. Assuming that transport of assimilates through the phloem follows Poiseuille's law, the resistance of C flow down the root would be proportional to the root's length. Thus, the root tips of longer roots might be expected to receive less photoassimilate than younger, shorter roots, due to their length rather than their age or axial position. Phloem sap viscosity is known to be an important limitation to C export from photosynthesizing *Pinus sylvestris* L. leaves [96], suggesting a similar phenomenon might occur in grass roots, although this remains speculative.

In *Lolium perenne* and *Paspalum dilatatum* (Poir.), 48%–64% of the carbon incorporated into new tissues was assimilated within the preceding 24 h [97]. In contrast, for nitrogen, these figures were 3%–17%, with over 80% of N having been supplied by an intermediate storage pool. This intermediate storage pool undoubtedly refers to internal remobilization from senescing to newly growing organs. Carbon older than 2–3 days contributed little to new tissue growth, and shaded plants tended to grow more slowly, further reinforcing the close linkage between photosynthesis and growth rate. Short-term labelling studies show that photoassimilates can travel 2–3 cm per minute in *Vicia faba* L. [98], potentially allowing C to reach leaf growth zones within 10 to 20 min from assimilation, and reinforcing the close linkage between photosynthesis and growth. Similarly to new tissues, when we look at root exudates, we find that approximately 58% of the C in *L. perenne* root exudates derives from recent assimilation [99].

Reciprocal translocation of C and N between tillers appears to be common, and is a potential mechanism by which mature tillers can support the growth and development of daughter tillers. Daughter

tillers represent a potential C source for root growth and development [100]. However, the degree of resource sharing between tillers appears to vary between species and varieties, and presumably has an environmental component. In *Panicum maximum*, young daughter tillers have a tight vascular connection with their parent and a high degree of photoassimilate sharing is apparent, while secondary tillers (those connected to primary tillers) receive virtually no carbon from the parent stem, suggesting a low degree of vascular continuity [100]. In *Poa trivialis*, removal of an exogenous N supply effectively stopped N remobilization from mature leaves on the main stem to developing daughter tillers [46]. Clearly, tillering has important implications for plant productivity, although factors influencing the degree of resource sharing between the main stem and daughter tillers remain poorly understood. Furthermore, since each tiller can be split off and regenerate a whole plant, this raises a philosophical question about what constitutes the plant, and whether tillers which remain physically attached but have lost vascular connection can be meaningfully said to be the same plant.

It is generally understood that photosynthate partitioning is “local” in grasses, with upper leaves feeding the developing panicle in reproductive wheat plants, while lower leaves provide carbon for roots [101]. Carbon fixed by primary tillers was allocated first to the tiller itself, and secondly to main stem axial roots. However, carbon fixed by secondary tillers tended to be retained almost wholly in those tillers, with secondary tillers having little role in supporting main stem roots [101]. Work on the interaction between tillers of different ages within a plant are at a rudimentary level, and the balance between cooperation and competition between stems in a single plant as it develops remains completely unstudied, yet clearly of enormous importance. Osaki *et al.* [102] has pointed out the need for continued carbon investment in roots as the plant matures to maximise plant production. As grasses shift from vegetative to reproductive growth, we see a shift in the relative mass of leaves as carbon is allocated to stem elongation [3]. As the tillers increase in size, it seems likely that the shading of older leaves deeper in the canopy leads to a decline in carbon flux to the roots and therefore root activity, and perhaps the characteristic drying out we see in maturing cereal crops.

7. Conclusions

While much progress has been made over the last few decades in certain aspects, a great deal of work remains to be done before a comprehensive understanding of the factors controlling plant carbon allocation and plant productivity emerges. Key questions remain regarding the potential diminishment of C₃ grass nutritive value under elevated CO₂ conditions, and whether C₄ plants will gain in importance as a target for forage plant improvement due to their potentially superior stress tolerance under high CO₂ conditions. Recent work in rice has unequivocally demonstrated the importance of Rubisco turnover for plant growth, and that Rubisco turnover rates appear to have a high degree of genetic variability. However, linking leaf behaviour to whole plant performance has yielded little so far, and a more comprehensive approach to the problem seems necessary. The factors controlling tiller formation remain unclear, with most studies in the area relying heavily on correlation and few causative links demonstrated. Both red/far-red ratio and carbon/nitrogen balance appear important, but there is little clarity on the relative importance of these factors. Similarly, the drivers of carbon allocation to differing classes of roots remains a key question, which will probably require a combined modelling/experimental approach. Finally, cooperation and competition between tillers in a single plant for N and C remains

almost completely unexplored, and represents an important avenue for future research efforts. Although many questions remain, much work has been done since the heyday of forage research in the 1970s. We continue to hope that a more complete understanding of plant carbon metabolism will help us develop higher-yielding, fertilizer-efficient plants.

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Conflicts of Interest

The author declares no conflict of interest.

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