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Implementing a framework of carbon and nitrogen feedback responses into a plant resource allocation model

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Abstract. The allocation of resources to roots and shoots can greatly alter total plant mass. Allocation is thought to be the consequence of uptake rates, transport rates, and growth rates and the communication between them via signalling mechanisms. Feedbacks that alter resource uptake and use are induced in nature by changes in the internal pools of carbon and nitrogen, but how these function together to define allocation remains unclear. We introduce a framework model of internal feedback responses to changes in plant carbon and nitrogen concentrations. We evaluate how well the model responds to changes in carbon and nitrogen availability by simulating external environmental perturbations that influence the uptake of carbon and nitrogen. The model reflects experimental results when looking at the effect of atmospheric CO_2 and soil nitrogen concentrations on total plant mass and replicates observed responses to leaf defoliation events. Overall, this shows that a combination of known signalling mechanisms are sufficient to reproduce experimentally observed responses to external resource availability. Model simulations highlight key areas of uncertainty where more empirical data are needed. In particular, quantitative data are needed to establish the strengths and rates at which feedback responses to carbon and nitrogen substrate concentrations alter growth and uptake rates.

KEYWORDS: allocation; carbon; feedback; model; nitrogen; plant growth

1. INTRODUCTION

Changes in the allocation of resources to different plant tissues (e.g. leaves, roots, stem, and seeds) greatly impact total biomass and crop yield and arise when plants react to changes in the environment. In particular, the responses of biomass allocation to changes in atmospheric CO_2 and soil nitrogen (i.e. C and N source activities) are thought to help balance the uptake of carbon and nitrogen. Carbon and nitrogen assimilation and the use of their products are entirely interdependent (Moorby, 1977; Paul and Foyer, 2001; Kaschuk et al., 2010). For example, the energy required for nitrogen assimilation is provided via photosynthesis (Stitt et al., 2002) and byproducts of nitrogen assimilation are required for photosynthesis to occur (Zhu et al., 2008). Gaining a quantitative understanding of how carbon and nitrogen behave together in plant metabolism and signalling can therefore elucidate how resource allocation can be optimized to enhance plant growth.

Environmental variation alters key processes within the plant (respiration, photosynthesis, nutrient uptake, etc.) to differing extents, meaning that different internal processes become more or less limiting under different environmental conditions. This imbalance necessitates a balancing of energy producing and utilizing processes which is modulated by molecular regulation (Paul and Foyer, 2001). In particular, intermediate products from carbon and nitrogen assimilation such as nitrate, sugars, and amino acids reflect the carbon:nitrogen status of the plant and act as signals (or feedbacks) for gene expression to affect many cellular processes (Fig. 1). This leads to important interactions between the signalling pathways for carbon and nitrogen. However, thousands of genes respond to changes in sugar concentrations (Lastdrager et al., 2014). A simplification of these

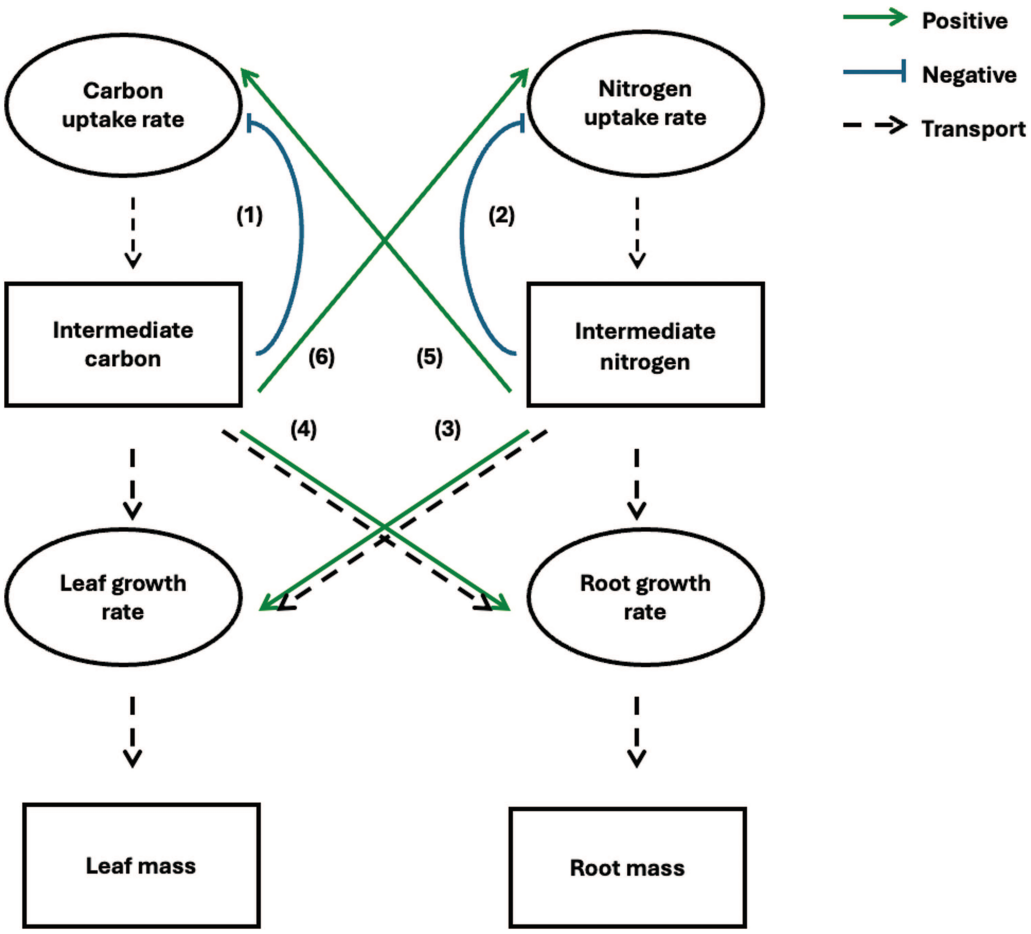

Figure 1. Summary of experimentally observed feedback responses to carbon and nitrogen concentrations during vegetative growth, as incorporated in the modeling framework. Ovals represent the internal processes (uptake and growth) and rectangles represent the products of internal processes (soluble pools and structural mass). Dashed lines represent the transport of resources between compartments. Blue arrows are negative feedbacks and green are positive feedbacks. Evidence for each of these is reviewed in the text. 1. High leaf carbon concentrations decrease carbon uptake rate. 2. High root nitrogen concentrations decrease nitrogen uptake rate. 3. High leaf nitrogen concentrations increase leaf growth rate. 4. High root carbon concentrations increase root growth rate. 5. High leaf nitrogen concentrations increase carbon uptake rate. 6. High root carbon concentrations increase nitrogen uptake rate.

processes is therefore needed to understand how they interact at a whole plant scale. The aim of this paper is to determine whether the unification of certain carbon and nitrogen feedback responses can reflect whole plant allocation patterns via modelling. Some key feedback responses on carbon and nitrogen uptake and growth are discussed in the following subsections along with routes for simplifying them for inclusion in mathematical models.

1.1. Influence of carbon and nitrogen pools on carbon assimilation

Photosynthesis is sensitive to leaf carbon concentrations. During the day, starch and other carbohydrates build up in the leaf as carbon are assimilated from the atmosphere. Via a number of mechanisms that sense leaf carbohydrate status, this triggers an immediate reduction in RuBisCO activity which is an important constraint on carbon assimilation (Paul and Foyer, 2001). A large body of evidence (Paul and Foyer, 2001; Smith and Stitt, 2007; Kelly et al., 2013; White et al., 2016) shows that high carbon concentrations have a negative feedback on carbon uptake (Feedback 1, see Fig. 1). For example, sucrose has been shown to reduce the transcription of photosynthesis related genes in *Arabidopsis*, rice, and tomato (Graham, 1996; Koch, 1996; Chan and Yu, 1998; Sheen et al., 1999; Smith and Stitt, 2007). Furthermore, excess sucrose is sensed by hexokinase which triggers the closure of stomata, leading to a reduction in photosynthesis (Kelly et al., 2013). High nitrogen status in the roots increases photosynthesis (Feedback 5, see Fig. 1). Cytokinins in the root are very sensitive to nitrogen supply and the transport of this hormone from roots to leaves promotes the expression of genes linked to photosynthesis (Paul and Foyer, 2001).

1.2. Influence of carbon and nitrogen pools on nitrogen uptake and assimilation

Nitrogen assimilation is carbon-dependent because energy is required for the synthesis of glutamate and glutamine and is a key

stage where carbon metabolism and nitrogen metabolism interact (Hodges, 2002). Plant sugars can induce the genes responsible for nitrate reductase (NR) in the leaves, increasing nitrogen uptake rate via the conversion of nitrate into ammonia in *Arabidopsis* and maize (Cheng et al., 1992; Klein et al., 2000; Iglesias-Bartolomé et al., 2004; Reda, 2015). Furthermore, Reda (2015) shows increased NR activity following sugar treatments of 8 hours (Feedback 6, see Fig. 1). The location where nitrogen derived signals are sensed can alter the type of feedback on nitrogen uptake rate. In the leaves, the products of nitrogen assimilation (glutamine and glutamate) act as signals for the expression of genes responsible for the inhibition of NR and therefore reduce nitrate uptake in barley, soybean, and *Arabidopsis* (Feedback 2, see Fig. 1) (Siddiqi et al., 1990; Clarkson and Lüttge, 1991; Muller and Touraine, 1992; King et al., 1993; Rufty et al., 1993; Imsande and Touraine, 1994; Reda, 2015). However, in the roots, glutamine and glutamate induce NR activity in maize, tobacco, and *Arabidopsis* (Shaner and Boyer, 1976; Wray, 1993; Gojon et al., 1998; Reda, 2015). Nitrate induces genes responsible for NR within 30 minutes, but this is only when photosynthesis is active.

1.3. Influence of carbon and nitrogen pools on growth rates

High concentrations of carbon intermediates have a positive feedback on root growth (Feedback 4, see Fig. 1). Sucrose is important in the regulation of plant growth, as it induces the production and transport of auxin (hormone responsible for growth), therefore increasing sink activity (Paul and Foyer, 2001; Lilley et al., 2012; Sairanen et al., 2012; Stokes et al., 2013). Xiong et al. (2013) found that root glucose activates TOR protein kinase, promoting root meristem activity in *Arabidopsis*. High plant nitrogen content increases shoot:root ratio in *Arabidopsis* (Stitt and Krapp, 1999; Nunes-Nesi et al., 2010). Scheible et al. (1997) show that, in tobacco, the presence of nitrogen in the roots increases protein synthesis and root growth rate but shoot growth rate is higher, leading to a stronger allocation of growth toward the leaves. This identifies a further feedback to increase leaf growth when nitrogen concentrations are high (Feedback 3, see Fig. 1).

1.4. Modelling feedbacks

Many models simulate the dependence of growth on carbon and nitrogen supply (i.e. growth is substrate limited) but do not necessarily include the signalling feedbacks on uptake and use, which contribute toward allocation (Thornley, 1972; Hunt et al., 1998; Bartelink, 1998; Ågren et al., 2012; Cheeseman, 1993; Siddiqi and Glass, 1986; Shaw and Cheung, 2018). Table 1 lists a variety of plant growth models and how they simulate resource allocation. The models which do simulate resource dependencies of uptake rates or growth are limited by either not considering above and below ground material (Dunbabin et al., 2002; Ågren et al., 2012; Pao et al., 2018) or using a functional balance assumption (Bartelink, 1998; Hunt et al., 1998; Shaw and Cheung, 2018). Though some previous models have simulated the dependency of source activity on carbon and nitrogen concentrations, no previous models have attempted to simultaneously simulate feedbacks on source and sink activity by altering carbon and nitrogen uptake rates and growth rates with changes in internal carbon and nitrogen. It is important for crop models to be able to accurately simulate biomass partitioning in response to environmental change, as it is an essential component in predicting future yields. Addressing these limitations by incorporating a more dynamic representation of source–sink interactions will improve predictions, particularly as crops experience increasing abiotic stressors under climate change. It is unknown how the unification of multiple feedbacks alters growth allocation individually and collectively.

This paper explores the mechanisms responsible for allocation with three objectives. First, to evaluate whether the combination of known signals is sufficient to reproduce empirically observed responses to imbalances in carbon/nitrogen supply. Secondly, to formulate a stable, working C and N allocation model that is capable of reproducing whole-plant signalling and allocation behaviour in response to variable carbon and nitrogen supplies, and evaluate the extent to which the parameterized model can reproduce observed behaviors when qualitatively compared with multiple experimental studies of source–sink manipulations. Thirdly, to sharpen questions about the unknown aspects of signalling, e.g. rates, thresholds, and the nature of signals. The model results are compared qualitatively against the results from experimental papers since the model is not calibrated for one specific plant species but instead is parameterized generally for herbaceous plants. This paper shows that the model mostly reacts to changes in CO_2 and nitrogen availability in the same way as experiments carried out on plants. This model provides a framework that can be adopted for use in crop and vegetation simulation models to further investigate the dynamics between internal feedback mechanisms underpinning allocation.

2. MATERIALS AND METHODS

There are typically two main approaches to simulating allocation of resources. The first focuses on reconstructing entire metabolic networks and the flows of energy and materials within them (i.e flux balance analysis models (Shaw and Cheung, 2018; Moreira et al., 2019)) but lacks the feedbacks on metabolism described above, since the details of the molecular mechanisms are not fully known. The second takes a much higher level approach, aiming to reproduce the outcome of the feedbacks in terms of allocation to roots and shoots, and the rates of carbon and nitrogen assimilation, in relation to environmental limitation. This second class of models is phenomenological or teleonomic (Thornley and Johnson, 1990; Buckley and Roberts, 2006; Feller et al., 2015). This approach does not explicitly consider the internal feedbacks that give rise to the behaviour. The model described here operates at an intermediate level. Since the detailed molecular mechanisms are not fully known, we approach this problem by explicitly simulating the behaviour of experimentally observed physiological feedbacks based on their relative trends. The model operates mechanistically at the physiological scale–incorporating feedback mechanisms for photosynthesis, nitrogen uptake, and growth rates–but not at the molecular scale, such as gene regulation.

Table 1. Overview of plant growth models and their allocation assumptions during vegetative growth

Model name	Authors	Species	Scale	Resource	Feedbacks or dependencies
Nwheat	Asseng et al. (2017)	Wheat	Crop	C and N	Partitioning is defined as a function of phenological stage.
APSIM	Brown et al. (2019)	Maize, wheat, chickpea, mungbean, cowpea, soybean, pigeonpea, fieldpea, stylo, navybean, lucerne, peanut, fababean, lupin, mucuna, canola, sugarcane, sorghum, cotton, barley	Crop	C and N	Partitioning is defined as a function of phenological stage. Photosynthesis and growth is dependent upon C and N availability.
BioCro	Lochocki et al. (2022)	Soybean, miscanthus	Crop	C	Allocation is determined by partitioning tables.
Grapevine XL	Zhu et al. (2021)	Grape	Crop	C and water	No C or N feedback mechanisms included.
CPlantBox	Zhou et al. (2020)	-	Plant	C and water	No C or N feedback mechanisms included.
WACNI	Chang et al. (2023)	Rice	Crop	C and N	Positive and negative feedbacks on C and N uptake but not on growth rates.
-	Feller et al. (2015)	Petunia	Plant	C and P	High C inhibits photosynthesis and promotes C storage. P inhibits photosynthesis.
QUINCY	Thum et al. (2019)	-	Ecosystem	C, N, P, water	Photosynthesis is dependent upon N and is downregulated when water or nutrient availability is low. Growth is dependent on available C, N and P.
CROPGRO	Boote et al. (2021)	Soybean, peanut, dry bean, faba bean, velvet bean, tomato, canola, carinata, maize, wheat, rice, sorghum, pasture grass, cassava, potato	Crop	C and N	Partitioning is defined as a function of phenological stage. N stress increases root growth.
-	Shaw and Cheung (2018)	*Arabidopsis*	Plant	C and N	Allocation relies on a functional balance assumption.
WOFOST	De Wit et al. (2019)	Potato, sugar beet, rapeseed sunflower, maize, wheat, barley	Crop	C and N	Allocation is determined by partitioning tables.
QualiTree-MappleT	Pallas et al. (2016)	Apple tree	Plant	C	Feedback inhibition of leaf storage carbohydrate on photosynthesis.
DSSAT	Jones et al. (2003)	Soybean, peanut, dry bean, faba bean, velvet bean, tomato, canola, carinata, maize, wheat, rice, sorghum, pasture grass, cassava, potato	Crop	C, N, water, P	Partitioning is defined as a function of phenological stage.
STICS	Brisson et al. (2003)	Wheat, barley, maize, soybean, sorghum, rapeseed, flax, tomato, sunflower, beetroot, potato, forage grasses, lucerne, lettuce, carrot, banana, sugar cane, mustard	Crop	C, N, water	Partitioning is defined as a function of phenological stage.
This work		-	Plant	C and N	Positive and negative feedbacks of C and N on photosynthesis and N uptake. Positive feedbacks of C and N on leaf and root growth rates. Leaf N is remobilized to the root when leaf N concentration is higher than root N. Similarly, root C is remobilized to the leaf when root C is higher than leaf C.

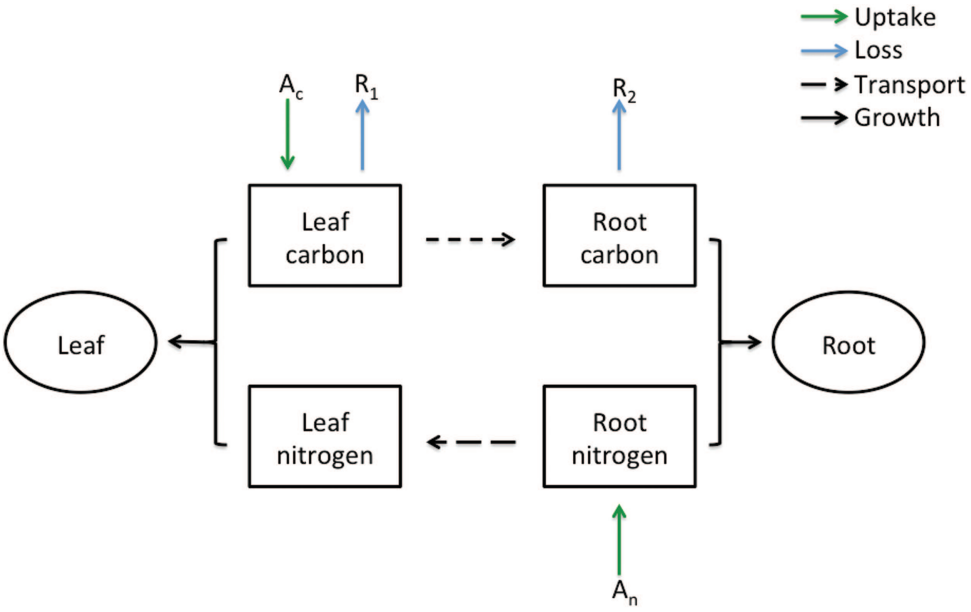

Figure 2. Diagram of the Thornley (1972) model with maintenance respiration. The boxes represent intermediate carbon and nitrogen concentration per unit leaf or root mass and the circles represent total leaf or root mass. Leaf carbon and root nitrogen concentrations increase via carbon (A_C) and nitrogen (A_N) uptake rates per unit leaf or root mass (green arrows). Leaf carbon and root carbon are depleted via leaf (R_1) and root (R_2) maintenance respiration (blue arrows). The black dashed arrows represent transport of resources and the black solid arrows represent the use of resources for growth of leaf and root mass.

The model used here is a unification of a widely used and tested transport resistance model (Thornley, 1972) and a new framework of feedbacks of internal carbon and nitrogen concentrations on C and N uptake and growth rate. The Thornley (1972) model is used since it is an excellent framework to investigate source–sink dynamics and has been used for a variety of different plant species and environmental conditions (Wann and Raper Jr, 1984; Rastetter and Shaver, 1992; Minchin et al., 1994; Dewar et al., 1994).

The model is represented by a system of six first-order ordinary differential equations (ODEs) (See Methods S2). The first four equations represent the four pools of substrate: non-structural carbon (i.e. sugars, starch) and nitrogen (i.e. nitrate, ammonium, amino acids) in the leaf and root and the final two equations represent the masses of leaf and root tissue (Fig. 2). These intermediate products of carbon and nitrogen assimilation are transported between the leaf and root, may accumulate in tissues, and are utilized by tissue growth processes and increase in size when the amounts of carbon or nitrogen taken up into the plant are higher than the amount required for growth. All figures in this paper are produced via MATLAB and the code is available through github (Github repository).

The model is based on a combination of the assumptions from Thornley (1972) and additional assumptions to simulate a plant which is sensitive to external and internal fluctuations of carbon and nitrogen.

2.1. Use of substrate and allocation

The rate of use of substrate for growth is derived from bisubstrate enzyme kinetics (Dixon and Webb, 1964 - taken from Thornley (1972)). It assumes that the amount of carbon and nitrogen used for the growth of new plant tissue is determined by the amount available. This allows the growth of leaves and roots to depend upon both carbon and nitrogen, such that allocation of growth to above or below ground biomass is a consequence of changes in intermediate concentrations. The rates of carbon and nitrogen use for leaf growth are simulated in the same way to Thornley (1972), but for simplicity, the number of parameter values is reduced by one

$$G(C_l, N_l) = v\frac{C_l}{C_l + k_1}\frac{N_l}{N_l + k_2}; \quad (1)$$

and for root growth

$$G(C_r, N_r) = v\frac{C_r}{C_r + k_1}\frac{N_r}{N_r + k_2}, \quad (2)$$

where v is the maximum rate of substrate use for growth, k_1 and k_2 are the Michaelis-Menten constants for carbon and nitrogen, C_l and N_l are leaf carbon and nitrogen concentration, and C_r and N_r are root carbon and nitrogen concentration. These values are parameterized to achieve leaf and root RGR close to $0.3d^{-1}$, equivalent to fast growing annual grasses (Garnier, 1992). These values are used throughout this paper ($v = 3600(kgmol)^{-1}m^3s^{-1}$ and $k_1 = k_2 = 1000kgmolm^{-3}$). Although this means that the maximum possible RGR is unfeasibly high (using Eq. (S2.11) in Methods S2), with the potential to reach $21.6d^{-1}$ (as C and $N \to \infty$), this value is never reached due to source limitations and is typically less than $0.3d^{-1}$. For simplicity, the Michaelis-Menten constants for carbon and nitrogen and v are assumed to be equal in both the leaf and the root.

2.2. Continuous growth and respiration

There is no litter production (i.e. tissue turnover) within the model; the only loss term is maintenance respiration which reduces leaf and root carbon pools. Maintenance respiration is simulated as a linear function of plant mass. As plant mass increases, maintenance respiration increases, such that R_1 is leaf respiration ($15 nmol g^{-1} s^{-1}$) and R_2 is root respiration ($10 nmol g^{-1} s^{-1}$) (Tjoelker et al., 2005). Growth respiration is accounted for by an efficiency constant ($Y_g = 0.66$ (dimensionless) (Kira, 1975)), assuming that a proportion of carbon and nitrogen resources utilized for growth are lost when constructing new biomass. The carbon consumed by leaf respiration is subtracted from the intermediate pool of leaf carbon and root respiration from root carbon. The production of leaf and root mass is expressed as exponential growth and therefore does not reach steady state. This represents a stage of vegetative growth which will only stop when relative growth rate (RGR) becomes zero. For this to occur in the model, carbon or nitrogen pool sizes in the leaves must be zero and carbon or nitrogen pool sizes in the roots must be zero. The model represents to an early stage of vegetative growth when resource availability is high, and growth is not yet constrained by nutrient limitations or ontogenic transitions, to ensure that feedback effects can be investigated without the influence of intrinsic plant development.

2.3. Carbon and nitrogen uptake rates

Thornley (1972) assumes that carbon and nitrogen uptake rates are constant per unit leaf or root volume. Here, this assumption is modified so that carbon uptake rate is also dependent upon atmospheric CO_2 (Eq. 2) and nitrogen uptake rate upon soil nitrogen concentration (Eq. 3).

Originally a constant rate (Thornley, 1972), carbon uptake rate ($A_{C,env}$) becomes

$$A_{C,env} = \frac{V_c \rho c_a}{\rho c_a + k_c}, \tag{3}$$

where V_c is maximum carbon uptake ($40 \mu mol m^{-2} s^{-1}$ (Sage, 1994)), ρ is the conversion factor of atmospheric to intercellular carbon (0.7 unitless (Katul et al., 2000)), c_a is atmospheric CO_2 ($\mu mol mol^{-1}$), and k_c is the concentration of CO_2 at half of V_c ($200 \mu mol mol^{-1}$ (Farquhar et al., 1980)). This simple model for photosynthesis assumes that the plant is fully light saturated. Although there are more comprehensive models of photosynthesis (Farquhar et al., 1980; Von Caemmerer, 2000), which include factors such as light and temperature, here, a simpler form is selected that predominantly focuses on carbon flux since the effects of carbon and nitrogen are the primary focus for this study. This equation could easily be swapped with another form to further investigate the effects of light or temperature in future work.

Nitrogen uptake rate can also be described using the Michaelis-Menten equation (Youngdahl et al., 1982), such that it becomes dependent upon soil nitrogen availability, and therefore, nitrogen uptake rate (A_N) becomes

$$A_N = \frac{V_n N}{N + k_n}, \tag{4}$$

where V_n is maximum nitrogen uptake rate ($61 \mu mol kg^{-1} s^{-1}$ (Youngdahl et al., 1982)), N is soil nitrogen concentration (μM), and k_n is soil nitrogen concentration at half of V_n ($103 \mu M$ (Youngdahl et al., 1982)).

2.4. Transport of substrate

The transport of substrate is assumed to follow Münch mass flow such that the amount of intermediate carbon or nitrogen transported between leaves and roots is determined by the difference in their concentrations in source and sink (Münch, 1930). This means that, typically, carbon and nitrogen are transported from source to sink. However, if the concentration in the sink becomes higher than in the source, nutrient remobilization can occur. For example, if leaf nitrogen levels exceed those in the root, nitrogen will be transported from the leaf back to the root. Transport resistance is scaled with plant size (Münch, 1930). As the plant increases in size, the level of transport resistance increases. Thornley (1972) assumes that it takes ~1 day for intermediates to be transported from source to sink. This is reduced to roughly 3 hours in the modified model to increase RGR. Given that most angiosperms have an approximate phloem velocity of 1 $cm min^{-1}$, this is corresponds to a proportional transport resistance of angiosperms up to 180 cm tall (De Schepper et al., 2013).

2.5. Internal feedback mechanisms

A framework of internal feedback mechanisms (Fig. 1) is applied to the Thornley (1972) model. Internal feedbacks on growth are simulated by making key processes of resource uptake and consumption depend on the internal concentrations of metabolic intermediates which are known to cause such feedbacks. Within this framework, uptake rates, consumption rates, and allocation to source and sink tissues are responsive to changes in internal carbon and nitrogen concentrations (Fig. 1). The types of feedback mechanisms were chosen to balance each other symmetrically such that, if a feedback is applied to nitrogen, the same type of feedback is implemented to carbon. In order to simplify the system, processes are assumed to be dependent upon local concentrations, e.g. carbon uptake rate would be sensitive to changes in leaf carbon and nitrogen but not root carbon and nitrogen. Although there is some experimental evidence for the specific resource status of a compartment and a remote feedback (e.g. root nitrogen status and leaf growth rate (White et al., 2016)), transport fluxes in the model mean that leaf and root nutrient status are closely coupled.

2.6. Developing a framework of feedbacks

The feedbacks (Fig. 1) enable a plant to: increase growth towards sinks when source strength is high for both carbon and nitrogen (Feedbacks 3 and 4); reduce source activity when source strength is high (Feedbacks 1 and 2), and increase carbon source activity when nitrogen source strength is high (Feedback 5) and similarly, for high carbon source strength, increase nitrogen source activity (Feedback 6).

These feedbacks are internal responses which occur with fluctuations in carbon and nitrogen concentration and affect the processes of uptake and growth. Each feedback can be implemented

mathematically by making the affected process dependent upon the carbon or nitrogen concentration responsible for such a feedback. For instance, feedback 1 alters carbon uptake rate when carbon concentration is high and feedback 5 increases carbon uptake rate with high leaf nitrogen. This means that carbon uptake rate must become dependent upon leaf carbon and leaf nitrogen concentrations. Currently, without any internal feedbacks, carbon uptake rate is assumed to be solely dependent upon atmospheric CO_2 concentration (Eq. 3). Including the feedbacks, carbon uptake rate would become

$$A_C = A_{C,env}\left(1 + \frac{1}{4(1 + 100000e^{-100(N_l - w)})}\right) - \alpha C_l. \quad (5)$$

αC_l is a negative linear feedback on carbon uptake rate when leaf carbon is high (feedback 1), $\frac{A_{C,env}}{4(1+100000e^{-100(N_l-w)})}$ is fractional increase in the carbon uptake rate when shoot nitrogen exceeds a threshold value w (feedback 5). Nitrogen uptake rate is modelled in the same way, with the same linear function of root nitrogen (feedback 2: high nitrogen reduces nitrogen uptake rate) and with a fractional stepwise function of root carbon (feedback 6: high carbon increases nitrogen uptake rate). Since the magnitude of the feedbacks and threshold values are not often known, an arbitrary value of $w = 400nmolmg^{-1}$ was chosen for shoot nitrogen and root carbon.

Leaf RGR (G_l) is dependent upon carbon and nitrogen concentration such that

$$G_l = G(C_l, N_l) \times N_l, \quad (6)$$

where $G(C_l, N_l)$ is the rate of use of C and N for growth (see equations 5 and 6) C_l is leaf carbon, N_l is leaf nitrogen. This scales growth rate based on shoot nitrogen to implement a positive feedback on leaf growth rate when nitrogen is high (feedback 3). Similarly, for root growth rate, Eq. (6) is multiplied by root carbon to simulate a positive feedback on root growth when carbon concentration is high (feedback 4).

Methods S1 provides a detailed explanation of how these feedback functions were chosen.

2.7. Parameterization

Parameter values were chosen based on experimental data using a variety of plant species, primarily taken from studies on multiple grasses (Garnier, 1992; Garnier and Laurent, 1994; Tjoelker et al., 2005; He et al., 2006). They are generally relatively fast growing herbaceous species and they would be most appropriate for a model plant species or a crop. This model represents not one particular species but the behaviour of a "generic" plant. See Methods S4 for the parameter values used. Further information on model sensitivity can be found in Methods S1, Fig. S1.

2.8. Simulating source-sink manipulations

The model output is compared to patterns observed in experiments using two soil nitrogen and two atmospheric CO_2 treatments (Coleman et al., 1993; Farage et al., 1998; Rogers et al., 1998; Ainsworth et al., 2003; Butterly et al., 2015). This allows us to determine if the simulated plant with imposed internal feedback mechanisms of carbon and nitrogen responds to changes carbon and nitrogen source availability. The combined high and low carbon and nitrogen treatments allow the exploration of combined effects in the model. Plant growth is simulated for two CO_2 concentrations: 350 and 700 ppm for a high soil nitrogen treatment ($400\mu M$) and a low soil nitrogen treatment ($200\mu M$). These concentrations were chosen based on the studies mentioned above. Growth simulations were run for 40 days to facilitate comparison with the experimental studies mentioned above, which were conducted over the same timespan, focusing primarily on the vegetative growth stage.

Defoliation is simulated to test the model response to a reduction in source size. To simulate a defoliation experiment, in line with Rogers et al. (1998), the model was run for 10 days from the same initial conditions used throughout this paper ($l_0 = r_0 = 0.01g$, $C_{l0} = 92.8nmolmg^{-1}$, $C_{r0} = 63nmolmg^{-1}$, $N_{l0} = 0.1nmolmg^{-1}$ and $N_{r0} = 7.54nmolmg^{-1}$) with soil nitrogen of $400\mu M$. At 10 days, total leaf mass was halved, total internal concentrations and root mass were used as initial conditions, and the model was run for another 10 days. This simulation was run for two levels of atmospheric CO_2 (350 and $700ppm$).

A/c_i curves were produced by substituting final leaf carbon and nitrogen concentrations at $t = 40$ days for both CO_2 and nitrogen treatments into Eq. (3) to plot carbon uptake rate against intercellular CO_2 between 0 and $1000\mu molmol^{-1}$. This aids the comparison of responses to varied atmospheric CO_2 and soil N since A/c_i curves are commonly used.

3. RESULTS

3.1. Atmospheric CO_2 and nitrogen experiment

Elevating atmospheric CO_2 increased total plant mass in both soil nitrogen treatments (Fig. 3). Higher soil nitrogen concentrations increased both total plant mass and the effect of CO_2 on total plant mass by 11%. As a consequence, high CO_2 increased plant mass by 30% in high soil nitrogen whilst in low soil nitrogen the change was 19%.

Naturally, plants growing in higher soil nitrogen have a higher percentage of nitrogen in the whole plant (Fig. 4). Initially, plant nitrogen percentage was lower in elevated atmospheric CO_2 but became higher than the ambient control treatment as the plant continued to grow. After 40 days, elevated CO_2 slightly increased plant nitrogen percentage from 3.1% to 3.4%. Similarly, soil nitrogen treatment had little effect on plant nitrogen percentage. High soil nitrogen treatment reduced nitrogen percentage for low (3%) and high (3.4%) CO_2. The same behaviour occurred when comparing nitrogen percentage against total plant mass (Figs. 4c and Figs. d) and the lower nitrogen treatment produced a smaller plant. This implies that higher soil nitrogen concentrations increased total plant mass and the weak reduction in nitrogen percentage is a dilution effect arising from a greater plant size.

Both treatments had a positive effect on carbon uptake rate. High atmospheric CO_2 levels and soil nitrogen increased carbon and nitrogen uptake. When soil nitrogen is low ($200\mu M$), atmospheric CO_2 only marginally altered the shape of the A/c_i curve, while increased soil nitrogen maximized the effect of CO_2 on the shape of the A/c_i curve. The effect of atmospheric CO_2

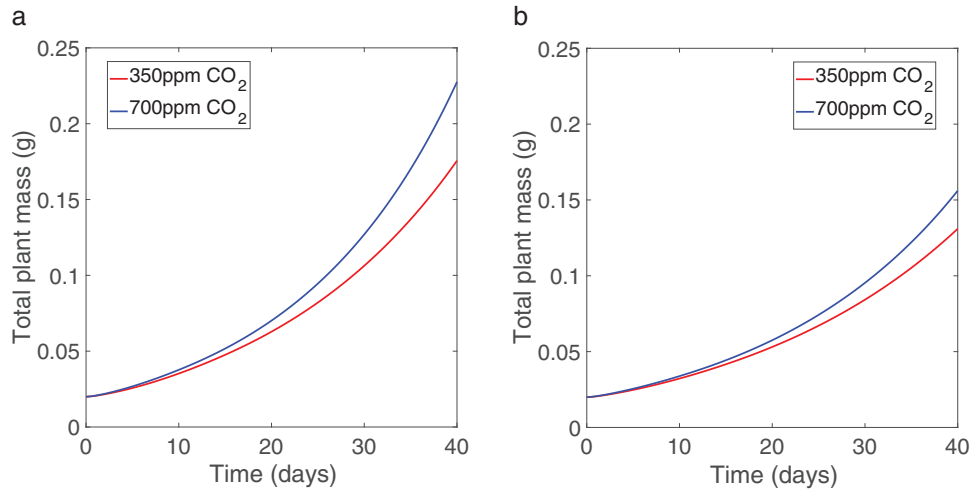

Figure 3. a) The relationship between plant mass over time and two atmospheric CO_2 treatments (350ppm and 700ppm) with a high soil nitrogen (N=400 μM) treatment. b) The relationship between plant mass over time and two atmospheric CO_2 treatments (350ppm and 700ppm) with a low soil nitrogen treatment (N=200 μM). Both ran with initial leaf mass $l_0 = 0.01$ and root mass $r_0 = 0.01$ and initial concentrations $C_{l0} = 92.8 nmol mg^{-1}$, $C_{r0} = 63 nmol mg^{-1}$, $N_{l0} = 0.1 nmol mg^{-1}$, $N_{r0} = 7.54 nmol mg^{-1}$.

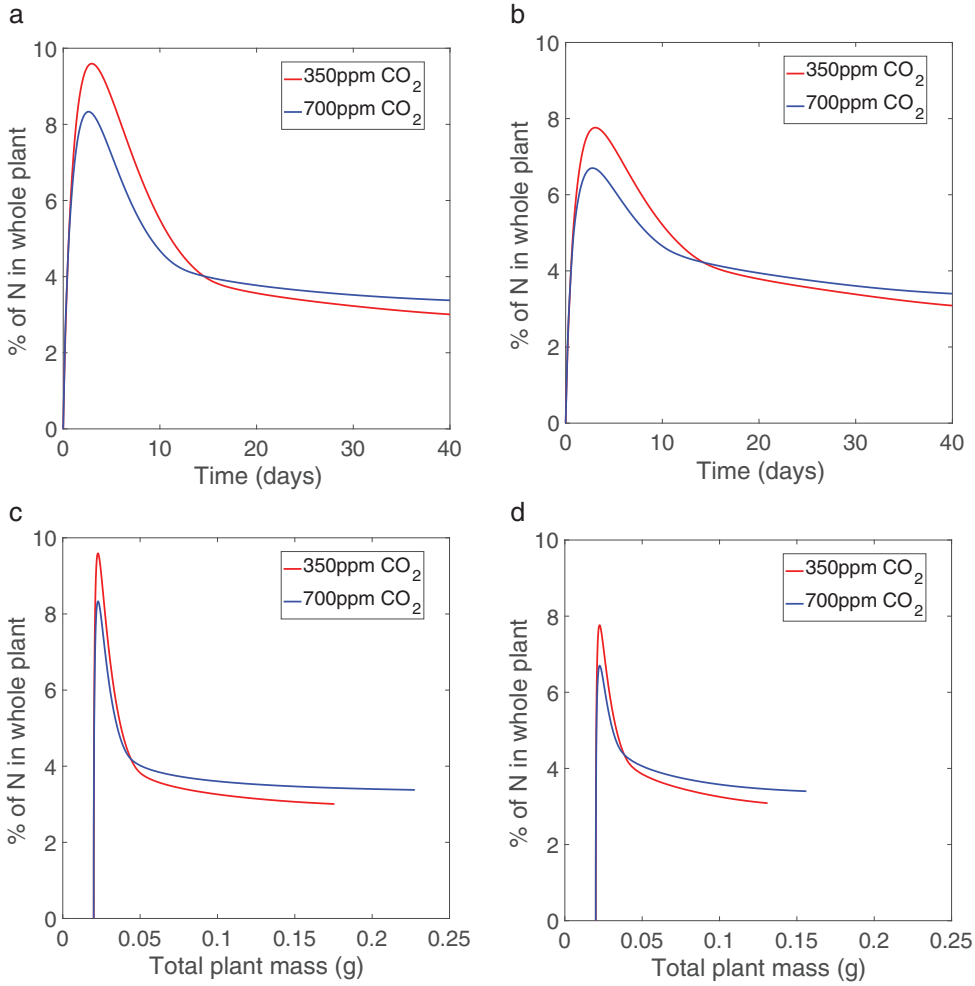

Figure 4. The relationship between nitrogen percentage of total plant mass over 40 days when varying CO_2 treatment (350ppm and 700ppm) with a) high soil nitrogen ($n = 400 \mu M$) b) low soil nitrogen ($n = 200 \mu M$). The relationship between nitrogen percentage of total plant mass against plant mass when varying CO_2 treatment (350ppm and 700ppm) with c) high soil nitrogen ($n = 400 \mu M$). d) low soil nitrogen ($n = 200 \mu M$). The model ran for 40 days with initial leaf mass of 0.01g and root mass of 0.01g and initial concentrations $C_{l0} = 92.8 nmol mg^{-1}$, $C_{r0} = 63 nmol mg^{-1}$, $N_{l0} = 0.1 nmol mg^{-1}$, $N_{r0} = 7.54 nmol mg^{-1}$.

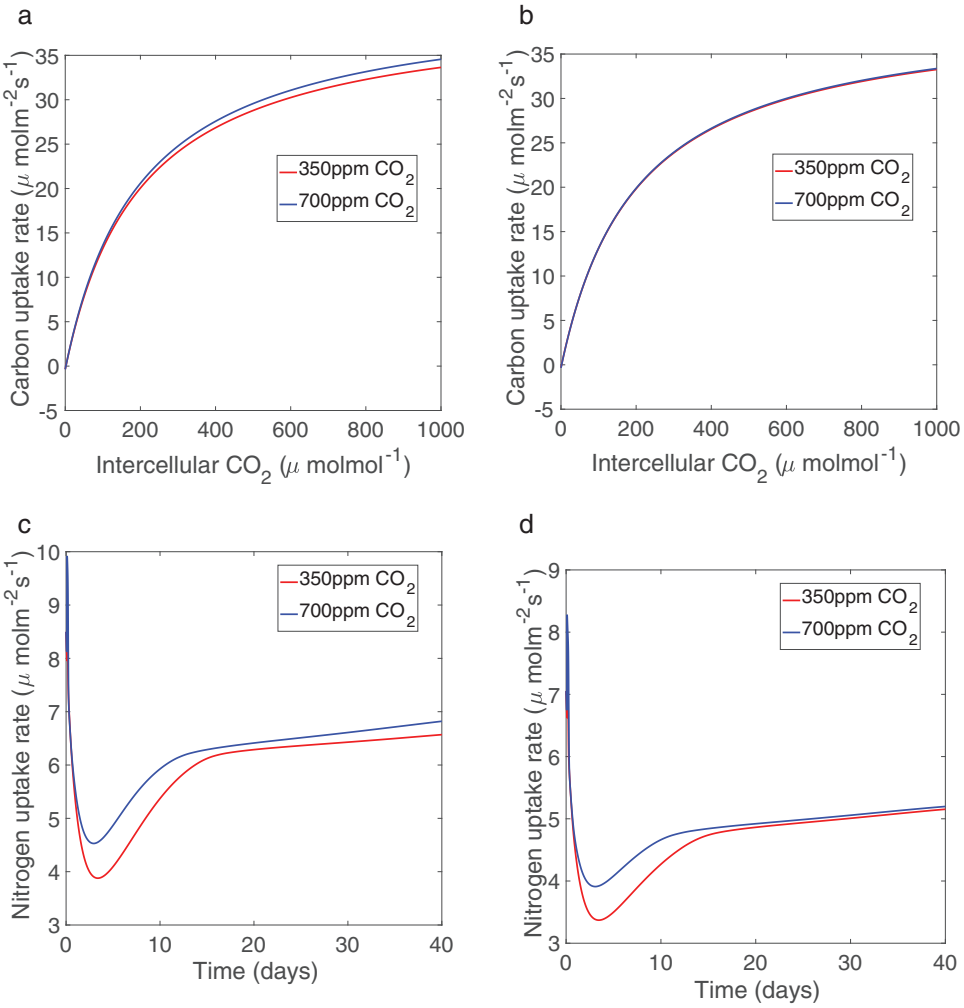

Figure 5. The relationship between carbon uptake rate and intercellular CO_2 for high $(700ppm)$ and low $(350ppm)$ atmospheric CO_2 a) when soil nitrogen is high $(400\mu M)$. b) when soil nitrogen is low $(200\mu M)$. These curves are created by substituting final leaf carbon and nitrogen concentrations at $t = 40days$ into Eq (3) to plot carbon uptake rate against intercellular CO_2 between 0 and $1000nmolmol^{-1}$. The relationship between nitrogen uptake rate and two atmospheric CO_2 concentrations over 40 days c) when soil nitrogen is high $(400\mu M)$ d) when soil nitrogen is low $(200\mu M)$. The model was run for 40 days with initial leaf mass of $0.01g$ and initial root mass of $0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}, C_{r0} = 63nmolmg^{-1}, N_{l0} = 0.1nmolmg^{-1}, N_{r0} = 7.54nmolmg^{-1}$.

was stronger with a high soil nitrogen treatment for both carbon and nitrogen uptake rate. In the first 15 days, CO_2 had a large effect on nitrogen uptake rate. This effect on uptake rate diminished with time. For a low nitrogen treatment, the effect of CO_2 after 15 days became much smaller than when $n = 400\mu M$ (Figs. 5c).

Root to shoot ratio (R:S) reached close to 0.3 when soil nitrogen is high and CO_2 is low (Fig. 6a). Initially for high nitrogen conditions, increasing atmospheric CO_2 had little effect on R:S. High atmospheric CO_2 increased the proportion of roots in relation to leaf mass after 5 days. Low nitrogen treatments increased root growth and the effect of CO_2 treatment emerged sooner than when soil nitrogen is high. Low nitrogen treatment overall increased R:S when compared with a higher nitrogen treatment (Fig. 6b). This reflects the environmental plasticity of the feedback model since when nitrogen availability was high, less roots were produced and when it was lower, more roots were produced. Changes in atmospheric CO_2 had the same effect on R:S under both high and low nitrogen conditions.

The relationships between carbon and nitrogen availability on R:S are a consequence of their relationship with leaf and root RGRs. When soil nitrogen was high, increased atmospheric CO_2 simply increased both leaf and root RGR, slightly shifting RGRs. Since the effect of CO_2 is stronger on root RGR than leaf, it led to an increased R:S (Fig. 6a). When soil nitrogen was low, atmospheric CO_2 also increased individual growth rates, but leaf growth was only slightly increased and root growth was increased greatly, rectifying the difference in R:S.

The model was run without any of the internal feedbacks to determine whether it was in fact the feedbacks or the Thornley (1972) model which is able to respond to changes in environment (Figs. S2–Figs. S7). The effect of CO_2 and soil nitrogen treatments on total plant mass remains (Fig. 3) when running the experiment on the model without any feedbacks (Figs. S2).

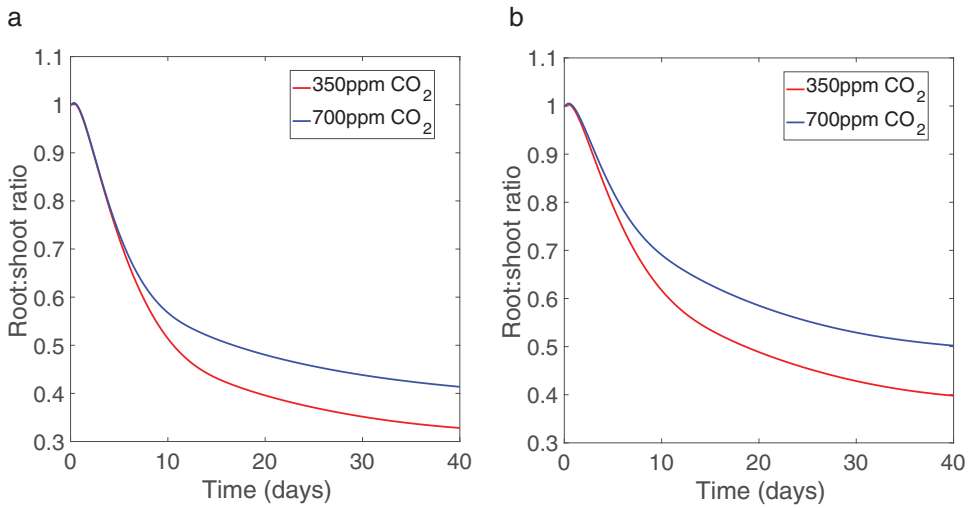

Figure 6. a) The relationship between root:shoot ratio over 40 days and two CO_2 treatments ($350ppm$ and $700ppm$) when soil nitrogen is high ($400\mu M$). b) The relationship between root:shoot ratio over time and two CO_2 treatments ($350ppm$ and $700ppm$) when soil nitrogen is low ($200\mu M$). The model was run with initial leaf and root mass of $0.01g$ respectively and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$, $C_{r0} = 63nmolmg^{-1}$, $N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

However, the removal of internal feedbacks increased intermediate nitrogen concentration and, therefore, nitrogen initially accounted for a much higher proportion of total plant mass than with feedbacks, reaching an unrealistic maximum percentage of 50%. Along with high percentages, increasing CO_2 treatment reduced nitrogen percentage within the plant. Removing the feedbacks produced a similar R:S for all combinations of soil nitrogen and CO_2 treatments such that R:S was between 0.2 and 0.4. Initially the lower CO_2 treatment had a higher R:S but by 40 days, high CO_2 increased R:S when compared with a lower CO_2 treatment. Due to the lack of internal feedbacks, carbon and nitrogen uptake rate remained constant throughout nutrient availability manipulations (Figs. S4).

3.2. Defoliation and CO_2 experiment

Defoliation reduced total plant mass but enhanced the effect of elevated CO_2 on growth (Fig. 7a). At 20 days, elevated CO_2 increased total plant mass by 12% without any defoliation (Fig. 3a), whereas plant mass was increased by 15% with defoliation (Fig. 7a). Therefore, defoliation increased the positive effect of high CO_2 by 3%, implying that the model with feedbacks is reacting to a halving of the carbon source size (defoliation). Before defoliation, lower CO_2 treatment increased R:S compared with a high atmospheric CO_2. After defoliation, both treatments invested into leaf growth at similar rates (Fig. 7b).

Leaf carbon was reduced and leaf nitrogen increased for both CO_2 treatments, 7 days after defoliation (Fig. 7c and Fig. d). For both CO_2 treatments, carbon uptake rate increased initially due to an imbalance between nitrogen and carbon use for growth and respiration and soon reached a plateau (Fig. 8a), and 7 days after defoliation, carbon uptake rate increased very slightly, with a difference of 0.3% when CO_2 was low and 0.2% when CO_2 was high. Defoliation had a much stronger effect on nitrogen uptake rate than for carbon, and 7 days after defoliation, nitrogen uptake rate decreased by 12% when CO_2 was low and when CO_2 was high (Fig. 8b).

Carbon concentration decreased 7 days after defoliation when simulating the experiment without any internal feedbacks on growth and uptake. This also applied for intermediate nitrogen concentration such that after 7 days, nitrogen was higher. Removing the feedbacks on intermediate concentration therefore did not alter the effect of defoliation on concentration. However, without feedbacks defoliation did not alter carbon and nitrogen uptake rates since in the absence of feedbacks, they are only dependent upon external CO_2 and nitrogen, respectively (Figs. S5–Figs. S7).

4. DISCUSSION

The aim of this paper was to determine whether a framework model of feedback mechanisms sensitive to changes in carbon and nitrogen responds to changes in source and sink manipulations in a similar way to experimental data (Coleman et al., 1993; Farage et al., 1998; Rogers et al., 1998; Ainsworth et al., 2003; Butterly et al., 2015) and to highlight areas of uncertainty where more empirical data are needed. This was carried out by simulating two levels of atmospheric CO_2 and two soil nitrogen treatments in factorial combination and a defoliation experiment where the total above ground biomass was halved after 10 days and growth was simulated for an additional 10 days. These simulations showed that the model qualitatively reproduces the CO_2 and nitrogen interaction often observed experimentally, whereby the positive effect of atmospheric CO_2 on growth is magnified with high nitrogen and diminished by low nitrogen (Coleman et al., 1993; Demmers-Derks et al., 1998). Additionally the model reproduces the experimental finding that defoliation amplifies the positive effect of high CO_2 on

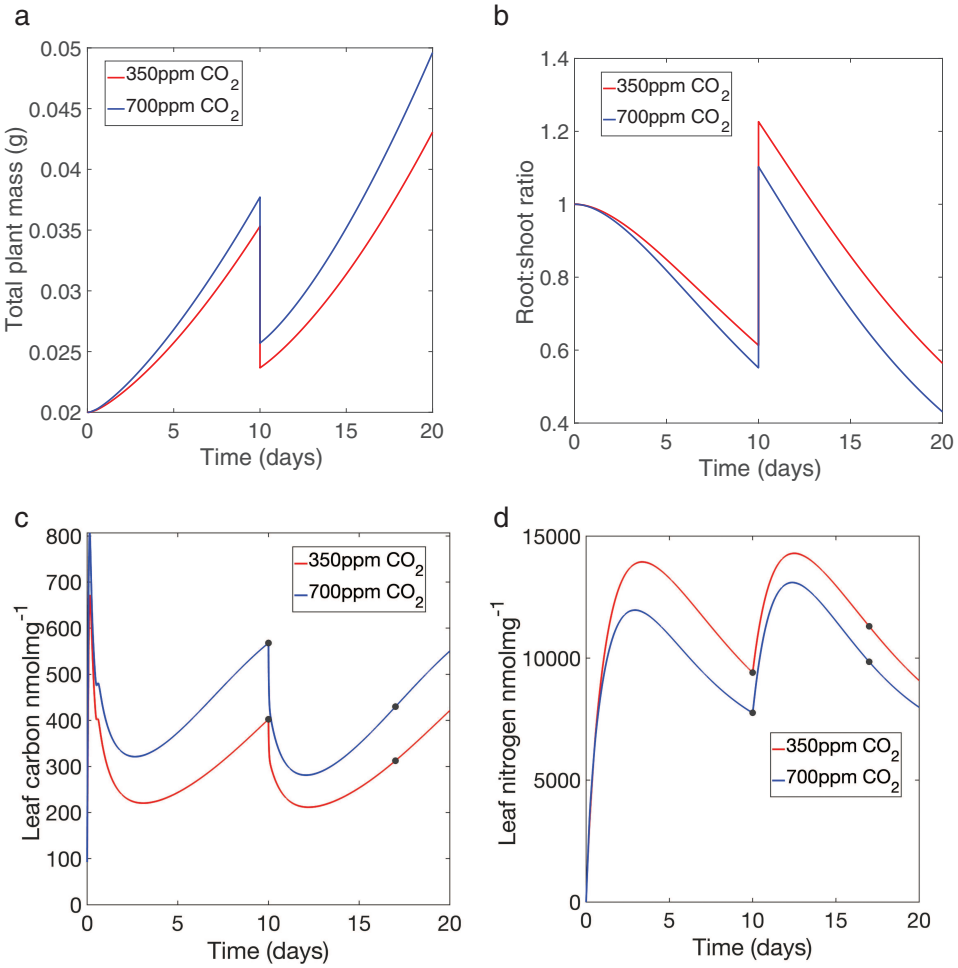

Figure 7. The effect of defoliation (when total leaf mass is halved at day 10) with all internal feedbacks and a high (700*ppm*, blue lines) and low (350*ppm*, red lines) CO_2 treatment on a) Total plant mass over 20 days of growth. b) Proportion of leaf mass compared to root (shoot:root) over 20 days. c) Intermediate leaf carbon concentration for 20 days. d) Intermediate leaf nitrogen over 20 days. Markers signify concentrations of carbon and nitrogen in the leaves at day 10 and day 17 for both carbon and nitrogen plots. All run with soil nitrogen $400\mu M$ and initial leaf and root mass of $0.01g$ respectively and $C_{l0} = 92.8nmolmg^{-1}$, $C_{r0} = 63nmolmg^{-1}$, $N_{l0} = 0.1nmolmg^{-1}$ and $N_{r0} = 7.54nmolmg^{-1}$.

growth (Ryle and Powell, 1992; Wand and Midgley, 2004). Moreover, increased atmospheric CO_2 leads to a larger simulated root:shoot ratio and increased soil nitrogen leads to a lower proportion of roots than either ambient CO_2 and nitrogen treatments, respectively. Imposing defoliation in the model produces higher leaf nitrogen, lower leaf carbon, and higher carbon uptake rates as observed experimentally (Von Caemmerer and Farquhar, 1984; Rogers et al., 1998; Eyles et al., 2013). Therefore, a model which simulates internal feedbacks on source and sink strengths with changes in carbon and nitrogen is able to reflect key behaviours observed in experiments that manipulate source strength. This model takes us one step closer towards modelling the mechanisms responsible for allocation.

The model accurately replicates a general result from CO_2 and nitrogen experiments, such that both nitrogen and CO_2 availability have a positive relationship with plant growth and there is an interaction between CO_2 and nitrogen treatments (Coleman et al., 1993; Curtis and Wang, 1998; Demmers-Derks et al., 1998; De Graff et al., 2006). This arises because low soil nitrogen imposes a limit on how much biomass can be produced (i.e. sink limitation); however, this does not feed back onto photosynthetic rate (Figs. 5a and Figs. b). The model simulates a slight raising of the A/c_i curve in elevated compared with ambient CO_2 in high N, contradicting experimental observations (Coleman et al., 1993; Ainsworth et al., 2003). However, this is something that is theoretically predicted at high CO_2 (Sage, 1994; Woodrow, 1994). The increased availability of CO_2 increases nitrogen uptake rate (via feedback 5) and consequently nitrogen availability. Since carbon and nitrogen concentrations drive leaf and root RGR, the immediately available concentrations of carbon and nitrogen are used for new tissue production. Therefore, the other feedback mechanisms have acted in place of feedback 1 (high carbon reduces carbon uptake rate) and the intermediate carbon pool has not been able to accumulate high enough to trigger a reduction in carbon uptake rate.

The model simulations in this paper show that high levels of atmospheric CO_2 promote an increase in root:shoot ratio, while increasing soil nitrogen reduces root:shoot ratio. This is to

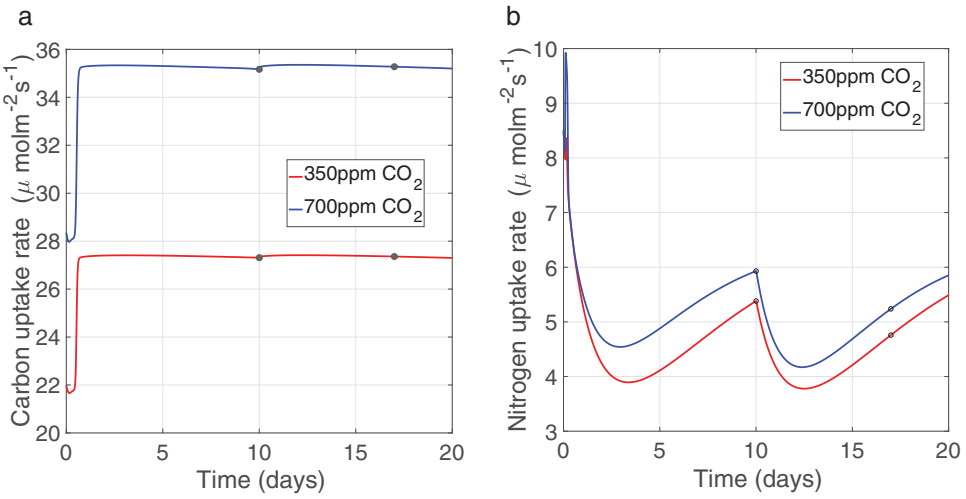

Figure 8. The effect of defoliation (when total leaf mass is halved at day 10) with all internal feedbacks and a high (700*ppm*, blue lines) and low (350*ppm*, red lines) CO_2 treatment on a) Carbon uptake rate over 20 days of growth. b) Nitrogen uptake rate over 20 days of growth. Markers signify carbon and nitrogen uptake rate at day 10 and day 17. All ran with soil nitrogen 400μM and initial leaf and root mass of 0.01g respectively and $C_{l0} = 92.8 nmolmg^{-1}$, $C_{r0} = 63 nmolmg^{-1}$, $N_{l0} = 0.1 nmolmg^{-1}$ and $N_{r0} = 7.54 nmolmg^{-1}$.

be expected within the model as high levels of carbon increase root growth and conversely high levels of nitrogen increase leaf growth via the internal feedback mechanisms. Individual studies have shown increases (Lacointe, 2000) and decreases (Butterly et al., 2015) in root:shoot ratio with elevated CO_2. However, a meta-analysis by Ainsworth et al. (2002) found no change in root:shoot ratio. Butterly et al. (2015) also found that increasing nitrogen availability increases root:shoot ratio, whilst Vicente et al. (2015) and Dybzinski et al. (2011) found that increasing nitrogen reduces root:shoot ratio. Therefore, empirical evidence conflicts on whether root:shoot ratios should increase or decrease from changes in CO_2 and soil nitrogen availability. An emergent property of the model is the decline in root:shoot ratio with age and is seen commonly in plants (Poorter et al., 1988; Negrini et al., 2020). Another emergent property of the model is that low N availability produces small plants with reasonable intermediate concentrations of carbon and nitrogen (Wu et al., 2007) rather than the alternative of producing larger plants with a diluted nitrogen concentration and associated costs for functioning (Vos et al., 2005). Reducing the ratio of nitrogen to carbon atoms that make up plant tissue (λ) in the model should allow this alternative growth strategy to occur.

Defoliation enhances the positive effect of high atmospheric CO_2 within the model. Removing half of the total leaf mass after 10 days makes the plant source limited, reducing carbon source strength via a reduction in photosynthesis whilst also reducing leaf nitrogen. Although nitrogen remobilization is considered in the model, this only occurs when leaf nitrogen is higher than root nitrogen. This consequently leads to an increase in leaf nitrogen concentration due to higher availability and a reduction in leaf carbon. The high levels of leaf nitrogen triggers the feedbacks of high N to increase carbon uptake rate whilst reducing nitrogen uptake rate and an increase in leaf growth rate. These responses entirely match experimental observations (Ryle and Powell, 1992; Wand and Midgley, 2004; Von Caemmerer and Farquhar, 1984; Rogers et al., 1998; Eyles et al., 2013). Furthermore, the framework model is able to respond to defoliation in a robust way. In the case of senescing leaves, nitrogen is typically retranslocated to other plant organs prior to defoliation (Gordon and Jackson, 2000). Although this is not simulated in our model, this could be implemented by adding a proportion of the mass lost into the nitrogen pools when enforcing a defoliation event. This is unlikely to alter the estimated responses to defoliation but perhaps slightly the magnitude of the response. In addition to senescence, extending the model to include reproductive growth will provide valuable insights into how carbon and nitrogen status influences overall yield. For example, carbon:nitrogen status has been shown to impact reproduction. Specifically, earlier flowering time is promoted by high carbon status and carbon derived signals such as sucrose and T6P regulate flowering times and grain filling (Cho et al., 2018; Tsai and Chang, 2022). The remobilization of nitrogen from senescing leaves is important for grain filling (Masclaux-Daubresse et al., 2008). This presents additional carbon and nitrogen feedbacks which could be added in conjunction with senescence and seed production in a future model.

A number of assumptions were made in order to simulate source–sink interactions, highlighting areas of focus for further experimental testing. Firstly, the rate of tissue growth is assumed to depend on carbon and nitrogen concentration in the model; however, this may not necessarily be the case. Additional data are required on the rate of carbon and nitrogen use for growing plant tissue and how this changes over a growing period. Secondly, the form of each feedback function used in the model were determined mathematically based on (Holland, 2019). However, the way in which carbon or nitrogen concentrations trigger feedbacks on growth and resource acquisition is largely unknown. In many cases, part of the mechanism has been elucidated, but the complete sequence of events needed to simulate the genetic interactions is unknown. Even when mechanisms are known,

further data would be needed to parameterize model functions. Most published work on the mechanisms compare gene expression and/or changes in enzyme activity for a plant with and without sugar or amino acid treatments. Some acknowledge the time it takes for a gene to be expressed (within 30 minutes (Reda, 2015)) or the time taken for resources to accumulate (Paul and Foyer, 2001) and most acknowledge a time length of the treatment (Reda, 2015). Some work does provide a timescale. For example, Xiong et al. (2013) show that meristem activation occurs within 24 hours of treating seedlings with glucose. There is a clear lack of knowledge on the time it takes from when concentrations are sensed, to gene expression for the induction of enzymes for a reaction, to the change in shoot:root ratio. Without this information, it is not possible to compare the rates at which the various feedbacks operate in a parameterized model. This raises questions such as: (1) How fast is the feedback? (2) Is this a feedback that is turned on or off or does it happen incrementally? (3) If it does work like a switch, what threshold values cause this feedback?

Nunes et al. (2013) show that when imposing sink limitation by reducing temperature or low nitrogen and supplying *Arabidopsis* with sucrose, T6P accumulates which increases the use of carbon for growth. T6P inhibits the expression of SnRK1 in order to increase growth processes. These authors present a starvation threshold for sucrose of $3\mu molg^{-1}$ (fresh weight) and T6P of $0.3-0.5nmolT6Pg^{-1}$ (fresh weight). This paper determines a threshold value that sugar must surpass in order for the rate of use of carbon for growth to be promoted. To better understand these mechanisms, not only do threshold values need to be obtained experimentally, but also the rates at which these feedbacks occur. It is unclear whether these threshold values represent a triggered switch-like behaviour or whether the response is more gradual and continuous. Furthermore, the strength of the feedback responses on growth processes is also unknown and is simulated to be dependent upon carbon or nitrogen concentration within the model.

This model can be extended to include other known feedback mechanisms. For instance, sugars have been shown to negatively influence the loading of sugars, altering the rate of transport of substrate between leaves and roots (Chiou and Bush, 1998; Vaughn et al., 2002; Ainsworth and Ort, 2010). The feedbacks chosen here only simulate responses to growth and uptake rates with high levels of carbon and nitrogen, whereas other known behaviours are in response to low concentrations. For instance, when sugars are scarce meristem growth stops (Lastdrager et al., 2014). SnRK1 protein kinase is present when sugars are low and this is responsible for suppressing growth (Baena-González et al., 2007; Polge and Thomas, 2007; Halford and Hey, 2009; Baena-González, 2010; Ghillebert et al., 2011), but sucrose can also stimulate SnRK1 (Baena-González, 2010). Low sugars can also stop the transcription of NR (Stitt and Krapp, 1999; Klein et al., 2000; Kaiser et al., 2002; Reda, 2015). Additionally, leaf nitrogen concentration sets a limit to the maximum capacity of carbon assimilation through a relationship with carboxylation rate (Walker et al., 2014). These known mechanisms represent other feedbacks which could be incorporated in the model. Further detailed analysis is needed on which feedbacks are likely to contribute the most towards allocation responses and will be essential for adding in more feedbacks into the model in future. For example, what is the smallest number of feedbacks required to simulate reasonable responses to changes in environment? Are additional feedbacks required to make the model's results align more closely with the experimentally observed responses? To better understand the mechanisms governing allocation, it is necessary to incorporate a complex network of feedback mechanisms into a model. However, since this may not be the primary goal of certain crop models, a balance must be struck between complexity and usability. As our understanding of allocation mechanisms improves, this knowledge can help refine crop models in a way that captures essential dynamics without requiring overly intricate feedback networks and over parameterization. In this modelling framework, we demonstrate that incorporating a limited set of carbon and nitrogen dependencies–such as CO_2 uptake, nitrogen uptake, and shoot and root growth rates–can still enable a dynamic partitioning response.

The model was parameterized using multiple herbaceous species to create a flexible framework that can be adapted for a wide range of species or integrated into crop models for improved partitioning responses. To apply the model to a particular species, species-specific parameters related to photosynthesis rate, nitrogen uptake rate, and maximum RGR are required. This model can be a useful tool to compare how different species' allocation responses function, provided that data are collected for the questions highlighted in this discussion. The model simulates the physiological responses to carbon and nitrogen derived feedbacks by altering the pools of leaf and root carbon and nitrogen. With additional experimental data, the model could be adapted to test the effect of gene regulatory mechanisms on biomass partitioning. An extension of this work could involve combining the model with a metabolic model to link feedbacks to precise metabolites involved. The work of Kannan et al. (2019) is an example of integrating gene regulation into a metabolic model.

5. CONCLUSION

Overall, the results of this paper show that the model with feedback mechanisms based on internal carbon and nitrogen concentrations is able to qualitatively reproduce most of the behaviours seen in experiments varying carbon and nitrogen availability. It reproduces more of the observed behaviours than a model without feedbacks and is critically able to regulate internal concentrations of carbon and nitrogen. The framework presented here extends the work of other models which have simulated dependency of source or sink activity on carbon and nitrogen (Bartelink, 1998; Hunt et al., 1998; Dunbabin et al., 2002; Ågren et al., 2012; Pao et al., 2018; Shaw and Cheung, 2018) by including more types of feedbacks which alter both source and sink activity. By incorporating more of the observed feedbacks, this work provides a closer representation of allocation processes. This model is a tool for investigating the dynamics of internal feedback mechanisms that control biomass allocation and can be adapted to a range of species by substituting a few species-specific parameters. We argue that this model provides an improved method for allocation which can be implemented into crop models to give process-based mechanistic feedbacks

between carbon and nitrogen resource availability. Furthermore, this paper sharpens questions about the physiological mechanisms underpinning resource allocation and highlights new research directions. Understanding the mechanisms behind allocation can provide new areas of focus to manipulate the net primary productivity of plants.

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AUTHOR CONTRIBUTIONS

Bethany L. Holland: Conceptualization, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review & editing
Nicholas A.M. Monk: Conceptualization, Supervision, Validation, Writing–review & editing
Richard H. Clayton: Conceptualization, Supervision, Validation, Writing–review & editing
Colin P. Osborne: Conceptualization, Funding acquisition, Supervision, Validation, Writing–review & editing

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

The data underlying this article are available in GitHub at https://github.com/bop15bh/Feedback_Model. No new experimental data were generated in this study; model parameters were derived from previously published literature as cited in the manuscript.

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LITERATURE CITED

Ågren GI, Wetterstedt JM, Billberger MF. Nutrient limitation on terrestrial plant growth–modelling the interaction between nitrogen and phosphorus. New Phytol 2012;194:953–60.
Ainsworth E, Davey P, Hymus G et al. Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under free air CO_2 enrichment (face). Plant Cell Environ 2003;26:705–14.
Ainsworth EA, Davey PA, Bernacchi CJ et al. A meta-analysis of elevated CO_2 effects on soybean (Glycine max) physiology, growth and yield. Glob Chang Bio 2002;8: 695–709.
Ainsworth EA, Ort DR. How do we improve crop production in a warming world? Plant Physiol 2010;154:526–30.
Asseng S, Kassie BT, Labra MH et al. Simulating the impact of source-sink manipulations in wheat. Field Crops Res 2017;202:47–56.
Baena-González E. Energy signaling in the regulation of gene expression during stress. Mol Plant 2010;3:300–13.
Baena-González E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. Nature 2007;448:938.
Bartelink H. A model of dry matter partitioning in trees. Tree Physiol 1998;18:91–101.
Boote KJ, Seepaul R, Mulvaney MJ et al. Adapting the CROPGRO model to simulate growth and production of *Brassica carinata*, a bio-fuel crop. GCB Bioenergy 2021;13:1134–48.
Brisson N, Gary C, Justes E et al. An overview of the crop model stics. Eur J Agron 2003;18:309–32.
Brown HE, Huth NI, Holzworth DP et al. A generic approach to modelling, allocation and redistribution of biomass to and from plant organs. in silico Plants 2019;1:diy004.
Buckley TN, Roberts DW. Despot, a process-based tree growth model that allocates carbon to maximize carbon gain. Tree Physiol 2006;26:129–44.
Butterly CR, Armstrong R, Chen D, Tang C. Carbon and nitrogen partitioning of wheat and field pea grown with two nitrogen levels under elevated CO_2. Plant Soil 2015;391:367–82.
Chan MT, Yu SM. The 3′ untranslated region of a rice α-amylase gene mediates sugar-dependent abundance of mrna. Plant J 1998;15:685–95.
Chang TG, Wei ZW, Shi Z et al. Bridging photosynthesis and crop yield formation with a mechanistic model of whole-plant carbon–nitrogen interaction. In Silico Plants 2023;5:diad011.
Cheeseman J. Plant growth modelling without integrating mechanisms. Plant Cell Environ 1993;16:137–47.
Cheng CL, Acedo GN, Cristinsin M, Conkling MA. Sucrose mimics the light induction of *Arabidopsis nitrate* reductase gene transcription. Proc Natl Acad Sci 1992;89:1861–4.
Chiou TJ, Bush DR. Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci 1998;95:4784–8.
Cho LH, Pasriga R, Yoon J et al. Roles of sugars in controlling flowering time. J Plant Biol 2018;61:121–30.
Clarkson, D.T. and Lüttge, U., 1991. Mineral nutrition: inducible and repressible nutrient transport systems. In Progress in Botany: Structural Botany Physiology Genetics Taxonomy Geobotany/Fortschritte der Botanik Struktur Physiologie Genetik Systematik Geobotanik (61–83). Berlin, Heidelberg: Springer Berlin Heidelberg.
Coleman J, McConnaughay K, Bazzaz F. Elevated CO_2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? Oecologia 1993;93:195–200.
Curtis PS, Wang X. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 1998;113:299–313.
De Graff MA, Van Groenigen KJ, Six J et al. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Glob Chang Biol 2006;12:2077–91.
De Schepper V, De Swaef T, Bauweraerts I, Steppe K. Phloem transport: a review of mechanisms and controls. J Exp Bot 2013;64: 4839–50.
De Wit A, Boogaard H, Fumagalli D et al. 25 years of the WOFOST cropping systems model. Agric Syst 2019;168:154–67.
Demmers-Derks H, Mitchell R, Mitchell V, Lawlor D. Response of sugar beet (*Beta vulgaris* l.) yield and biochemical composition to elevated CO_2 and temperature at two nitrogen applications. Plant Cell Environ 1998;21:829–36.
Dewar, Roderick C., Anthony R. Ludlow, and Phillip M. Dougherty. Environmental Influences on Carbon Allocation in Pines. Ecological Bulletins, 43 (1994): 92–101.
Dunbabin VM, Diggle AJ, Rengel Z, Van Hugten R. Modelling the interactions between water and nutrient uptake and root growth. Plant Soil 2002;239:19–38.
Dybzinski R, Farrior C, Wolf A et al. Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for

light and nitrogen: an analytically tractable, individual-based model and quantitative comparisons to data. Am Nat 2011;177:153–66.
Eyles A, Pinkard EA, Davies NW et al. Whole-plant versus leaf-level regulation of photosynthetic responses after partial defoliation in *Eucalyptus globulus* saplings. J Exp Bot 2013;64:1625–36.
Farage PK, McKee IF, Long SP. Does a low nitrogen supply necessarily lead to acclimation of photosynthesis to elevated CO_2? Plant Physiol 1998;118:573–80.
Farquhar GD, von Caemmerer S, Berry JA. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 1980;149:78–90.
Feller C, Favre P, Janka A et al. Mathematical modeling of the dynamics of shoot-root interactions and resource partitioning in plant growth. PLoS One 2015;10:e0127905.
Garnier, E. Growth Analysis of Congeneric Annual and Perennial Grass Species. Journal of Ecology 1992, 80(4), 665–75.
Garnier E, Laurent G. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. New Phytol 1994;128:725–36.
Ghillebert R, Swinnen E, Wen J et al. The AMPK/SNF1/SnRK1 fuel gauge and energy regulator: structure, function and regulation. FEBS J 2011;278:3978–90.
Gojon A, Dapoigny L, Lejay L et al. Effects of genetic modification of nitrate reductase expression on $^{15}NO_3^-$ uptake and reduction in Nicotiana plants. Plant Cell Environ 1998;21:43–53.
Gordon WS, Jackson RB. Nutrient concentrations in fine roots. Ecology 2000;81:275–80.
Graham I. Carbohydrate control of gene expression in higher plants. Res Microbiol 1996;147:572–80.
Halford NG, Hey SJ. Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants. Biochem J 2009;419:247–59.
He JS, Fang J, Wang Z. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of china. Oecologia 2006;149:115–22.
Hodges M. Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J Exp Bot 2002;53:905–16.
Holland BL. Modelling plant growth by investigating source-sink dynamics. Ph.D. Thesis, University of Sheffield, 2019.
Hunt H, Morgan J, Read J. Simulating growth and root-shoot partitioning in prairie grasses under elevated atmospheric CO_2 and water stress. Ann Bot 1998;81:489–501.
Iglesias-Bartolomé R, González CA, Kenis JD. Nitrate reductase dephosphorylation is induced by sugars and sugar-phosphates in corn leaf segments. Physiologia Plantarum 2004;122:62–7.
Imsande J, Touraine B. N demand and the regulation of nitrate uptake. Plant Physiol 1994;105:3.
Jones JW, Hoogenboom G, Porter CH et al. The DSSAT cropping system model. Eur J Agron 2003;18:235–65.
Kaiser WM, Weiner H, Kandlbinder A et al. Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction. J Exp Bot 2002;53:875–82.
Kannan K, Wang Y, Lang M et al. Combining gene network, metabolic and leaf-level models shows means to future-proof soybean photosynthesis under rising CO_2. In Silico Plants 2019;1:diz008.
Kaschuk G, Hungria M, Leffelaar P et al. Differences in photosynthetic behaviour and leaf senescence of soybean (*Glycine max* [l.] merrill) dependent on N2 fixation or nitrate supply. Plant Biol 2010;12:60–69.
Katul G, Ellsworth D, Lai CT. Modelling assimilation and intercellular CO_2 from measured conductance: a synthesis of approaches. Plant Cell Environ 2000;23:1313–28.
Kelly G, Moshelion M, David-Schwartz R et al. Hexokinase mediates stomatal closure. Plant J 2013;75:977–88.
King BJ, Siddiqi MY, Ruth TJ et al. Feedback regulation of nitrate influx in barley roots by nitrate, nitrite, and ammonium. Plant Physiol 1993;102:1279–86.
Kira T. Primary production of forests. In: Cooper JP, ed. Photosynthesis and productivity in different environments . 1975. New York: Cambridge University Press, 5–40
Klein D, Morcuende R, Stitt M, Krapp A. Regulation of nitrate reductase expression in leaves by nitrate and nitrogen metabolism is completely overridden when sugars fall below a critical level. Plant Cell Environ 2000;23:863–71.
Koch K. Carbohydrate-modulated gene expression in plants. Ann Rev Plant Biol 1996;47:509–40.
Lacointe A. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Ann For Sci 2000;57:521–33.
Lastdrager J, Hanson J, Smeekens S. Sugar signals and the control of plant growth and development. J Exp Bot 2014;65:799–807.
Lilley JL, Gee CW, Sairanen I. An endogenous carbon-sensing pathway triggers increased auxin flux and hypocotyl elongation. Plant Physiol 2012; 160(4):2261–2270.
Lochocki EB, Rohde S, Jaiswal D et al. Biocro ii: a software package for modular crop growth simulations. In Silico Plants 2022;4:diac003.
Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M. Leaf nitrogen remobilisation for plant development and grain filling. Plant Biol 2008;10:23–36.
Minchin P, Thorpe M, Farrar J. Short-term control of root: shoot partitioning. J Exp Bot 1994;45:615–22.
Moorby J, 1977. Integration and regulation of translocation within the whole plant, in: Jennings, D.H. (Ed.), Integration of activity in the higher plant. Society of Experimental Biology Symposium 31, Cambridge University Press, Cambridge, England, 425–454.
Moreira TB, Shaw R, Luo X. A genome-scale metabolic model of soybean (Glycine max) highlights metabolic fluxes in seedlings. Plant Physiol 2019;180:1912–29.
Muller B, Touraine B. Inhibition of NO_3^- uptake by various phloem-translocated amino acids in soybean seedlings. J Exp Bot 1992;43:617–23.
Münch E. Die Stoffbewegungen in der Pflanze. Fischer, Jena, Jena, Germany: Gustav Fischer 1930.
Negrini ACA, Evans JR, Kaiser BN et al. Effect of n supply on the carbon economy of barley when accounting for plant size. Funct Plant Biol 2020;47:368–81.
Nunes, C., O'Hara, L.E., Primavesi, L.F., Delatte, T.L., Schluepmann, H., Somsen, G.W., Silva, A.B., Fevereiro, P.S., Wingler, A. and Paul, M.J., 2013. The trehalose 6-phosphate/SnRK1 signaling pathway primes growth recovery following relief of sink limitation. Plant physiology, 162(3), 1720–1732.
Nunes-Nesi A, Fernie AR, Stitt M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant 2010;3:973–96.
Pallas B, Da Silva D, Valsesia P et al. Simulation of carbon allocation and organ growth variability in apple tree by connecting architectural and source–sink models. Ann Bot 2016;118:317–30.
Pao, YC, Chen, TW, Moualeu-Ngangue, DP and Stützel, H, Environmental triggers for photosynthetic protein turnover determine the optimal nitrogen distribution and partitioning in the canopy. J Exp Bot 2019; 70(9), 2419–2434.
Paul MJ, Foyer CH. Sink regulation of photosynthesis. J Exp Bot 2001;52:1383–400.
Polge C, Thomas M. SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? Trend Plant Sci 2007;12:20–8.
Poorter H, Pot S, Lambers H. The effect of an elevated atmospheric CO_2 concentration on growth, photosynthesis and respiration of Plantago major. Physiologia Plantarum 1988;73:553–9.
Rastetter EB, Shaver GR. A model of multiple-element limitation for acclimating vegetation. Ecology 1992;73:1157–74.
Reda M. Response of nitrate reductase activity and nia genes expression in roots of *Arabidopsis* hxk1 mutant treated with selected carbon and nitrogen metabolites. Plant Sci 2015;230:51–8.

Rogers A, Fischer BU, Bryant J et al. Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. perennial Ryegrass under free-air CO_2 enrichment. Plant Physiol 1998;118:683–9.

Rufty TWJ, Israel DW, Volk RJ et al. Phosphate regulation of nitrate assimilation in soybean. J Exp Bot 1993;44:879–91.

Ryle G, Powell C. The influence of elevated CO_2 and temperature on biomass production of continuously defoliated white clover. Plant Cell Environ 1992;15:593–9.

Sage RF. Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. Photosynthesis Res 1994;39:351–68.

Sairanen I, Novák O, Pěnčík A et al. Soluble carbohydrates regulate auxin biosynthesis via pif proteins in *Arabidopsis*. Plant Cell 2012;112. 24(12):4907–4916.

Scheible WR, Lauerer M, Schulze ED et al. Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. Plant J 1997;11:671–91.

Shaner DL, Boyer JS. Nitrate reductase activity in maize (Zea mays l.) leaves: I. regulation by nitrate flux. Plant Physiol 1976;58,:499–504.

Shaw R, Cheung C. A dynamic multi-tissue flux balance model captures carbon and nitrogen metabolism and optimal resource partitioning during *Arabidopsis* growth. Front Plant Sci 2018;9:884.

Sheen J, Zhou L, Jang JC. Sugars as signaling molecules. Curr Opin Plant Biol 1999;2:410–8.

Siddiqi MY, Glass AD. A model for the regulation of K+ influx, and tissue potassium concentrations by negative feedback effects upon plasmalemma influx. Plant Physiol 1986;81: 1–7.

Siddiqi MY, Glass AD, Ruth TJ, Rufty TW. Studies of the uptake of nitrate in barley: I. kinetics of $^{13}NO_3^-$ influx. Plant Physiol 1990;93:1426–32.

Smith AM, Stitt M. Coordination of carbon supply and plant growth. Plant Cell Environ 2007;30:1126–49.

Stitt M, Krapp A. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 1999;22:583–621.

Stitt M, Müller C, Matt P et al. Steps towards an integrated view of nitrogen metabolism. J Exp Bot 2002;53:959–70.

Stokes ME, Chattopadhyay A, Wilkins O et al. Interplay between sucrose and folate modulates auxin signalling in *Arabidopsis*. Plant Physiol 2013; 162(3):1152–1565.

Thornley J. A balanced quantitative model for root: shoot ratios in vegetative plants. Ann Bot 1972;36:431–41.

Thornley JHM, Johnson IR. Plant and Crop Modelling. Oxford: Clarendon, Oxford, 1990.

Thum T, Caldararu S, Engel J et al. A new model of the coupled carbon, nitrogen, and phosphorus cycles in the terrestrial biosphere (quincy v1. 0; revision 1996). Geosci Model Dev 2019;12:4781–802.

Tjoelker M, Craine JM, Wedin D et al. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytol 2005;167:493–508.

Tsai SS, Chang YCA. Plant maturity affects flowering ability and flower quality in phalaenopsis, focusing on their relationship to carbon-to-nitrogen ratio. HortScience 2022;57:191–6.

Vaughn MW, Harrington GN, Bush DR. Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proc Natl Acad Sci USA 2002;99:10876–80.

Vicente R, Pérez P, Martínez-Carrasco R et al. Nitrate supply and plant development influence nitrogen uptake and allocation under elevated CO_2 in durum wheat grown hydroponically. Acta Physiologiae Plantarum 2015;37:114.

Von Caemmerer S. Biochemical models of leaf photosynthesis. Collingwood, Australia: Csiro publishing, 2000.

Von Caemmerer S, Farquhar G. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced p (CO_2) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* l. Planta 1984;160:320–9.

Vos J, Van Der Putten P, Birch C. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* l.). Field Crops Res 2005;93:64–73.

Walker AP, Beckerman AP, Gu L et al. The relationship of leaf photosynthetic traits–vcmax and jmax–to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. Ecol Evol 2014;4:3218–35.

Wand S, Midgley G. Effects of atmospheric CO_2 concentration and defoliation on the growth of *Themeda triandra*. Grass Forage Sci 2004;59:215–26.

Wann M, Raper Jr C. A dynamic model for plant growth: validation study under changing temperatures. Ann Bot 1984;53:45–52.

White AC, Rogers A, Rees M, Osborne CP. How can we make plants grow faster? a source–sink perspective on growth rate. J Exp Bot 2016;67:447.

Woodrow IE. Optimal acclimation of the C3 photosynthetic system under enhanced CO_2. Photosynthesis Res 1994;39:401–12.

Wray JL. Molecular biology, genetics and regulation of nitrite reduction in higher plants. Physiologia Plantarum 1993;89:607–12.

Wu J, Wang D, Rosen CJ, Bauer ME Comparison of petiole nitrate concentrations, spad chlorophyll readings, and quickbird satellite imagery in detecting nitrogen status of potato canopies. Field Crops Res 2007;101:96–103.

Xiong Y, McCormack M, Li L et al.. Glc-TOR signalling leads transcriptome reprogramming and meristem activation. Nature 2013;496: 181.

Youngdahl L, Pacheco R, Street J, Vlek P. The kinetics of ammonium and nitrate uptake by young rice plants. Plant Soil 1982;69: 225–32.

Zhou XR, Schnepf A, Vanderborght J et al. Cplantbox, a whole-plant modelling framework for the simulation of water-and carbon-related processes. In Silico Plants 2020;2:diaa001.

Zhu J, Gou F, Rossouw G et al. Simulating organ biomass variability and carbohydrate distribution in perennial fruit crops: a comparison between the common assimilate pool and phloem carbohydrate transport models. In Silico Plants 2021;3:diab024.

Zhu XG, Long SP, Ort DR. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr Opin Biotech 2008;19:153–159.