Modelling plant growth by investigating source-sink dynamics

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Abstract

The allocation of growth between plant compartments can alter plant size greatly. Allocation arises from the balance between internal processes that define growth (i.e. resource uptake and export by source tissues and resource consumption by sink tissues), which are altered by changes in carbon and nitrogen availability. Models that simulate the allocation of growth between source and sink tissues use a range of different approaches but remain limited in the representation of source-sink feedbacks. Although many such feedbacks are observed experimentally, it is unclear how they work collectively to define allocation. This thesis begins by investigating how growth allocation between carbon sources and sinks influences plant growth. It shows that maintenance respiration greatly alters the sensitivity of plant mass to different allocation strategies, specifically enabling the situation where increasing allocation towards roots increases total plant mass when leaf maintenance exceeds root maintenance respiration. Next, this thesis aims to better understand how plants allocate resources in response to environmental heterogeneity. In particular, it uses non-dimensional modelling to evaluate how internal feedbacks on growth based on carbon and nitrogen concentrations should be modelled individually and collectively. The resulting system of model feedbacks enhances growth when compared to a model without any feedbacks and reacts to environmental limitations on source activity by increasing growth towards the respective compartment. Finally, the framework is extended by implementation within a previously validated model and by comparison of simulated changes in source activity (CO_2 and nitrogen availability) and source size (defoliation) against experimental results. This work shows that the model with feedbacks largely reflects plant behaviour observed experimentally. Overall, this thesis has developed a novel approach to simulating allocation via a framework of multiple complimentary feedbacks that alter source and sink strengths. It provides a tool for investigating and simulating the mechanisms responsible for allocation.

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly acknowledged.

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Publications

Included in this thesis is one paper currently under review for publication. I am the lead author and my supervisory team are co-authors.

Chapter 2: Holland, B. L., Monk, N., Clayton, R. H. and Osborne, C. P. (2019). A theoretical analysis of how plant growth is limited by carbon allocation strategies and respiration. Submitted to In Silico Plants.

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Chapter 1

General introduction

1 Improving crop yield

With the global population estimated to reach 9.8 billion by 2050 (United Nations, 2017), and the demand for crops exceeding the predicted yield (Beddington et al., 2011), it is essential to have measures in place to prevent world hunger. To meet such increasing global food demands, improving crop yield is considered to be vital (Fischer et al., 2009; Beddington et al., 2011). Recent advances in crop yield are slowing; for rice and wheat, the annual yield increase is just below 1%, and for maize, it is 1.6% (Fischer et al., 2009), therefore it is essential to understand and optimise plant growth to break through existing yield barriers. As atmospheric CO_2 continues to rise, and climate change intensifies, it has also become important to understand how crop yields might react to future CO_2 levels.

Crop production was initially maximised by increasing the use of arable land but as available land became limiting along with a greater focus on sustainability and increased urbanisation, yield became the focus. Cereals (wheat, rice, maize etc.) replaced less productive crops (Evans, 1997) and the use of irrigation and fertilisation was elevated. Modern crops have been selectively bred to have higher proportions of harvestable biomass in order to increase yield (Génard et al., 2008). Additionally, modern crops intercept 80–90% of all visible light through the optimisation of canopy development and architecture during growing seasons (Long et al., 2015). The remaining process that has not been bred to its maximum is the physiological conversion of intercepted light energy to crop biomass (Zhu et al., 2008). Out of all plant processes, photosynthesis is the best understood mechanistically, which aids the current research focus on the efficiency of photosynthesis in crops. However, increases in photosynthetic efficiency do not translate directly into biomass improvements, being limited by the carbon requirements of non-photosynthetic plant parts (e.g. roots and seeds) for growth and their maintenance (Long et al., 2006). For example, an analysis of seed production in soybean, maize and wheat showed that yield is more limited by storage than resource uptake (Borrás et al., 2004). Understanding how photosynthetic carbon gain is integrated into the more complex whole-plant processes leading to biomass production is therefore vital for achieving yield improvements.

2 What factors affect plant growth?

Plants are made up of distinct components with different functions (roots, leaves, stems, flowers, fruits and seeds). Leaves are responsible for the absorption and conversion of light and CO_2 into carbon compounds used for energy and tissue construction, whilst roots are responsible for the absorption of water and nutrients from the soil. Water is crucial for all chemical reactions to take place and to maintain tissue hydration (Larcher, 2003). Plant growth is determined by the balance between integral processes defining uptake, transport and use of resources such as water, light, CO_2 and nutrients. These processes include photosynthesis, water and nitrogen uptake via the roots, the transport of essential resources between plant components via the xylem and phloem, respiration, and the conversion of resources to plant material. Other external biotic factors also affect plant growth. These include: herbivory, competition from weeds or other plants, and disease.

Soil nutrients

Nutrients can greatly affect the growth of a plant. The primary nutrients required for growth are nitrogen, potassium and phosphorus. Nitrogen is an essential component of chlorophyll and all proteins, and nitrogen availability is therefore an important regulator of plant productivity. Typically, plant growth rates and nitrogen content are limited by soil nitrogen availability (Ingestad and Agren, 1991). Arguably this makes nitrogen a primary resource required for growth following CO_2, light and water. Another nutrient required for photosynthesis and also respiration is phosphorus, used in energy storage (ATP), biological membranes, DNA/RNA and protein regulation. It is therefore required in large quantities for fast growth. Potassium controls the opening and closing of stomata, which influences the fluxes of CO_2 and water within a plant (Larcher, 2003). High levels of potassium in the soil increase root growth (Drew, 1975). Additional nutrients such as calcium, magnesium and sulphur are also needed for growth and are responsible for cell wall composition, photosynthesis and amino acid synthesis respectively.

Relative growth rate, age and size

A plant's survival and reproduction depends upon its relative growth rate and size (Bigler and Bugmann, 2003; Shipley, 2006). Relative growth rate (RGR) is conceptualised as a size normalised measure of change of biomass over time. It is a useful measure of growth for comparisons among plant species since it is not scaled with size (Evans, 1972). Plant growth follows a sigmoidal curve, which can be described as two phases: the first is exponential growth, which is followed by a slower growth phase which reaches a plateau (Thornley, 1998). Annual plants reach this plateau based on a predetermined developmental pattern such that, after a distinct vegetative growth phase, the plant enters reproductive growth and invests all of its energy into filling seeds. For longer living plants (perennials), growth becomes limited by size (e.g trees). Tall trees are especially limited by the energy requirements needed to transport water to their furthest leaves (Gower et al., 1996; Murty et al., 1996; Hunt et al., 1999). As plant size increases, growth becomes limited by photosynthesis, such that average canopy photosynthesis declines with total plant size due to the overlapping of leaves, such that the absorption and use of solar irradiance approaches a limit (Pury and Farquhar, 1997). As leaves age, they become less efficient and senesce (Kikuzawa, 1995). Similarly, as individual roots age, the rate of nutrient uptake decreases (Waisel et al., 2002). Unlike photosynthesis, which isn't limited by a depletion of local CO_2, the rate of nitrogen uptake is limited by available nitrogen within the soil since, nutrients in the soil are less mobile and become depleted in rooting areas (Vitousek and Howarth, 1991; Craine et al., 2005).

Environment

Processes within the plant which are required for growth (respiration, photosynthesis, nutrient uptake) are sensitive to changes in environmental conditions. Due to their sessile nature, plants must be able to react to changes in their environment. When there is a lack of water, nitrogen, phosphorus or an increase in atmospheric CO_2, root growth is favoured over leaf growth (Priestley and Catlin, 1974; Bongarten and Teskey, 1987; Weinstein et al., 1991; Cannell, 1994; Maillard et al., 1999).

Conversely, plants invest energy into growth of leaves in areas of high light availability (Hutchings and de Kroon, 1994). The balance of resource uptake, consumption and release determined by processes defining growth is modulated by molecular regulation (Paul and Foyer, 2001) and the products of each process (i.e nitrate, sugars, and amino acids) act as signals for the expression of genes controlling various cellular processes. Changes in these processes lead to different growth strategies for above and below ground biomass, causing variation in the ratio of leaves to roots and tending to rebalance resource uptake, consumption and release. Long distance signals within the plant are mediated by hormones, whose production is influenced by changes in environment, and it is believed that hormones act like a switch for growth processes (Dewar et al., 1994). Key hormones involved in the regulation of growth include auxin, gibberelic acid, cytokinin and abscisic acid.

Allocation

Allocation of growth to individual plant compartments is a consequence of the supply, transport and use of resources (Thornley, 1998). The relationship between allocation of growth to above and below ground biomass can be described using sources and sinks. A source tissue is a net exporter of resources to other tissues in the plant. These resources have been acquired from the external environment or internal stores (e.g. carbon supply from the leaves, nitrogen supply from the roots, nutrient remobilisation like carbon from degraded starch stores) to the rest of the plant and a sink is a net importer of resources from internal sources (Doehlert, 1993). For example, leaves are a carbon source whilst roots are carbon sinks and conversely, roots are a nitrogen source whilst leaves are nitrogen sinks. The relationships between sources and sinks are complex due to numerous physiological feedback relationships within the plant (Génard et al., 2008). Information on these feedbacks are covered in more detail in Chapter 3. Optimising the relationship between sources and sinks can be beneficial for avoiding energy wastage arising from the costly nature of carbon and nitrogen uptake.

This thesis aims to develop novel approaches to modelling growth by focusing on the complex interactions linking source and sink behaviour. Firstly, the typical approaches to modelling growth are reviewed, followed by a comparison of how sources and sinks are usually considered in these models. This review identifies a particular gap in knowledge in the explicit consideration of signalling and crosstalk between sources and sinks.

3 Modelling plant growth

Whole-plant processes which affect growth are well defined in isolation (e.g. photosynthesis, Farquhar et al. (1980); respiration, Amthor (2000)), but there is a lack of quantitative knowledge about how these processes work together. This is where modelling plant growth can be beneficial. A set of equations can be used to represent some aspects of the way that the natural world behaves. Models by definition are representations of the real system and therefore they require assumptions and simplifications but they do not necessarily limit its usefulness. Modelling can provide a way of assessing how different mechanisms interact. This can be difficult to do by experiments alone because there is a lack of fine control over each process. Destructive measurements may be the only means of analysing certain processes, meaning that they can only be quantified at a discrete point in time. In contrast, modelling can provide a more detailed picture of how these processes behave over time. Modelling also requires less time and money than running a similar experiment in a lab, helps to refine the potential range of questions that are addressed with experiments, and allows more targeted hypothesis testing. It formalises quantitative predictions based on current understanding that can be tested via experimentation.

Below, I review the types of plant growth models there are, how they work and their uses, particularly focusing on those models which explicitly include source-sink relations. Interesting or useful techniques for modelling carbon allocation are identified and the limitations of such models are highlighted.

3.1 Top-Down Models

Types of models which start with total plant biomass and divide it into multiple components, are known as top-down models. Each component can represent a particular process or structural parameter which can limit growth. Top-down models can be used with experimental data to determine which processes vary among species, genotypes and treatments (Poorter et al., 2013).

Relative Growth Rate

The decomposition of RGR into its underlying constituents is an example of a top-down model. RGR represents the relative increase in biomass over time such that, if RGR is constant, plant mass follows an exponential curve against time. This can be shown as:

$$m_2 = m_1 e^{RGR(t_2 - t_1)}, \tag{1}$$

where m_2 and m_1 are plant masses at time t_2 and t_1 respectively (Blackman, 1919). Plant growth does not necessarily follow such a strict curve, for example, there are instances when RGR would decrease over night, but it represents a general trend of growth. Growth rate initially begins as an exponential curve and decreases in gradient until it reaches zero, therefore plant growth can be described as a sigmoidal curve (s-shaped) (Poorter et al., 2013).

The RGR can be decomposed among different defining mechanisms. Initially, RGR can be split into LAR (leaf area ratio) which represents the total leaf area per unit plant mass and ULR (unit leaf rate) which is defined as the increase in mass per leaf area (i.e growth rate normalised by leaf area). Again, LAR and ULR can be split further. LAR can be divided into the fraction of biomass allocated to the leaves (LMF) and SLA, which is the specific leaf area (the ratio of leaf area to leaf dry mass). Similarly, ULR can be fractioned into A which is the rate of assimilation per unit leaf area per day, CUE which signifies the carbon remaining after respiration and all maintenance processes have taken place, and C which represents the carbon concentration of new tissue. Again, A can be split into photosynthetic nitrogen use efficiency (PNUE) and leaf nitrogen concentration (LNC), while SLA can be split into leaf density and leaf thickness (Poorter et al., 2013).

The purpose of RGR models is to determine how the magnitude of variation in RGR scales with the variation in underlying processes. They provide insight into where the differences in the rate of growth originate when comparing species or genotypes. Unfortunately, this method does not provide a full picture of growth, and the analysis only focuses on carbon economy. It is well known that other factors should be included such as nitrogen or water. The scheme also assumes that the parameters are independent from each other, which is not often true. No-where in this scheme acknowledges that sink processes can control growth.

3.2 Bottom-Up Models

For bottom-up models, plant growth is simulated by combining the multiple physiological processes which define growth. Typically these include mechanisms at a certain level of integration to describe the behaviour of plant growth at a higher level. This is the opposite approach to top-down models. The problem with this approach is that combining component processes may fail to capture the emergent properties of the whole system through a lack of understanding of how it works.

Empirical Models

Empirical models or dose-response curves represent the type of models which are created by fitting equations against experimental data. They can give good predictions using a range of similar conditions to that used in the calibration experiment. Technically, these models do not contain any physiological mechanisms or detail, and they are often used to predict biomass. Environmental variables are required as input and growth rates or allocation fractions are typically produced using empirical models. Some examples of empirical models include BIOMASS and PnET. BIOMASS is used to investigate the influence of below ground resources on the growth and allocation patterns of tree stands (McMurtrie and Landsberg, 1992). PnET models carbon and water relationships of forests (Aber and Federer, 1992). Hirose (1986) presents an empirical model on growth and partitioning, showing that dry matter and nitrogen allocation have linear relationships with the concentration of nitrogen within the whole plant. These types of models do not explicitly model the relationship between sources and sinks.

Radiation Use Efficiency

Rather than modelling photosynthesis explicitly, radiation use efficiency (RUE) models make it possible to simulate plant growth as a function of intercepted light. They include a small level of mechanistic detail such that they calculate light interception by the plant. The following is an example of a radiation use efficiency model:

$$\frac{dm}{dt} = LUE(1 - e^{-kLAI})I, \tag{2}$$

where $\frac{dm}{dt}$ represents the change in plant mass over time, k is the coefficient for light extinction, LUE is the light use efficiency and I is the photosynthetic active radiation incident on the plant

(Poorter et al., 2013). These types of models use empirical conversion factors to represent the relationship between increase in mass and light interception. One model which includes an RUE component, is CASA by Potter et al. (1993). A benefit of using these types of models is that they are simple, in the case of CASA, it can be partially parameterised at large scales using remote sensing data. However, they lack any mechanistic detail to represent underlying processes, and do not include the energy requirements of sinks for maintenance and growth. These types of models can prove useful in a source-sink context if growth is assumed to be source limited. This enables the efficiency of carbon acquisition from the environment to be investigated. Any sink limitation is implied in the LUE term but there is no mechanism for implementing this.

3.3 Modelling Photosynthesis

Significant work on modelling photosynthesis within a leaf has been carried out by Farquhar et al. (1980). Their model simulates the specific factors affecting CO_2 assimilation such as the kinetic properties of rubisco, photosynthetic carbon reduction and carbon oxidation, and electron transport. Farquhar et al. (1980) state that the net rate of CO_2 assimilation is:

$$A = C - 0.5O - R_d, \tag{3}$$

where A is the assimilation rate, C is the rate of carboxylation, O is the rate of oxygenation and R_d represents non-photosynthetic respiration in the light (Farquhar et al., 1980). It also includes enzyme kinetics in the calculation of net CO_2 assimilation rate.

Another leaf-based expression of photosynthesis is derived by looking at stomatal conductance, activation of rubisco, the regeneration of ribulose-1, 5-biphosphate and the build-up of intermediates formed from photosynthesis (Kirschbaum et al., 1997). This derivation is formed on the theory formalised in the Farquhar et al. (1980) paper.

The work of Farquhar et al. (1980) can be seen in many papers on modelling photosynthesis and is widely accepted (Wullschleger, 1993; Maroco et al., 2002; Von Caemmerer, 2013). Most photosynthesis models are derived from the Farquhar et al. (1980) paper for an individual leaf and scale up to canopy level. There are two methods for creating canopy photosynthesis models. The canopy can be divided into multiple layers and for each layer, light interception and photosynthesis is considered (Mao et al., 2007). Alternatively, “big leaf" models can calculate integrated canopy values (De Pury and Farquhar, 1997). An example of such whole canopy photosynthesis models can be found in Werner et al. (2001). The purpose of this model is to investigate the effect of photo-inhibition on the assimilation of carbon.

3.4 Respiration models

Various processes within the plant require energy. This energy takes the form of ATP, synthesised via respiration. Much research delves into the theory that respiration can be described as a sum of the functional components (Amthor, 1986). Some of the functions which are considered to be respiratory processes (Amthor, 1986; Cannell and Thornley, 2000; Litton et al., 2007), include:

- Synthesis of new structural biomass;
- Translocation of photosynthate;
- Ion uptake;
- Nitrogen assimilation;
- Nitrate reduction;
- Sulphur assimilation;
- Protein synthesis and turnover;
- Ion gradient maintenance;
- Membrane repair.

One of the earliest respiration frameworks considered partitions the respiration processes (similar to the processes mentioned above) into above and below ground compartments (James, 1953; Lundegardh, 1960; Beevers, 1961). Additionally, some models separate respiration driven processes into two different compartments: growth and maintenance (Thornley, 1970; McCree et al., 1970). This is a logical compartmentalisation since some of these processes are responsible for synthesising new tissue and others are responsible for maintaining a functioning plant.

Amthor investigates plant growth by treating respiration as comprising of three components. These include respiration for maintenance, respiration for growth and waste respiration which occurs without providing any benefit to maintenance or growth (respiration related to ion transport)(Amthor, 1986). The idea of including waste respiration in a similar paradigm is discussed by Amthor (2000). This is proven to be difficult due to a lack of knowledge on quantifying waste.

Whether or not the cost of maintenance respiration differs within the leaves and roots is an important question in creating a function for respiration. Johnson (1983) suggests that the cost of respiration in the roots is higher than that in the leaves. This is due to nitrogen and anion assimilation being very expensive within the roots (Johnson, 1983).

Ryan (1991) also states that the importance of separating maintenance respiration and growth respiration into individual terms comes down to the cost of maintenance respiration being much more sensitive to environmental and internal changes than growth respiration, which depends on tissue composition (Ryan, 1991). This implies that a function consisting of separate terms for maintenance and growth respiration would be more accurate than a function which represents respiration as one combined term.

Ryan (1991) represents respiration using the following equation:

$$R = \left(\frac{1-Y_G}{Y_G}\right)\frac{dM}{dt} + m.M \tag{4}$$

where R is respiration, $\frac{dM}{dt}$ is the growth rate, m is the maintenance coefficient, Y_G is an efficiency coefficient and M is plant biomass (Ryan, 1991). The equation for respiration is represented as the sum of a growth term and a maintenance term. This clearly implies that respiration would follow a linear relationship with the mass of a plant. Equation (4) fixes the cost of respiration responsible for growth and enables the maintenance coefficient to vary with environmental conditions. This representation of respiration remains fairly simple. In contrast to the ideas mentioned above, Thornley and Cannell (2000) state that the common procedure of separating respiration components into maintenance and growth cannot be done as there is no distinct division between the biochemical processes required for growth and maintenance. Alternatively, Mori et al. (2010) use experimental data to investigate the relationship between the cost of respiration and amount of biomass of a plant. The graphs appear to show a positive linear correlation. This supports the theory involved in the work of Ryan (1991).

Respiration within this thesis is expressed as a rate of carbon used for the cellular processes associated with growth and maintenance per plant mass. From an energy balance perspective and when modelling carbon allocation, respiration is considered to be a cost to the plant, particularly maintenance respiration as carbon is used for cellular processes which do not contribute towards new tissue formation. However, respiration plays an essential role for the generation of carbon skeletons for the production of new plant material and for other cellular processes which generates energy for the plant such as nitrogen assimilation. When thinking about carbon, maintenance respiration can be seen as a net drain on energy and therefore the cost of respiration is equivalent to the rate of maintenance respiration throughout this thesis.

Functional Structural Plant Models

Functional-structural plant models (FSPM) (Vos et al., 2009) are models which combine plant architecture and physiology. These models have been heavily developed and are well defined. They consist of a structural compartment which looks at the development of the architecture of a plant and a mechanistic component which covers the more physiological aspects of plant growth.

One FSPM, in particular, investigates the branching and senescence of tillers in cereals using L-systems (Evers and Vos, 2013). Lindenmayer (1968) developed L-systems to describe branching architecture by creating strings using a set of rules. These rules are then implemented iteratively to create a complex structure. The architecture of a cereal consists of a tiller bud, an internode, a node, a sheath and a lamina (Evers and Vos, 2013). The FSPM uses the L-system rules consisting of sequences of these specific organs and simulates the 3D structure of a plant canopy (Evers and Vos, 2013).

The model created by Evers and Vos (2013) uses source-sink dynamics to investigate the effect of the environment on the emergence and senescence of tillers. In order to study these effects, methods of ray tracing are used in the model. These enable an accurate depiction of the amount of light hitting each tiller / leaf, and simulate the absorption of light by each leaf. Light directly affects the rate of photosynthesis which in turn affects the rate of growth. Evers and Vos (2013) also highlight the importance of environmental effects on tiller senescence.

Plant growth, in this modelling framework, can be affected by tiller production and senescence. The model is rule-based (Evers and Vos, 2013). For a new tiller to be formed, there must be enough carbohydrates available to support its growth. The required amount of available carbohydrate can be represented as a predetermined source-sink ratio. If the threshold source-sink ratio is not exceeded in the simulated plant, a tiller will not be produced.

The source-sink dynamics within this model are defined upon the assumption that a tiller, during its production, is a sink. Once the tiller has stopped growing, it is redefined as a source. It is assumed that all sources and sinks give and take resources from one central pool in order to keep the model simple. Evers and Vos (2013) also make some simplifying assumptions about there being no resistance to the transport of resources between the plants organs within the model. This means that there will be no metabolic cost of transport and all sinks will be equally likely to draw upon the resource pool.

The model described by Evers and Vos (2013) is a useful tool to investigate the effect of source-sink dynamics on a crop scale. It also has the ability to simulate plant growth using three different methods. Probability distributions are used to determine when a tiller will form or senesce. This is a descriptive approach which is problematic for cases when environmental conditions are not uniform across an area. Dose-response curves fix this problem as they relate the probability to form a tiller or to senesce with one or more environmental variables. Dose-response curves simply describe behaviour and are not able to explain it, therefore a third method is a mechanistic approach. This includes processes at one level of integration and produces information at a higher level, and involves branching responses to the red:far red light ratio, which varies in response to tiller crowding and shading.

Current limitations of the model presented by Evers and Vos (2013) include additional data requirements and costs when using the dose-response curves or mechanistic approaches in comparison to the probability distribution option. The model does not include the effects of water, which limits its application in water-limited situations. The model is also limited by its predetermined source-sink ratio for tillering as allocation is preset as opposed to a consequence of growth processes.

L-systems are also applied in the L-Peach and PEACH models (Grossman and DeJong, 1994; Allen et al., 2005). These models are developed from the work by Grossman and DeJong (1994), and are based around the hypothesis that the allocation of carbohydrate is determined by competition among organs' individual growth potential. Both L-Peach and PEACH simulate the growth of peach trees by including source and sink interactions. Allen et al. (2005) combine the framework of the PEACH model with L-systems to solve the system of equations, incorporating the effects of light, and updating the previous systems of equations used. L-Peach is used to investigate the effects of fruit thinning and water stresses on growth. A key theme in the Grossman and DeJong (1994) paper is that sink strength drives carbon allocation. This is a significant difference with the approach taken by Evers and Vos (2013).

GREENLAB is also a FSPM. Unlike the continuous model created by Evers and Vos (2013), GREENLAB is discrete, and simulates plant growth over finite time intervals. Continuous models are typically chosen to simulate the whole organism but lack detail, whereas simulating a collection of cells in detail is carried out with discrete models (Prusinkiewicz, 2004).

Plant architecture can be simulated using a stochastic automaton. This method is used in the GREENLAB model. An automaton is a simulator that contains specific rules or probabilities that a particular event will occur, in this case, the initiation of organ growth. It also contains a random number generator which creates probability distributions simulating multiple scenarios. These scenarios are then compared against the predetermined rules which decipher if the growth of a new organ is to be initiated or not. The aim is to develop an FSPM of organogenesis by taking a mathematical approach and a simple physiological basis (Yan et al., 2004). The model is based on one equation which incorporates the effects of multiple processes such as shading and the regulation of stomatal resistance. It does not have specific functions representing each mechanism.

Assumptions made to formulate the model include a non-linear relationship between the surface area of a leaf and the rate of assimilation (Yan et al., 2004). Functions are used to represent the sink strength of each type of organ at every ontogenetic step. Yan limits the morphology of leaves in the model such that they cannot vary in shape or length. Sink activity also must remain constant throughout growth. The organs are simply permitted to vary in size. A further assumption included by Yan et al. (2004) leads to biomass being dependent on surface area. The effects of processes such as shading, leaf age and nitrogen distribution are also present in the model.

Yan et al. (2004) uses the model to investigate the effect of pruning on the rate of growth. The level of plasticity within a plant with respect to sink competition for resources is also simulated using GREENLAB. An advantage of using stochastic processes to model plant growth with respect to its architecture is that it is easy to include experimental data.

The model is currently limited to a few similar structures, which are mainly trees. This implies that the model may not be as suitable for other plant species but the methodology remains relevant. GREENLAB is not entirely mechanistic in the sense that certain physiological processes are over simplified. This limits its ability to make precise predictions. The current version of the model also does not include water, which suggests that the model lacks enough detail to make precise predictions of growth under field conditions (Yan et al., 2004).

A Modular Plant

Unlike the FSPMs mentioned previously, the model created by Kaitaniemi and Honkanen (1996) does not take into account the location of a plant component with respect to other components. The structure of a plant is assumed to be modular and a plant is represented as a data structure tree. Such data structure trees consist of nodes and links (Kaitaniemi and Honkanen, 1996). The model is used to simulate the effect of carbon and nutrient translocation between plant components and determine their effects on the growth of new organs. Biotic factors such as herbivory can influence the rate of growth of a plant, and the effects of these factors on the model are considered (Kaitaniemi and Honkanen, 1996).

Kaitaniemi and Honkanen (1996) use the assumption that stronger sinks will receive a larger proportion of the resources available than weaker sinks. It is assumed that the translocation of carbon is linked to the translocation of nutrients, following a concentration gradient between sources and sinks.

The effect of source-sink activity on the rate of plant growth is assessed using this model but it does not include the influence of external factors such as light, nutrient limitation and water. This drawback to the model makes it less dynamic. The current model is primarily applied to trees and behaves in a largely qualitative manner. These sorts of models don't simulate the dependence of source and sink strengths on the availability of resources.

Goal Seeking Models

Goal seeking models use algorithms to represent optimising procedures or mechanisms involved in plant growth. These “goals" could be a ratio or a parameter value. Goal seeking algorithms are often required in circumstances where knowledge about a certain mechanism is limited or when computation is slow or expensive.

Teleonomic Models

Teleonomic models compare a range of computed parameter values against a specific target. These models allow the optimisation of plant functions, but can be limited since they are often simplified and could be rewritten in a mechanistic manner. Teleonomic models can be used to analyse different plant functions by assuming a particular balance between different types of growth (reproductive and vegetative) with functions such as: dispersal of seeds, ability to intercept light, availability for herbivory, mechanical support, and nutrient procurement (Thornley, 1995).

Thornley (1995) compares the outputs of a teleonomic model (Thornley and Johnson, 1990) against a transport resistance model for the allocation of carbon (C), nitrogen (N) and phosphorus (P). The first model (transport resistance model) contains eight state variables: shoot structure, shoot carbon, shoot nitrogen, shoot phosphorus, root structure, root carbon, root nitrogen and root phosphorus. The model contains functions for the assimilation of carbon via photosynthesis, root uptake of nitrogen and phosphorus and the transport of each resource. The teleonomic model contains five state variables: shoot structure, root structure, carbon, nitrogen and phosphorus. It assumes a single pool of resource for each substrate, therefore the movement of substrate is only involved in the growth of plant structure or the acquisition of such substrate. Thornley's approach takes a two-pronged teleonomic assumption. The first defines the goal which the system seeks and the second defines what method the system takes in order to reach this goal. The goal is defined such that the optimum specific growth rate for exponential growth is when the root and shoot fractions are:

$$f_{sh}^{*} = \frac{1 + \frac{f_c}{C_T}}{3 + \frac{f_C}{C_T} + \frac{f_N}{N_T} + \frac{f_P}{P_T}}, f_{rt}^{*} = \frac{2 + \frac{f_N}{N_T} + \frac{f_P}{P_T}}{3 + \frac{f_C}{C_T} + \frac{f_N}{N_T} + \frac{f_P}{P_T}}, \tag{5}$$

where f_C, f_N, f_P are the proportions of C, N, P in the structure and C_T, N_T, P_T are the concentrations of C, N and P in the plant.

Thornley (1995) concludes that teleonomic models may be of use when the system is in equilibrium but are not useful for dynamic behaviour. The teleonomic model is unable to directly represent some of the well-observed ontogenic effects on partitioning. In contrast, it is shown that this is not a problem for the transport resistance model. The teleonomic model could result in large errors and the optimising goal may be insufficient for determining allocation dynamics (Thornley, 1995).

The pipe model is another example of a teleonomic model. It is based on a functional relationship between vascular tissue and leaves (Shinozaki et al., 1964) and assumes that the structure of a tree can be comprised of a series of pipes where steady state growth occurs. Functional relationships are often used in teleonomic models. They make an assumption about the allocation strategy within the model which overlooks the underlying mechanistic detail. Much work has been done on the use of functional relationships (Charles-Edwards, 1976).

Evolutionary Algorithms & Game Theory

Evolutionary algorithms can be described as optimisation procedures. They are goal seeking algorithms which aim to find the optimum value for a particular process or mechanism. Zhu et al. (2007) use evolutionary algorithms to investigate a series of components involved in photosynthesis. They demonstrate the potential of incorporating metabolic pathway models with evolutionary algorithms to identify groups of changes most likely to increase productivity. This work concludes that resource partitioning between proteins is suboptimal in terms of maximising productivity. Game theory can also be used for goal seeking purposes. These models differ by using the inclusion of other plants to influence the evolution of the growth rate or strategies within a single plant. Light competition is a clear example which is present within a community of plants (Weiner, 1990).

Transport Models

Early ideas about modelling the allocation of carbon to sources and sinks appear in the work of Thornley (1971). Thornley assumes that all leaves sequester carbon into a single pool of carbon substrates. His model is formulated using the idea that these substrates are translocated between the leaves, shoots and roots of a plant. Therefore the movement of substrates is modelled between each of the three compartments.

The model is based on two main processes: 1. The passage of photosynthate between plant compartments and its dependence on the substrate concentrations in each compartment; 2. The consumption of substrate for growth and how substrate concentration affects this. The scheme makes the assumption that substrate moves between three main compartments of a plant: the leaf, the stem and the root, and that photosynthate either remains in the existing pool of resources or is used for growth. Processes of maintenance and wastage are not included.

The model simulates vegetative steady state growth. This is formulated by using Michaelis-Menten kinetics which leads to the following definition:

$$G_l = \frac{V_l k_l S_l}{K_l + S_l}, \tag{6}$$

where G_l is the rate of substrate consumption for growth in the leaves, S_l is the amount of substrate in the leaves, V_l is the volume of leaf tissue, k_l is the rate constant and K_l is the Michaelis-Menten constant (Thornley, 1971). This equation ensures that the use of substrate is dependent upon substrate levels and allows for saturation of substrate consumption to be implemented.

This scheme becomes completely reliant on the quantity of substrate entering the system through the leaves and therefore simulates only source limitation. A preferable framework would incorporate interdependency between the two compartments, for example, the roots acquiring nitrogen or water. This would enable the model to respond to environmental changes in a more balanced way.

Carbon & Nitrogen Model

Thornley refines the theory presented in his previous work by creating a two-compartment model (Thornley, 1972). Instead of analysing growth by simulating the movement of carbon across three individual plant compartments, this second model simulates the movement of carbon and nitrogen between the roots and the shoots of a plant. The “shoot" refers to parts of the plant which can acquire carbon via photosynthesis and is divided into structure, carbon and nitrogen components, and the root is divided similarly. Figure 1 represents the movement of resources between each compartment (Thornley, 1998). This model is very similar to the previous allocation model and makes the same assumptions as before. In this new model, carbon is acquired in the shoot, where it can then be consumed in the shoot or transported and consumed in the roots. Similarly, nitrogen is acquired in the roots and can be consumed in the roots or transported and consumed in the shoot.

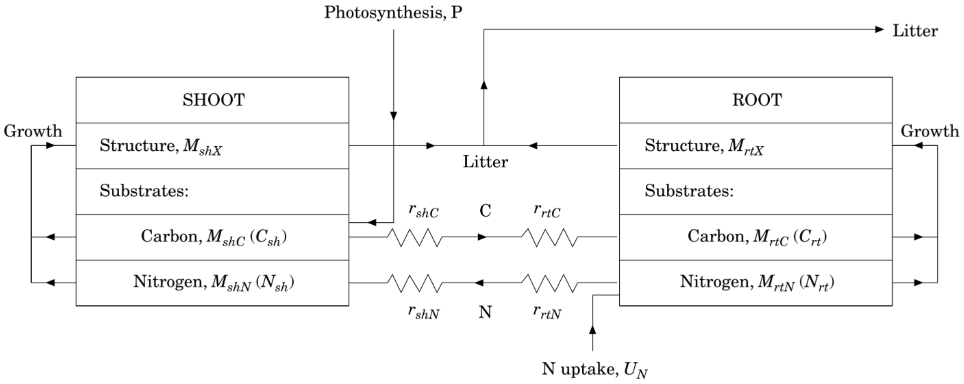


Fig. 1 Diagramatic representation of the transport of carbon and nitrogen between the roots and shoots, including uptake, transport, growth and litter processes (Thornley, 1998).

The model is based on six ordinary differential equations (ODEs). The first four ODEs are used to simulate the change in carbon or nitrogen in each compartment respectively (shoot carbon, shoot nitrogen, root carbon, root nitrogen) and the final two equations are used to simulate the conversion of substrate to new plant tissue.

Thornley asserts that all allocation models should be created using the transport framework. His model has been widely applied in a range of contexts, including: tobacco growth (Wann and Raper Jr, 1984), forest and ecosystem modelling (Rastetter and Shaver, 1992), water transport (Dewar, 1993) and can effectively represent various source-sink interactions (Minchin et al., 1993). Further extensions to the Thornley (1972) model investigate how carbon uptake is limited by factors such as temperature, soil moisture, solar radiation and shoot nitrogen (Buitenwerf and Higgins, 2016). All instances of the model's applications have been successful. Dewar et al. (1994) agree with Thornley that the transport model is an optimal method of controlling carbon allocation.

The transport mechanism within the Thornley (1972) model is assumed to follow the Münch pressure flow model for phloem transport (Münch, 1930). This states that the translocation of solute is induced by an osmotic pressure gradient. The pressure gradient is created by the loading and unloading of sugars from source to phloem sieve elements and from sieve elements to sink respectively (Taiz and Zeiger, 1998). The Münch transport mechanism has been validated mathematically (Henton et al., 2002).

Another transport model is a simple model of phloem transport created by Minchin et al. (1993). Minchin et al. extend the work of Thornley and Johnson (1990), which simulated the transport between a source and a sink by including an additional sink. This is to investigate the dynamics of sink priority. The model is made simpler than some physiologists would like but it still simulates observed source-sink behaviour. The later work of Thornley strengthens the idea presented in Minchin et al. (1993) that the use of minimal detail to simulate phloem transport is sufficient to represent observed behaviour in whole plants (Thornley, 1995). Further work is seen in the SWT model which creates a more detailed version of the Münch pressure flow theory by including water transport (Dewar et al., 1994).

A Framework Model

An alternative method of modelling plant growth is attempted by Chew et al. (2014). A key area of research in modelling plant growth is determining how metabolic processes and regulatory networks affect the rate of growth on a whole plant scale. Chew et al. (2014) investigate the effects of internal and external regulators on growth, using similar approaches to those presented in Evers and Vos (2013). Chew et al. (2014) investigate plant growth by combining four individual plant models, which characterise different aspects of functioning. The purpose of this is to combine models originating from different research domains to provide a mechanistic level of detail at a whole plant or organ scale, and is focused on the model plant *Arabidopsis*. This differs from the model by Evers and Vos (2013) which looks at crops.

Details relating to the allocation of carbon are included in the first part of the framework model (Chew et al., 2014), which simulates processes within a cell and includes photosynthesis and sugar partitioning. Despite mechanistic detail in photosynthesis, its treatment of sinks is relatively crude. This part of the model works on the assumption that 12.5% of the carbon assimilated from photosynthesis is converted into starch, and also assumes that 84% of this starch is broken down overnight based on experimental data (Gibon et al., 2004, 2009).

The second section of the framework model consists of a functional structural element. Similarly to the other FSPMs mentioned previously, this model simulates the growth of individual plant organs and analyses how each organ affects the overall structure of the plant for light interception. The processes included in this part of the model are goal seeking algorithms, which are represented as mathematical functions which simulate the behaviour but do not contain all the mechanistic details underpinning them.

The development part of the framework model is used to predict the time taken to flower using thermal time. It includes the effects of processes such as the photoperiod-sensing pathway, the vernalisation pathway and developmental responses to warm ambient temperature (Chew et al., 2014). This part of the model uses goal seeking algorithms.

Finally, the photoperiodism compartment is a gene dynamic model of the circadian clock. This is represented as an ODE.

The model attempts to cover the processes involved in each model component but does not contain a high level of mechanistic detail within each. The creation of the framework model is laborious in terms of converting units and combining models together. The high level of parameterisation enables the application of the model to *Arabidopsis*, which is well known experimentally, but would be more difficult to apply in other systems.

3.5 Source-Sink Models

Models simulating plant growth are inconsistent with each other regarding source-sink assumptions and this is evident from looking at the variation among assumptions used in such models. This points to a lack of knowledge with respect to the internal mechanisms responsible for partitioning. Some source-sink assumptions which are typically used in plant growth models are presented below:

1. Allocation is dependent upon respective sink strengths. Thus the strongest sinks are prioritised over weaker sinks (Ford and Keister, 1990; Luxmoore, 1991);
2. Allocation is directed to the areas in which rectify a deficiency. For example, if there is a deficiency of water or nutrients, carbon would be allocated towards the roots (Ewel and Gholz, 1991) and if there is a reduction in carbon fixation, carbon would be allocated towards the leaves (Hogsett et al., 1985);
3. The plant maintains a functional balance between the acquisition of carbon from the leaves and the uptake of nitrogen and other resources from the roots. For example, the allocation to the roots is directly proportional to the allocation to the leaves, which can be defined by an allocation coefficient (Davidson, 1969) ;
4. Allocation is based on a first-come, first-served scheme. This implies that the sinks in close proximity to the source will be provisioned first and the furthest sinks will be the last to receive carbon. This is also applied to the roots with nitrogen and water such that root sinks will receive resources first (Weinstein et al., 1991);
5. Allocation is dependent upon a particular goal such as optimising the total growth rate of a plant or the net carbon increase (McMurtrie, 1985);
6. Allocation is split equally between all sinks.
7. Allocation is dependent upon transport resistances, where further sinks with greater resistance have the lowest priority.

The second and third allocation strategies mentioned above are teleonomic and are stunted in their ability to represent the multiple processes involved in allocation.

Clearly much more work can be done on analysing the effect of source-sink activity on growth. Table 1 shows a comparison of features within some of the models described. All models within the table follow assumption 1, which is driven by sink strength. Most of the work mentioned above uses assumptions which determine how allocation is prioritised to different sinks. However, given that there are known feedbacks on source and sink activity implies that allocation does not always prioritise sink growth. The effect of varying the source-sink allocation ratio on plant growth is a theme which isn't explicitly visited in the work of these models, nor how growth processes such as photosynthesis or respiration constrain the allocation of growth to above and below ground biomass.

Model	Mechanistic	Structure	Environment	Water / N	Transport	Disadvantages	Advantages
Evers and Vos (2013)	Yes	Yes	Yes	No	No	Rule based, laborious data requirements	Crop scale
L-Peach	No	Yes	Yes	Yes	Yes	Rule based, partly empirical	Includes roots
GREENLAB	Yes	No	Yes	Yes	Yes	Lack of structure	Includes nitrogen
Chew et al. (2014)	Not fully	Yes	Yes	No	No	Complex, many variables	Detailed
Kaitaniemi and Honkanen (1996)	No	Yes	No	No	No	Focuses on herbivory	Allocation is based on light and nutrients

Table 1 A comparison of previous models simulating sources and sinks. It compares the models against: type of source-sink assumption used; if it contains mechanistic details; if plant structure is modelled; whether environmental factors are considered; if there is any inclusion of water or nitrogen allocation in the model and the inclusion of transport between sources and sinks.

Much work has been done on the modelling of source-sink allocation of carbon but an interesting extension to this framework is the inclusion of nitrogen allocation and root dynamics. This is important, since nitrogen is an essential element used in photosynthesis. Key resources needed for growth such as nutrients and water, are acquired from the roots. Thus a detailed model including the behaviour of the roots creates a much more accurate representation of growth. The resources obtained via the roots influence the allocation of carbon to sources and sinks and also to overall plant growth (Running and Gower, 1991). Such issues are presented in the Thornley papers which suggest that there should be crosstalk between root and shoot models. It is not sufficient for the roots to be completely reliant on the resources transported from the leaves (Thornley, 1971). Many plant growth models simulate the allocation of carbon and nitrogen but they do not necessarily address the dependencies of each resource on their uptake and use. Details of these models are discussed in the proceeding chapters.

The only interaction allowed between sources and sinks in most of the models here is the flow of materials, implying that a sink either receives the materials it needs to grow or it doesn't. Source and sink capacity are also fixed and their strengths depend respectively on the external or internal availability of resources and their strengths are not dependent upon the levels of carbon and nitrogen within plant compartments. None of these models involve crosstalk between sources and sinks, which feeds back on their respective strengths to balance resources. In addition, there are no further consequences of source-sink imbalances brought about by crosstalk between carbon and nitrogen signalling. Therefore, to be able to improve growth through the optimisation of resource allocation, the mechanisms responsible need to be investigated. This is where modelling provides a useful tool to better understand resource partitioning, since it is difficult to measure the levels of carbon and nitrogen within leaves and roots non-destructively when imposing several resource conditions.

4 Overview of thesis

This thesis investigates how the coordination of sources and sinks can be used to increase growth and attempts to gain a better understanding of how the allocation of growth to sources and sinks arises through a combination of internal responses to carbon and nitrogen within the plant. This is done by simulating growth at the whole plant scale whilst incorporating the overall behaviour of the intercellular processes which define growth. Throughout, the general approach is to keep plant growth as simple as possible. Mostly, a non-dimensional approach is used to explore the sensitivity of plant growth and its processes to changes in carbon and nitrogen in a broader sense than what might otherwise be heavily constrained by parameterisation.

Chapter 2 addresses the limits to possible allocation strategies imposed by carbon balances, specifically looking at the direct effect of changing carbon allocation strategies between above and below ground tissue on total plant mass. The extent to which allocation between carbon sources and sinks affect overall plant size during vegetative growth is investigated whilst the energy requirements of processes such as photosynthesis and respiration are varied. Additionally, the chapter investigates how vegetative allocation strategies and flowering time alter reproductive output (i.e. seed yield in the case of crops). This study finds that maintenance respiration has a big effect on total plant mass and enables a certain level of plasticity in the allocation of growth between leaves and roots. Altering the energy requirements for maintaining source and sink tissue can lead to situations where increasing the allocation of carbon towards root growth (which are considered only as carbon sinks in this framework) can increase total plant size. This work argues that maintenance respiration should be considered in crop improvement strategies.

Chapter 3 refines the constraints on allocation imposed solely by carbon balance by looking at the relationship between sources and sinks of carbon and nitrogen. This work focuses on simulating an environmentally responsive plant by developing a framework of feedback mechanisms on growth when intermediate carbon and nitrogen concentrations fluctuate. This framework enables feedbacks between sources and sinks to balance their respective strengths with resource availability as the plant grows. The balanced framework is developed by selecting a set of behaviours which have been observed experimentally and implementing them within a simple model of carbon and nitrogen. How the feedbacks should be simulated is investigated and their combined effect on plant growth is determined by simulating three environmental scenarios (equal carbon and nitrogen availability, reduced carbon availability and reduced nitrogen availability). This work showed that this framework of feedbacks allowed the model to allocate growth towards plant compartments where there is a deficiency in resource. It also showed that six feedbacks operating simultaneously can lead to stable behaviour whereby the plant responds to internal imbalances among resources to reach a steady state.

Chapter 4 builds on from the work in the previous chapter by testing the framework of internal feedback mechanisms under two source-sink manipulation experiments with a parameterised version of the model. This is done by implementing the framework from Chapter 3 within a model previously validated by experimental data (Thornley, 1972) whilst varying atmospheric CO_2 and soil nitrogen availability and imposing a pruning event on above ground biomass. This work shows that the model is able to reflect in qualitative terms most of the observed behaviours in experiments manipulating source-sink strengths. Consequently, this presents a useful tool which can be used to continue to investigate the dynamics of the feedback mechanisms responsible for biomass partitioning.

Chapter 5 brings together the findings from each chapter and identifies further research questions that arise from this work.

Chapter 2

A theoretical analysis of how plant growth is limited by carbon allocation strategies and respiration

Abstract

Improving crop yield is essential to meet increasing global food demands. Boosting crop yield requires the coordination of carbon acquisition by leaves and carbon utilisation by roots and seeds. Simple modelling approaches may be used to explain how this coordination is achieved within plant growth. Here, the limits to allocation strategies and the influence of maintenance costs are explored by analysing the sensitivity of a simple root-shoot carbon allocation model for vegetative and reproductive growth. The model is formulated based on fundamental constraints on plant growth and therefore can be applied to all plants. This general but quantitative approach shows that the relative costs of root and leaf respiration alter the relationship between carbon allocation and final plant size, enabling a range of allocation strategies to produce a similar total amount of plant material during vegetative growth. This plasticity is enhanced by increasing assimilation rate within the model. Results show that high leaf allocation during vegetative growth promotes early reproduction with respect to yield. Having higher respiration in leaves than roots delays the optimal age to reproduce for plants with high leaf allocation during vegetative growth and increases the restrictions on flowering time for plants with high root allocation during vegetative growth. It is shown that, when leaf respiration is higher than root respiration, reallocating carbon toward the roots can increase the total amount of plant material. This analysis indicates that crop improvement strategies should consider the effects of maintenance costs on growth, a previously under-appreciated mechanism for yield enhancement.

1 Introduction

Improving crop yield is considered vital for meeting increasing global food demands (Fischer et al., 2009; Beddington et al., 2011), and new approaches are needed to break through existing yield barriers. Modern crops have been selectively bred to have increased proportions of biomass in harvestable material in order to maximise yield (Génard et al., 2008). Additionally, light interception has been maximised to allow 80–90% of all visible light by a crop over a growing season (Long et al., 2015). Out of all plant processes, photosynthesis is the best understood at the mechanistic level, which facilitates the current focus on investigating the efficiency of photosynthesis in crop research. However, the benefits gained from such increases in photosynthetic rates are not only limited by nutrients, temperature and water, but also the energy requirements for growth of non-photosynthetic plant parts (e.g. roots and seeds) and their maintenance.

Crop yields depend on the accumulation of biomass via growth and its allocation (partitioning of resources) to harvestable plant parts. Environmental trade-offs and metabolic costs can impact the allocation of carbon between leaves and roots. For example, a lack of water or nutrients within the soil can lead to a plant allocating more carbon into roots (Bongarten and Teskey, 1987; White et al., 2016). Conversely, if there is a lack of light or a loss of leaves, more carbon will be partitioned towards the leaves (Kozlowski, 1949; Barney, 1951; Nelson, 1964; Murthy, 1990). A plant's size can impose limitations on carbon acquisition when considering light-limitation. Growing surplus leaves per unit ground area (increasing leaf area index) leads to self-shading and reduces potential light absorption by the lower leaf layers. Self-shading can be partially overcome by optimising leaf angle to increase the level of light interception on the lower canopy levels (Long et al., 2006; Burgess et al., 2015). Additionally, a large plant requires more energy for respiration and tissue turnover than a small plant. For example, in the case of forest stands, a decline in net primary productivity with stand age and size is thought to arise from high levels of respiration compared to photosynthesis, restrictions on water transport, and nutrient limitations on photosynthesis (Hunt et al., 1999).

The carbon costs of maintenance respiration may differ between leaves and roots. Johnson (1983) suggests from a theoretical analysis that the cost of respiration in the roots is higher than that in the leaves, which is a consequence of nitrogen and anion uptake and assimilation being very expensive within the roots. Conversely, Tjoelker et al. (2005) show empirically that leaf respiration varies between 1 and 2.25 times higher per unit mass than root respiration for 39 grassland and savannah species. These examples show that the cost of maintaining a leaf may not always be equal to that required to maintain a root. Amthor et al. (2019) argue that respiration should be a focus in optimising crop productivity. Knowing that there are species where respiration in the leaves is more than twice the cost of respiration in the roots (Tjoelker et al., 2005), it becomes important to investigate the limitations on carbon allocation with different ratios of leaf and root maintenance.

Whole plant processes which affect plant growth are well defined in isolation (e.g. photosynthesis (Farquhar et al., 1980) and respiration (Amthor, 1986)), but there is a lack of knowledge about how such processes defining growth work together to determine the allocation of growth to above and below ground biomass. Here, modelling plant growth can be beneficial. Modelling can provide a way of assessing how different mechanisms interact, which can be limited by experiments alone due to a lack of fine control over each process. It also helps to refine the potential range of questions that are addressed with experiments, and allows more targeted hypothesis-testing. In particular, it formalises quantitative predictions based on current understanding that can be tested via experimentation. A modelling approach is therefore adopted for the specific research questions in this chapter.

It remains unclear what internal mechanisms are responsible for biomass partitioning, since plant growth models use a variety of different assumptions for allocation (Ewel and Gholz, 1991; Luxmoore, 1991; Weinstein et al., 1991; Dewar et al., 1994). Some more recent models assume allocation based on a functional balance of resources to leaves and roots and is calibrated to maximise plant relative growth rate (Zerihun et al., 2000; Buckley and Roberts, 2006; Feller et al., 2015). Most carbon allocation or plant growth models investigate the effects of environmental conditions, herbivory, senescence and/or pruning on plant growth (Hogsett et al., 1985; Ford and Keister, 1990; Luxmoore, 1991; Weinstein et al., 1991), and ignore the effects of different potential allocation strategies between leaves and roots on growth and how cellular processes such as photosynthesis and respiration alter this. However, any strategy for increasing productivity must balance the allocation of carbon to the growth of source and sink tissues. Here, a carbon source is defined as a net exporter of carbon to the rest of the plant, where carbon is acquired from the environment (i.e. mature leaves via photosynthesis), while sinks are net importers of carbon from internal sources (Doehlert, 1993), and include young leaves, seeds and roots.

Understanding the developmental stage at which a plant should reproduce can bring insights into optimal strategies that a plant should adopt when faced with environmental or biotic hazards and has a limited amount of time to reproduce before it is killed by an external factor. Equally, when considering crop production in a seasonal climate, development must be completed during a growing season of limited duration. Many have investigated the effects of flowering time on yield (Cohen, 1971; King and Roughgarden, 1982; Guilbaud et al., 2015) and specifically focused on environmental heterogeneity (Paltridge and Denholm, 1974; Ledder et al., 2004), multiple reproductive phases (Chiariello and Roughgarden, 1984) and photosynthetic rates (Schaffer et al., 1982). Yet, the effects on yield when varying allocation between leaves and roots during vegetative growth were not addressed.

This chapter explores the following questions: 1. How does varying the allocation of carbon for growth between leaves and roots alter total plant biomass during vegetative growth? 2. How do the processes of respiration and photosynthesis constrain possible strategies of allocation between leaves and roots? 3. Does increasing allocation towards the roots always lead to a decrease in overall plant size? 4. How does varying allocation between leaves and roots during vegetative growth and flowering time alter reproductive output (which is equivalent to seed yield in the case of crops)? These are addressed by analysing the sensitivity of a simple carbon allocation model for vegetative and reproductive growth. All values within this chapter are dimensionless in order to look at the general behaviour of the model.

2 Model description

The model considers only carbon sources and sinks, with no soil or other environmental interactions. Sink or source strength can be defined as the combination of source or sink size and activity, which relates to the uptake or export rate of a particular resource. By combining two alternative, but complementary, perspectives, the model simultaneously accounts for net carbon export rate from the leaves (source strength), internal carbon allocation to leaves for growth (growth of source size) and roots or seeds for growth (growth of sink size), and the development of leaves, roots and seeds (White et al., 2016).

The *physiological perspective* defines growth by the acquisition and loss of carbon via photosynthesis and respiration respectively (Lambers et al., 1990). This expresses carbon gain as the difference between carbon acquired via photosynthesis (source strength) and the loss of carbon through respiration:

$$\text{Net carbon gain} = Al - R_1 l - R_2 r, \tag{1}$$

where Al represents the rate of carbon assimilation (A is assimilation rate per unit leaf tissue, and l is the amount of leaf tissue) and $R_1 l + R_2 r$ is the rate of use of carbon for maintaining plant material (R_1 and R_2 are leaf and root respiration, r is the amount of root tissue (dimensionless)). This formulation accounts for the fact that not all carbon assimilated is used for the growth of new plant tissue; some is used for the maintenance of existing tissue and other metabolic processes.

The second perspective is the *development* of source and sink tissues. Here, plants are conceptualised as modular structures, where a module is one of the repeating units from which a plant is constructed (e.g. leaves, roots or cells) and growth is assumed to be a function of module initiation rates (the rate at which each module is constructed). Growth in this case is defined as the development of individual leaves and roots (Pritchard et al., 1999), which can be expressed as the combination of organ initiation rates:

$$Growth = \mu_1 M_l + \mu_2 M_r, \tag{2}$$

where μ_1 and μ_2 are the module initiation rates of leaves and roots and M_l and M_r are the sizes of leaf and root modules, respectively. Individually, these perspectives bring limited insights. The physiological approach is resource driven and the developmental perspective is driven by the carbon requirements for the growth of new organs. By combining these perspectives, the model simulates plant growth based on fundamental constraints (White et al., 2016), allowing the exploration of how physiological rates, allocation and developmental rates interact to control growth.

2.1 Assumptions

Simplifying assumptions mean that the model is best interpreted as a simulation of monocarpic species growing in warm, fertile conditions, with strong competition for light within a closed leaf canopy. This is closest to the situation for annual crops. Although the developmental pattern of these plants means that the limits simulated by the model are never reached in nature, these limits set boundaries that development cannot exceed. Roots are modelled as carbon sinks, which account for all non photosynthetic plant parts. The model does not simulate soil nutrient limitations or capture other environmental limitations such as temperature or drought. This allows the effects of carbon allocation on growth to be directly analysed.

Photosynthesis

The rate of carbon assimilation by photosynthesis is assumed to be the sole mechanism of carbon acquisition and is modelled as the average canopy gross photosynthetic rate, which is a decreasing function of total leaf area. As leaf area index (LAI) increases, the average unit rate of photosynthesis (per unit of leaf area) decreases due to self-shading (Pury and Farquhar, 1997). Assimilation rate is chosen to be:

$$A(l) = \frac{\theta A_0}{\theta + l}, \tag{3}$$

where θ is the shading coefficient, A_0 is the unit rate of photosynthesis for an unshaded leaf and l is the amount of leaf tissue. When $l = \theta$, the rate of photosynthesis per unit leaf area is half of its maximum (unshaded) value. Increasing the shading coefficient (θ) reduces the effect of shading on assimilation rate.

Determinate growth

Acquired carbon can either be allocated for growth and its associated cost (growth respiration) or used for maintenance respiration, where the cost of maintenance is linearly dependent upon the size of the plant (Ryan, 1991). The carbon allocated towards growth encompasses the cost of growth respiration since this is a dimensionless system. A necessary condition for growth is that assimilation rate must always be greater than respiration rate. This provides a natural constraint on appropriate parameter values. In the model, plants grow until canopy photosynthesis is exactly counterbalanced by total plant respiration, which sets a final plant size. Once this size is reached, no further growth occurs. Although a maximum size set by source-sink balance has been hypothesised for forest trees (Hunt et al., 1999; Day et al., 2001), this idea has been superseded by hypotheses relating to water and nutrient limitation of growth in tall trees and reductions in photosynthetic efficiency (Gower et al., 1996; Murty et al., 1996). However, despite being inappropriate for trees, this approach may be suitable for plants which do not grow indefinitely (determinate growth) such as monocarpic, herbaceous species, including annual crops, where plants reproduce once before dying and can be assumed to maximise reproductive output (i.e. seeds) using available resources. For many crops the harvestable plant parts are the roots, therefore within this framework seeds are used to describe any reproductive organ which stores carbon.

For annual crops the cessation of vegetative growth is brought about through a predetermined developmental pattern. However, a useful approximation for this is when resource uptake and use become balanced (due to self-shading) given that there is no senescence of leaves or roots in the model. It can be argued that the decline in plant relative growth rates is caused by plants becoming less efficient as they grow due to self-shading and tissue ageing (Evans, 1972; Rees et al., 2010), while others suggest it is due to increased allocation of growth towards non-photosynthetic plant compartments (roots and stems) and reductions in soil nutrients (Paine et al., 2012; Philipson et al., 2012). Simulating steady-state plant growth is commonly used for plant growth models (Thornley, 1972; Charles-Edwards, 1976; Reynolds and Thornley, 1982; Hirose, 1986; Yin and Schapendonk, 2004) and it can be argued that they can be applied to non-steady state conditions (Hirose, 1986; Ågren and Ingestad, 1987; Hirose et al., 1988; Van Der Werf et al., 1993).

Reproductive growth

Roots are the only sink organ modelled during vegetative development, and roots and leaves both stop growing after a plant transitions from vegetative to reproductive growth. During reproductive development, the only sinks are seed growth and the respiration required to maintain existing roots and leaves. This transition to reproductive growth is imposed at numerous stages of a plants life. These assumptions approximately replicate what happens during the development of annual crops such as wheat and rice (Atwell et al., 1999).

Senescence

There is no turnover or senescence of plant tissues in the model, and source strength is maintained during reproductive development. This is a simplification that ignores the decline in source activity that occurs in annual crops during seed growth, which is linked to the remobilisation of nitrogen from leaves to seeds (Masclaux et al., 2001).

Resource allocation

The balance of carbon allocation between sources and sinks is assumed to be fixed such that the model simulates one allocation strategy throughout determinate growth. This ensures easy analytical comparisons between allocation strategies, but ignores ontogenic drift in allocation. Allocation strategy is dependent upon module initiation rates of leaves and roots, where the root module initiation rate is a fixed proportion of leaf initiation rate:

$$\mu_2 = \alpha\mu_1, \tag{4}$$

where α is the allocation coefficient. For example, when $\alpha = 2$, twice as much carbon is allocated to roots than leaves and when $\alpha = 1/2$, twice as much carbon is allocated to leaves than roots.

3 Model behaviour

3.1 Vegetative growth

Leaf and root growth can be individually expressed as:

$$\frac{dl}{dt} = \mu_1 M_l, \tag{5}$$

and

$$\frac{dr}{dt} = \mu_2 M_r, \tag{6}$$

where M_l and M_r are leaf and root module size respectively. Using Eq (4), root and leaf growth are related by

$$\frac{dr}{dt} = m\alpha\frac{dl}{dt}, \tag{7}$$

where $m = M_r/M_l$.

Therefore, growth trajectories are given by

$$\frac{dr}{dl} = \frac{dr/dt}{dl/dt} = m\alpha \tag{8}$$

As a consequence of assuming a constant allocation strategy, the growth trajectories are straight lines with gradient $m\alpha$. Each growth trajectory represents the duration of plant growth from a seedling to the point, in which plant material reaches steady state as a function of leaf and root tissue and varies dependent upon initial seedling size.

The physiological perspective (Eq (1)) defines growth rate as the difference between the rates of photosynthesis and respiration. Carbon assimilated by the plant via photosynthesis, is distributed between maintenance respiration and growth (including growth respiration):

$$A(l)l = R_1 l + R_2 r + \frac{dl}{dt} + \frac{dr}{dt}, \tag{9}$$

Substituting from Eq (7):

$$A(l)l = R_1 l + R_2 r + [1 + m\alpha]\frac{dl}{dt}, \tag{10}$$

and therefore

$$\frac{dl}{dt} = \frac{1}{1+m\alpha}\big(A(l)l - R_1 l - R_2 r\big) \tag{11}$$

Using Eq (7) again gives

$$\frac{dr}{dt} = \frac{m\alpha}{1+m\alpha}\big(A(l)l - R_1 l - R_2 r\big) \tag{12}$$

The model is given by Eqs (11) & (12). Steady states of the model satisfy the equation

$$r = \frac{A(l)l - R_1 l}{R_2}, \tag{13}$$

which corresponds to a continuous curve in the (l, r) phase space. $A(l)$, which represents the effect of self-shading on photosynthesis, is a decreasing function of l. Using the functional form given in Eq (3), the general form of the steady state curve is illustrated Fig. 1. The curve has a peak at $l = \theta(\sqrt{A_0/R_1} - 1)$, $r = \frac{\theta R_1}{R_2}\left[\sqrt{A_0/R_1} - 1\right]^2$ and maximum leaf tissue is determined by $l = \theta(A_0 - R_1)/R_1$. Therefore the qualitative shape of the blue curve is independent of R_2, which only determines the height of the curve. The location of the peak of the curve is determined by A_0/R_1, therefore, optimising the ratio between the unshaded rate of photosynthesis and leaf respiration increases final plant size within this framework, which is to be expected. The steady state curve (black line) begins with a steep increase, which is the ratio of root tissue to leaf tissue increasing because a much higher quantity of carbon is required for root respiration to balance out assimilation from the leaves. As leaf tissue increases, the level of self-shading increases. Once assimilation rate reaches its maximum, additional leaves are costly and less root tissue is required for respiration to balance the canopy assimilation rate. This explains the decline in the steady state curve. For the purposes of behaviour illustration, the following default parameters: $A_0 = 10$, $\theta = 10$, $m = 1$, $\alpha = 1$, $R_1 = 2$, $R_2 = 2$, with initial conditions of $l = 0.01$ and $r = 0$ will be used.

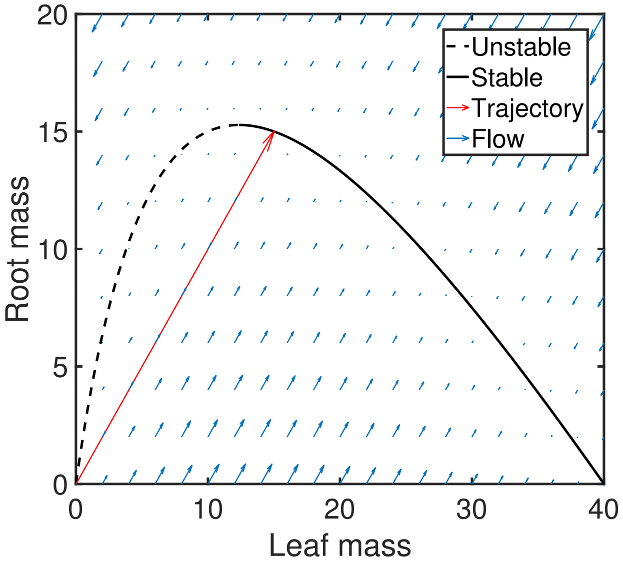


Fig. 1 The range of steady-state solutions (Eq (13)) (given by values of l and r for which the ratio of respiration and photosynthesis are equal) (black line) with a plot of the vector field $(\frac{dl}{dt}, \frac{dr}{dt})$ showing the local direction of growth trajectories. An example trajectory is also shown(orange line). Using $A_0 = 10$, $\theta = 10$, $R_1 = R_2 = 2$, $\alpha m = 1$ with an initial seedling size of $l = 0.01, r = 0$. Assumes vegetative growth only (no reproduction). All model parameters are dimensionless

The orange line (Fig. 1) is the vegetative growth trajectory which represents the growth of a plant from seedling to a steady state at which the rates of photosynthesis and respiration are equal. The growth trajectories are straight lines in the l, r plane with gradient αm. Allocation is a combination of balance between allocation and module size. The straight line signifies a constant ratio of root to leaf tissue allocation as the plant grows. The root to leaf ratio at steady state depends on αm, initial leaf and root tissue, and the black steady state curve. Figure 1 also shows the vector field $(\frac{dl}{dt}, \frac{dr}{dt})$. This illustrates the fact that only the portion of the steady state curve with negative gradient (solid line) corresponds to steady states that are stable. Biologically, this represents plants in which the roots account for the majority of total plant material with very few leaves. These situations are unrealistic given the assumption that roots are only considered as carbon sinks. The maximum stable allocation strategy is defined as:

$$\alpha_{max} = \frac{R_1}{R_2}\left(\sqrt{A_0/R_1} - 1\right). \tag{14}$$

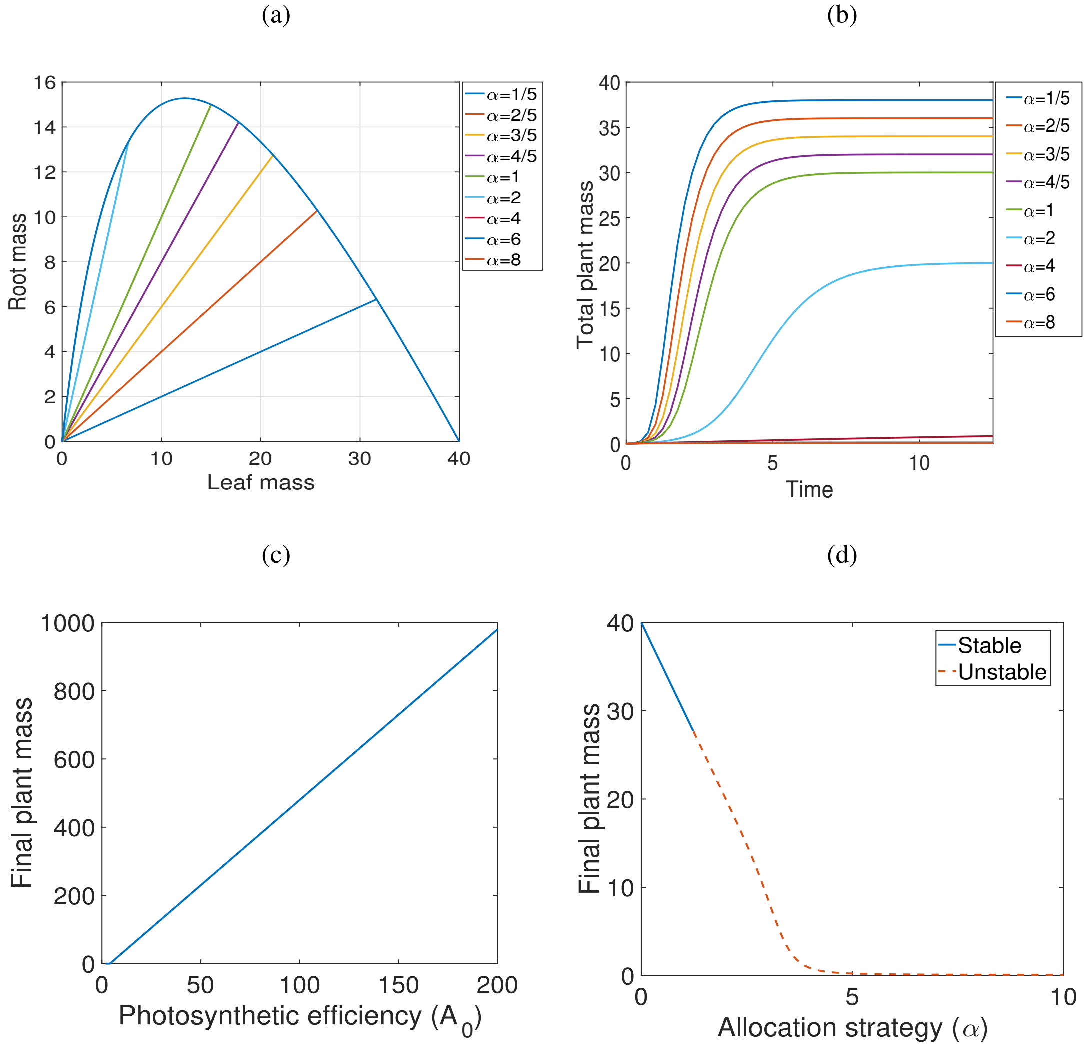

(a)
(b)
(c)
(d)

Fig. 2 a) The range of steady state solutions (blue line) with multiple growth trajectories for different allocation strategies. b) Growth curves for multiple allocation strategies when $A_0 = 10$, $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 2$. c) The relationship between photosynthetic efficiency (A_0) and final plant size when $\theta = 10$, $m = 1$, $\alpha = 1$, and leaf and root respiration is equal ($R_1 = 2 = R_2 = 2$). d) The relationship between allocation strategy and final plant size when $A_0 = 10$, $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 2$. All simulations were performed with initial conditions of $l = 0.01$ and $r = 0$, and all model parameters are dimensionless.

Solutions to the model are calculated numerically using Ode45 in MATLAB, which is a form of the Runge-Kutta method for numerical integration. Figure 2a shows several growth trajectories for different allocation strategies (α) when the cost of leaf and root maintenance respiration are equal ($R_1 = R_2$). The choice of allocation strategy determines the point at which the growth trajectory intersects the steady state curve. This determines the ratio of root and leaf material and total plant size at steady state. These final plant sizes can be related to yield as a larger plant produces a greater yield than a smaller one. For these parameters, steady state total plant size is a decreasing function of allocation strategy. In other words, strategies which favour leaf growth result in larger steady state total plant size than those favouring root growth, when roots are only considered as sinks. The same effects also apply to growth rates (Fig. 2b). The constant allocation ratio implies that the intersection between the vegetative growth line and the steady state curve (final plant size) is dependent upon the choice of initial seedling size and the steepness of the growth trajectory (allocation strategy). Varying the allocation strategy can alter the point at which the growth trajectory reaches the steady state curve.

The effects of shading are a decreasing function of the shading coefficient θ thus steady state total plant size is a decreasing function of θ, since both the peak of the steady state curve and maximum leaf tissue depend upon shading. Increasing A_0 has a positive impact on final plant size as expected (Fig. 2c). As the shading coefficient tends towards infinity, assimilation rate becomes unconstrained and plant material continuously increases. Conversely, imposing a very small shading coefficient highly constrains assimilation rate and leads to a very small final plant material.

These behaviours imply that the model represents the growth of a plant in a reasonable manner reproducing well-known phenomena.

3.2 Reproductive growth

Formulated in terms of the current model, a plant undergoes vegetative growth from an initial seedling size using Eqs (11) & (12) with a predetermined allocation strategy. The plant can then decide to reproduce at any developmental stage. Once the plant has made the life history decision to reproduce, no further vegetative growth occurs, total leaf and root mass become fixed at this point in time and the reproductive sink increases in size until the steady state is reached. Since the growth of leaves and roots is set to zero, all carbon made available from the plant minus respiratory costs at its chosen developmental stage is invested into seed production. Leaf and root biomass from the chosen developmental stage are the new initial conditions for the reproductive stage of growth. Within this framework, the seeds themselves have the same respiratory cost as roots and simply represent an additional sink. The model then becomes:

$$\frac{ds}{dt} = A(l)l - R_1 l - R_2 r - R_2 s, \tag{15}$$

where s is the amount of seed material, A is assimilation rate, l is leaf tissue, r is root tissue and R_1 and R_2 are maintenance respiration for leaves and roots & seeds respectively. Within this framework, there are two decisions that a plant can make which alter potential seed yield: 1. The allocation strategy during vegetative growth; 2. The developmental stage at which a plant reproduces.

4 How do maintenance costs limit carbon allocation?

Within the model, growth depends on the allocation of carbon to plant compartments, the assimilation of carbon via photosynthesis and loss of carbon via maintenance respiration. How these processes interact together can provide insight into the limitations on growth. In particular, how do maintenance costs limit the range of carbon allocation strategies which alter final plant size?

4.1 When leaf and root maintenance costs are equal, unshaded assimilation rate reduces limitations to carbon allocation

In the circumstances when the costs of leaf and root maintenance respiration are equal, increasing allocation towards the leaves increases final plant size. Figure 2d shows the effect of varying allocation strategy on final plant size. With default parameters, final plant size decreases as α increases, until $\alpha = 4$, then the plant does not have enough carbon to grow when allocation favours the roots. The maximum allocation strategy for stable steady state solutions is $\alpha_{max} = 1.2361$. This implies that carbon allocation is limited by an upper bound on root strategies, therefore only plants which allocate up to 1.2361 times more carbon to root growth than leaves are realistic given the parameter set. A sensitivity analysis shows how this upper bound is changed by alternative model parameterisations (see appendix).

There are two parameters within the model that vary the effect of assimilation rate; the unshaded assimilation rate and the shading coefficient. Increases in the unshaded assimilation rate cause both the maximum final plant size and the upper bound of root allocation to increase. For example, when increasing unshaded assimilation to $A_0 = 20$, the limit to root allocation is reached approximately when $\alpha_{max} = 2.1623$. Figure 3 shows the relationship between allocation strategy and final plant size with multiple unshaded assimilation rates. The gradients of these lines are not altered when varying A_0. Therefore, the model behaves reasonably, confirming that that having a higher unshaded assimilation rate ensures that the plant has more carbon available to allocate towards new modules, enabling a plant to allocate more towards the roots.

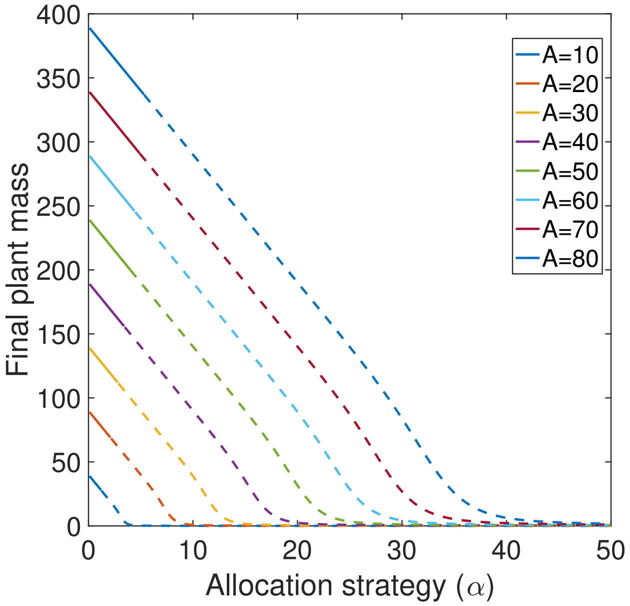


Fig. 3 The relationship between allocation strategy and final plant size with multiple unshaded assimilation rates, when maintenance respiration is equal in the roots and leaves ($R_1 = 2, R_2 = 2$), $m = 1$, $\theta = 10$, and initial conditions $l_0 = 0.1$ and $r_0 = 0$. When $\alpha < 1$, more carbon is allocated to the leaves and when $\alpha > 1$, more carbon is allocated to the roots. All model parameters are dimensionless.

In contrast, increasing the effect of shading on growth has no effect on the limits to carbon allocation. Increasing the shading coefficient (i.e. reducing the effect of shading) increases the maximum final plant size but the upper bound on root allocation remains the same. This is because α_{max} is independent of θ (Eq (14)). The shading coefficient (θ) simply delays the effect of shading on assimilation rate, therefore the cost is not applied until the total leaf canopy size is high (small α). This means that canopy architecture does not constrain the allocation of growth to roots within this framework. Further to this, using different initial conditions would alter the limits to carbon allocation since they alter the rates of photosynthesis and respiration.

4.2 When leaf respiration costs more than root respiration, increasing carbon allocation to the roots can increase final plant size

When leaf and root respiration are equal, increasing allocation towards the leaves increases final plant size and increasing allocation to the roots decreases final plant size. Only the negative slope of the steady state curve has stable solutions during vegetative growth (Fig. 1b). The gradient of this curve defines the relationship between allocation strategy and final plant size. If the gradient of the curve is less than -1, then when α is large (i.e. allocation favours roots over leaves), the trajectory intersects the steady state curve at a higher point (Fig. 4a). At this point, a larger plant is produced compared to the point of intersection for a smaller α. This is the opposite effect to when the gradient of the curve is greater than -1, when intersecting a higher point on the curve produces a smaller plant when compared to having a smaller α (Fig. 4b).

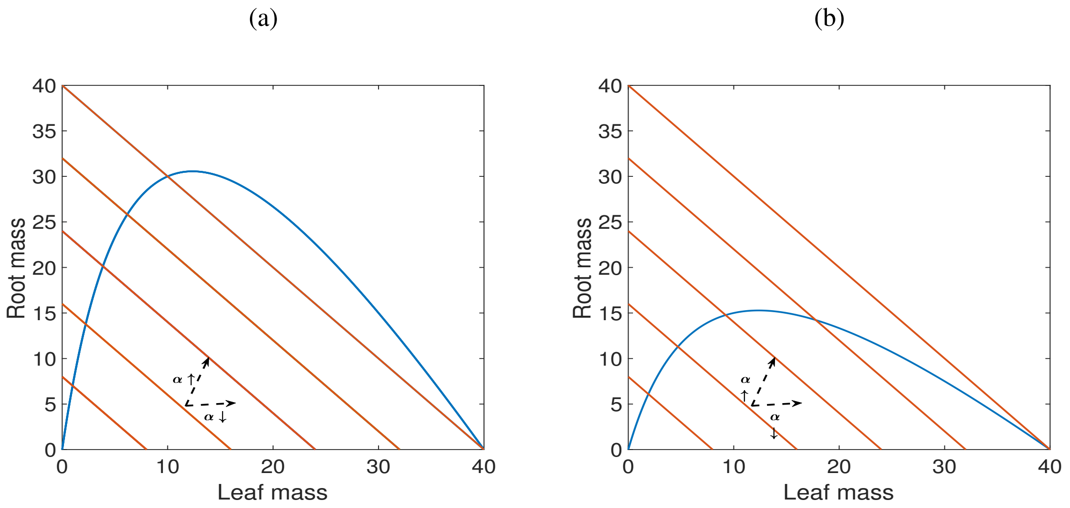


Fig. 4 a) Curve of steady states (blue line) when the gradient is less than -1 with lines of constant plant size (orange lines) when $A_0 = 10$, $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 1$. b) Curve of steady states (blue line) when the gradient is greater than -1 with lines of constant plant size (orange lines) when $A_0 = 10$, $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 2$ (Dimensionless).

When the cost of leaf respiration is higher than the cost of root respiration, decreasing leaf allocation reduces the amount of carbon lost via respiration, enabling a larger plant with a smaller leaf allocation strategy. When increasing allocation to a much larger root production strategy, there would be insufficient carbon assimilated to produce a larger plant. This means that, in the instance where the gradient of the steady state curve is less than -1, increasing allocation to the roots increases final plant size. But are there any realistic scenarios where this would happen? In order for this effect to occur within the model, the gradient of the steady state curve (differentiated Eq (13)) must be less than -1, which is equivalent to:

$$-\frac{R_1}{R_2}\left(1-\frac{R_1}{A_0}\right) < -1, \tag{16}$$

therefore

$$\frac{R_1}{R_2}\left(1-\frac{R_1}{A_0}\right) > 1. \tag{17}$$

A necessary condition for growth is that $A_0 > R_1$ therefore the cost of leaf respiration must be greater than the cost of root respiration.

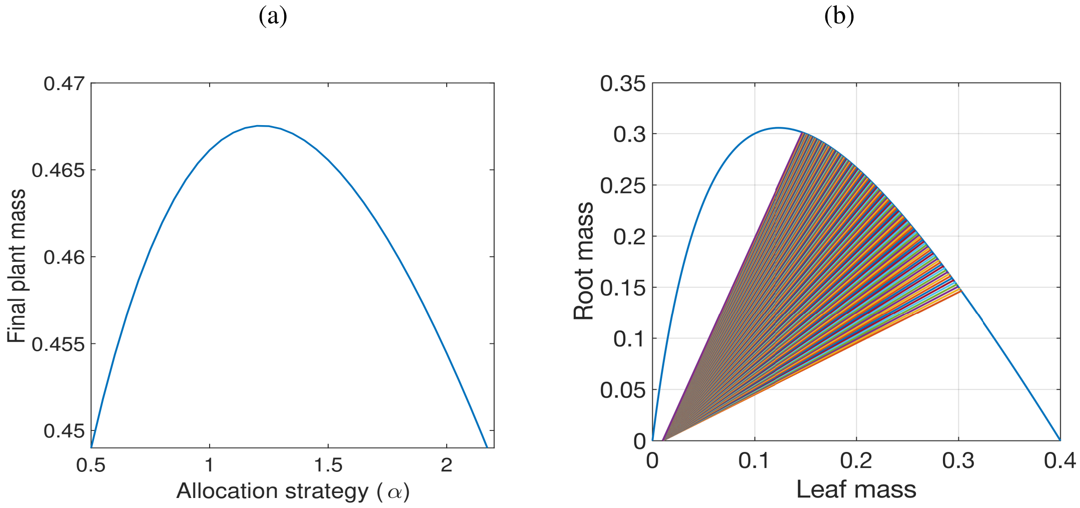


Fig. 5 a) The relationship between allocation strategy and final plant size when the cost of leaf maintenance respiration is twice the cost of root maintenance respiration. b) The ratios of leaf and root tissue at steady state with growth trajectories for a range of allocation strategies. Both produced with $A_0 = 10$, $\theta = 0.1$, $m = 1$, $R_1 = 2$, $R_2 = 1$ and initial leaf and root tissue of $l_0 = 0.01$ and $r_0 = 0$. $\alpha_{max} = 2.4721$, therefore all solutions are stable within the range of alpha plotted. All model parameters are dimensionless

The relationship between allocation strategy and final plant size changes substantially when making leaf respiration larger than root respiration. Figure 5b shows multiple trajectories intersecting the steady state curve with different allocation strategies. Higher allocation strategies (large α) intersect a higher point on the steady state curve and lower allocation strategies (small α) intersect a lower point on the curve. Towards the top of the steady state curve, the gradient is less steep than -1. Thus as α increases, the point of intersection moves further along the steady state curve, declining in plant size until the gradient reaches zero. This can be seen in Figure 5a. It shows the increase in final plant size as allocation tends towards favouring the roots. Once the α reaches 1.2, final plant size decreases. Therefore there is only a small region along the steady state curve, where increasing allocation strategy increases final plant size. This graph implies that allocating more carbon towards the roots when $\alpha < 1.2$ would improve yield. It is also important to note the small range of final plant size in Fig. 5a - i.e. when varying allocation between two times more carbon to the leaves and two times more carbon to the roots, there is only a change of 4% in final plant size when $A_0 = 10$, $\theta = 0.1$, and $m = 1$ (Fig. 5a). This implies that steady state plant size can be plastic with respect to allocation strategy, such that over a range of α, any allocation strategy can be implemented and achieve the same final plant size. This identifies two key questions: what range of parameter values defining carbon uptake (shading coefficient θ and unshaded assimilation rate per leaf A_0) allow this plasticity of final plant size to occur? Can altering these values limit or extend this plasticity within the model?

Varying the shading coefficient has no effect on the range of plasticity within the model

The scenario in which leaf respiration costs twice the amount of root respiration is fairly common across different plant species (Hansen and Jensen, 1977; Reich et al., 1998; Loveys et al., 2003; Tjoelker et al., 2005). This implies that there are a large range of parameter values which allow plasticity to occur with minimal change in final plant size. In the following sensitivity analysis, all parameters are varied within the model, while leaf and root respiration remain constant.

Increasing the shading coefficient has no effect on the region of allocation strategies which do not alter final plant size. For plasticity to occur, Eq (16) must be satisfied. Since this equation is not dependent upon θ, it cannot have any effect on the range of plasticity. When the shading coefficient is 0.5 (high shading), a plant can have any strategy of α between 0.5 and 1.5 (Fig. 6a). Within this framework, the range of allocation strategies which produce similar final plant biomass is not altered by shading as increasing shading only reduces the availability of carbon for allocation.

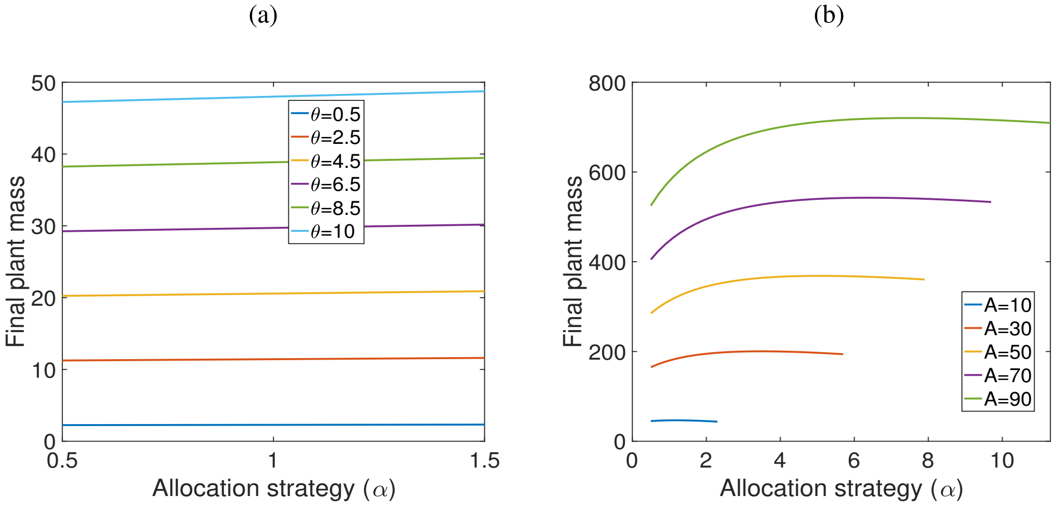


Fig. 6 The relationship between allocation strategy and final plant size a) whilst varying θ when leaf and root maintenance respiration is equal. Allocation strategy is varied from $\alpha = 0.5$ to the maximum stable α with $A_0 = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 1$. b) whilst varying assimilation rate when leaf and root maintenance respiration is equal. Allocation strategy is varied from $\alpha = 0.5$ to the maximum stable α with $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 1$. Using initial leaf and root tissue of $l_0 = 0.01$ and $r_0 = 0$. All model parameters are dimensionless.

As assimilation increases, there is more available energy to grow, therefore much more carbon needs to be allocated to the roots when growth stops in the model. This means that the range over which final plant size increases and then decreases (parabola-like curve in Figure 5a) is much larger. Though this is the case, there is a much larger change in final plant size under these scenarios. Figure 6b shows the relationship between final plant size and a range of allocation strategies which produce a stable steady state when varying A_0. When unshaded assimilation rate is $A_0 = 10$, plasticity occurs when α is between 0.5 and 2.47 (Fig. 6b), while as assimilation increases, this range shifts to root-favoured strategies. When $A_0 = 70$, plasticity occurs when α is between 4 and 9.83 (Fig. 6b). Therefore, increasing assimilation rate increases the parameter space which allows plasticity of final plant size to occur and promotes a root favoured allocation strategy.

5 How does the timing of reproduction influence yield?

The steady state condition when photosynthetic carbon gain is exactly balanced by respiratory carbon loss is a special case that may not be commonly achieved in nature. One situation when this may occur is in mature forest stands, as size becomes a limiting factor on growth. This balance of the rate of photosynthesis and respiration rate is one mechanism proposed to explain why forest biomass does not increase indefinitely, but tends to approach a plateau (Hunt et al., 1999; Day et al., 2001). The general problem with such a steady state is that, once this point is reached, the system is essentially “stuck" unless tissues turn over. One biological situation where this is not a problem is at the end of life in a monocarpic plant. Monocarpic species reproduce once at the end of their lives (Harper and White, 1974), and thus it might be assumed that they invest all remaining photosynthetic carbon into seed production at this point, so that growth approaches a steady state situation. If this argument is accepted, this is an interesting case to consider because many crop species are annuals (i.e. monocarpic), including all cereal and grain legume crops.

5.1 Seed production depends upon allocation strategy and developmental stage

Vegetative growth is simulated with a variety of allocation strategies. This produces multiple growth trajectories with different gradients. An initial root mass of 0.1 is used for each trajectory, and initial leaf tissue is determined by $r = \alpha ml$. This sets the initial seedling size for each vegetative growth trajectory. Reproductive growth is then simulated by taking a point along the growth trajectory with a distinct amount of leaf and root tissue as the initial conditions to solve Eq (17) (Figure 7a). The time taken to produce seed can be solved analytically using Eq (17) for a range of allocation strategies and developmental stages. The amount of seed produced is calculated and compared.

The dependence of seed production on the developmental stage of reproduction is shown in Fig. 7b for a range of allocation strategies when leaf and root respiration are equal. Each developmental stage represents total amount of leaf tissue as a percentage of the total amount of leaf tissue reached at steady state during vegetative growth (percentage of total age) or in other words, various stages along a plant’s lifespan if it didn’t reproduce. As the amount of leaf tissue increases during growth, the amount of energy available to produce seed increases and so does the level of self-shading. Once the plant produces a certain amount of leaf tissue, assimilation per unit leaf tissue begins to decrease (due to shading costs) while the cost of maintaining the plant is still increasing. This leads to the decline in the amount of seed tissue. A plant with an allocation strategy favouring leaf growth should reproduce at $25\% - 31\%$ of its total potential vegetative age to produce the maximum seed. A plant with an allocation strategy favouring root growth, should reproduce at $35\% - 49\%$ of its potential vegetative age. A plant favouring leaf growth can reproduce at an earlier ontogenic stage as it has already invested more energy into leaf growth

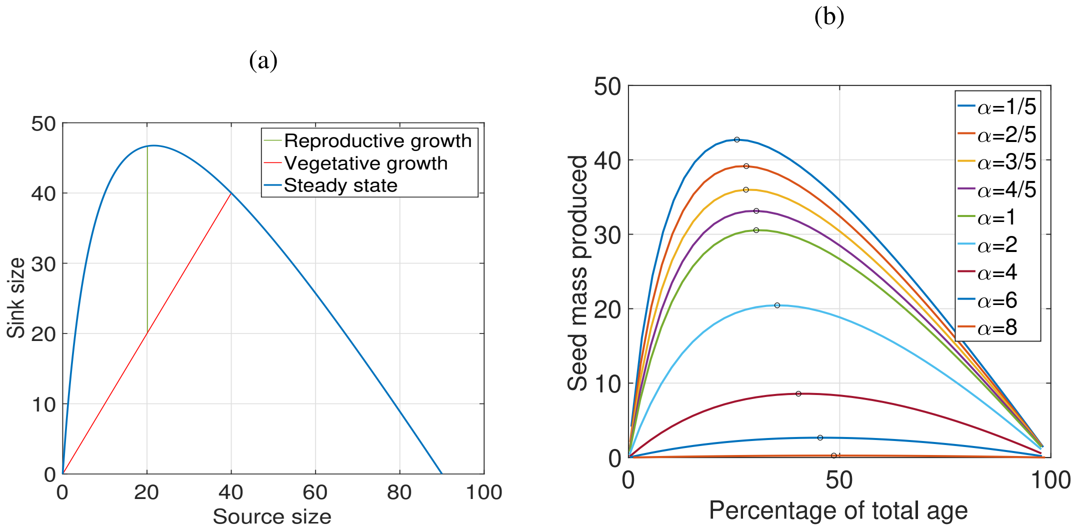


Fig. 7 a) The range of root to leaf allocation patterns when growth stops (blue line) with a vegetative growth trajectory (orange line) starting from an initial leaf and root tissue of $l = 0.1$, $r = 0.1$ and a reproductive growth trajectory (green line) starting from half of its total possible vegetative size ($l = 20$, $r = 20$), when $A_0 = 10$, $m = 1$,$\alpha = 1$, $\theta = 10$ and leaf respiration is equal to root respiration ($R_1 = 1 = R_2 = 1$). Sink size includes the combined tissues of roots and seeds, while source size is the amount of leaf tissue. b) The relationship between transition to reproduction at different percentages of total age (percentage of the amount of total plant material at vegetative steady state) and seed production, comparing multiple allocation strategies with an amount of initial root tissue of 0.1, and when $A_0 = 10$, $m = 1$, $\theta = 10$, $R_1 = 1$ and $R_2 = 1$. The data points (circles) represent the maximum amount of seed tissue produced for each allocation strategy. Developmental stage is a percentage of the amount of total plant material at vegetative steady state. All model parameters are dimensionless.

whereas a plant favouring root growth would need more time to develop enough leaves to create the most seed. High leaf allocation therefore promotes early reproduction. Although the timing of reproduction is affected by factors such as day length, temperature and stress, the balance of carbon between leaves and roots during vegetative growth imposes limits that all potential reproductive scenarios sensitive to these factors must stay within.

The optimal strategy for maximising reproductive output is defined by the maximum vertical distance between the vegetative growth trajectory and the steady state curve. This difference is the potential energy available to synthesise seed material. This potential energy (ΔE) can be expressed as:

$$\Delta E = \frac{A(l)l - R_1 l - R_2 r}{R_2}, \tag{18}$$

where A is assimilation rate, l is the amount of leaf tissue, r is the amount of root tissue, and R_1 is maintenance respiration for leaves and R_2 is maintenance respiration for roots and seeds. Equation (18) states that the potential energy for reproduction available at a given time is equivalent to the difference between the energy produced via photosynthesis and the energy required to maintain the leaves and roots.

With the largest potential energy producing the maximum yield, allocation strategies with the least steep trajectories have the largest potential energy. This implies that allocation strategies favouring leaves will have the largest potential energies for reproduction. Figure 7b confirms this, where it is clear that allocation strategies which favour leaves produce the largest amount of seed tissue. The strategy which allocates the largest amount of carbon to the leaves produces the most seed and as allocation towards the roots increases, yield decreases.

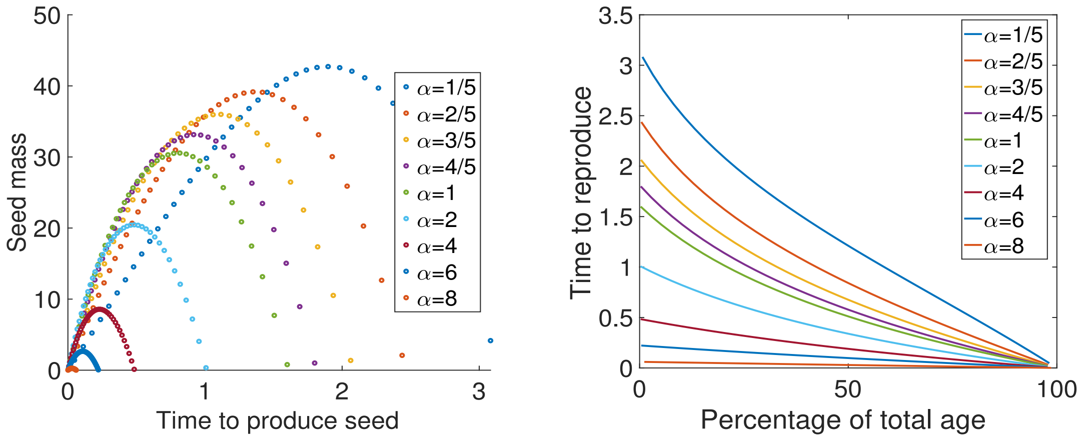
5.2 During vegetative growth, leaf favoured allocation strategies promote early reproduction

The time taken to produce seed can be calculated by integrating Eq (15) and rearranging to yield:

$$t = \frac{r_{ss} - r_0}{A(l)l - R_1 l - R_2 r}, \tag{19}$$

where t is the time taken to produce seed, r_{ss} is the total sink size (seed + root tissue) at steady state and r_0 is initial root tissue (amount of root tissue at the end of vegetative growth).

Time taken to produce seed decreases with developmental stage for plants favouring leaf allocation strategies during vegetative growth, implying that a plant reproducing later along the vegetative trajectory takes less time to reproduce. Figure 8a shows that there is a positive linear relationship between the amount of seed tissue and time taken to produce seed. The time to reproduce decreases as allocation towards the roots increases. This is because less carbon has been invested in leaf growth, so there is less energy available from photosynthesis to produce seed and it is exhausted more rapidly. For plants with a root favoured strategy during vegetative growth, the relationship between developmental stage and time taken to produce seed is much weaker (Fig. 8b). This is due to root allocation strategies ($\alpha > 1$) creating a much steeper vegetative trajectory, reducing the variation in potential seed production for the range of developmental stages along the trajectory. This suggests there is a trade-off between time taken to reproduce and yield. For a plant in a hazardous environment creating a reduced growing season, it may be beneficial to reproduce earlier and not obtain the maximum possible yield. For plants without constraint on the length of growing season, the optimal age to reproduce can be chosen based on total seed produced in Figure 7b.


(a)

(b)

Fig. 8 a) The relationship between time taken to produce seed during reproductive growth and the amount of seed tissue for multiple allocation strategies when $A_0 = 10$, $m = 1$, $\theta = 10$ and $R_1 = R_2 = 1$. Each data point represents a different developmental stage. b) The relationship between choice of reproduction at different percentages of total age and the time taken to produce seed for multiple allocation strategies. All model parameters are dimensionless.

5.3 Higher leaf maintenance costs delay reproduction for plants allocating more carbon to the leaves

When increasing the cost of leaf maintenance, there is a reduction in seed production and therefore also in time taken to produce seed. The maximum sink size (at the peak of the steady state curve) is equivalent to $r_{max} = \frac{\theta}{R_2}(\sqrt{A_0 R_1} - R_1)$. Therefore as the cost of respiration tends towards the amount of carbon assimilated via photosynthesis, the maximum sink size decreases. Not only does the maintenance cost affect yield, but it also affects the optimal age to reproduce in order to maximise yield. When maintenance costs are equal and $A_0 = 20$, for high leaf allocation strategies, it is beneficial to reproduce between $20\% - 23\%$ of the potential vegetative age if it didn't reproduce.

In contrast, for high root allocation strategies, it is beneficial to reproduce within $27\% - 41\%$ of the potential vegetative age for default parameters. However, when maintenance costs are unequal ($R_1 = 2R_2$), for high leaf allocation strategies it is beneficial to reproduce between $25\% - 28\%$ of the potential vegetative age and for high root allocation strategies, it is beneficial to reproduce within $30\% - 38\%$ of the potential vegetative age. This change in maintenance costs shifts the optimal region for leaf allocation strategies by 5% towards later reproduction and reduces the optimal region for root allocation strategies by 6%. This implies that having a higher maintenance cost for leaves than the roots, whilst allocating more carbon to the leaves, delays the optimal age to reproduce and further limits the optimal age to reproduce, when the plant is allocating more carbon towards the roots. This is because having a higher cost of leaf material reduces the amount of energy available to produce seed. A plant must therefore be more established in order to have the maximum energy available to reproduce (data not shown).

6 Discussion and conclusions

The aims of this chapter were to understand the consequences of different allocation strategies and costs of maintenance respiration for plant growth in a general but quantitative manner, and to investigate any possible limitations to carbon allocation. These were addressed by using a simple root-shoot carbon allocation model. The effects of varying the source-sink allocation ratio on plant growth are not explicitly explored in the work of most plant growth models. Typically, these incorporate an allocation assumption and investigate the effects of environmental conditions or perturbations on growth (Hogsett et al., 1985; Ford and Keister, 1990; Luxmoore, 1991; Weinstein et al., 1991). Ignoring the effects of environmental conditions made it possible to determine which underlying processes have the greatest influences on plant growth. In particular, the model suggested that maintenance respiration plays a significant role in the effect of allocation strategies on growth.

When maintenance costs are equal between leaf and root tissue, increasing allocation to the leaves increases growth rate and therefore final plant size. Many propose that the most efficient means for a plant to maximise its growth rate is to allocate just enough resources to the roots for nutrient assimilation and allocate the majority of resources to the leaves (Mooney, 1972; Wareing and Patrick, 1975; Russell et al., 1977; Reynolds and Thornley, 1982). In the case when maintenance costs are equal, there are limitations on carbon allocation to the roots and high assimilation rates alleviate these limitations. Thus high rates of photosynthesis enable both large plant size and high root allocation.

However, it is not necessarily the case that leaf and root maintenance respiration are equal and published data show that leaf respiration can be up to 2.25 times larger than root respiration (Hansen and Jensen, 1977; Reich et al., 1998; Loveys et al., 2003; Tjoelker et al., 2005). In the circumstance where leaf respiration is higher than root respiration, increasing root allocation can in theory lead to a larger plant. This effect is especially pronounced when canopy self-shading is high (i.e. the shading coefficient in the model is low), and contradicts the widely held assumption that carbon should be preferentially allocated to the leaves to optimise growth. Instead, it shows that maintaining a large number of shaded leaves within a canopy can be detrimental for production when those leaves carry a high maintenance cost. When leaves become too inefficient and costly to maintain, plants senesce their leaves.

Long-standing theory in vegetation modelling predicts that plants should add leaf layers until the lowest layers fail to make a positive net contribution to canopy carbon gain (Woodward et al., 1995). However, modern crops such as soybean seem to violate this prediction, producing very dense leaf canopies, especially when supplied with high atmospheric CO_2, and the dense shading of lower leaves means that they contribute little to canopy carbon assimilation (Drewry et al., 010a,b). Recent modelling and experimental manipulation of soybean crop canopies shows that these plants over invest in leaves, and that leaf removal can actually improve yields (Srinivasan et al., 2017). Under certain circumstances, crop plants therefore produce more leaves than is optimal for growth and yield, and the model shows how this behaviour can arise from the high cost of maintaining leaves and a declining rate of photosynthesis with shading. It has been hypothesised that the over-production of leaves evolves in wild plants from the benefits of shading out competitors in dense, competitive plant communities (Anten, 2005). This may increase the fitness of individual plants but, in the case of crops, breeders and farmers aim to maximise the yield from the whole population of plants within the field, and it is advantageous to reduce competition among individuals (Denison, 2012; Anten and Vermeulen, 2016). Respiration accounts for a large proportion of carbon loss within the plant (Gifford et al., 1984; Amthor, 1989, 2000; Cannell and Thornley, 2000), providing a key mechanism which can be manipulated to boost crop productivity. Advances in respiration research identify genes responsible for substrates, enzymes and transporters that are essential for protein turnover and transport, which can be targeted to optimise respiration (Amthor et al., 2019).

In the model, circumstances when leaf maintenance costs are higher than root maintenance costs also introduce a certain level of plasticity with respect to final plant size, such that a range of allocation strategies can lead to a similar final plant size (this occurs when the gradient of the steady state curve is less than -1). When maintenance costs are equal, leaves are much more beneficial for growth than roots, but when leaves cost more to maintain than roots, the benefits of growing leaves and roots become more equal. This allows for a range of root:shoot ratios to produce the same final plant size. Work has been done to investigate the effect of environmental conditions (Ericsson, 1995) or competition (Waite and Hutchings, 1982) on the plasticity of allocation but not necessarily how maintenance costs alter the plasticity of allocation. Increasing the shading coefficient has no effect on the range of allocation strategies where this plasticity occurs. However, increasing assimilation rate increases the range of allocation strategies where final plant size does not change.

It is assumed that respiration rates remain constant throughout a plants life however low levels of irradiance can reduce the rate of respiration in the leaves (Stoller and Myers, 1989; Hay et al., 2006). If maintenance respiration remains constant, growth reaches steady state when leaf maintenance respiration becomes equal to photosynthesis but in reality leaf respiration reduces in the shaded leaves. The reduction in maintenance respiration also accounts partially for the increase in net photosynthetic rate (Stoller and Myers, 1989). This also would only reduce the rate of maintenance respiration in the shaded portion of the leaf canopy. The magnitude to which self-shading reduces maintenance respiration would determine how much this inclusion would alter the model results. Including this effect would either reduce the difference between leaf and root maintenance respiration rates, producing either fewer scenarios in which increasing root growth increases final plant mass or leaf respiration would become lower than root respiration, eradicating this effect entirely. As long as the rate of leaf maintenance respiration remained higher than root maintenance respiration, the same effect of reducing leaf allocation on plant mass and plasticity of final plant mass would still occur.

When looking at reproductive growth, the optimal developmental stage for the plant to reproduce is defined by the carbon available to produce seeds or other reproductive organs such as tubers. Therefore, allocation strategies favouring leaf growth will have the largest potential energies for reproduction, implying that high leaf allocation during vegetative growth promotes early reproduction. This corroborates the work of Guilbaud et al. (2015) who suggest that high growth rates correlate with early flowering, since high leaf allocation leads to a higher growth rate. Their work extends that of Cohen (1971) by investigating how nitrogen dynamics alter the decision to flower. Cohen (1971) paved the way for reproductive growth models by investigating the effect of transition to flowering on yield. He determined that one transition which allocates all resources from vegetative growth to reproductive growth is the most beneficial for yield, and that flowering time is dependent upon growth season length. Other models build onto this by including loss terms (King and Roughgarden, 1982) or environmental conditions and hazard rates (Paltridge and Denholm, 1974; Ledder et al., 2004). Others investigate the effects of multiple reproductive phases (Chiariello and Roughgarden, 1984) or altering flowering time on yield (Fischer, 1985; Kantolic and Slafer, 2001; Poggio et al., 2005; Reynolds et al., 2009) and the effect of photosynthetic rate on reproduction (Schaffer et al., 1982). However, there has been little previous work investigating the effect of varying vegetative allocation strategy on yield. Having a higher leaf respiration than roots delays the optimal age to reproduce for leaf allocation strategies and increases the restrictions on flowering time for root allocation strategies.

The model results hinge upon the assumption that growth stops when carbon sources and sinks are balanced. The extent to which this situation arises in natural or crop systems is unclear, and three factors would tend to act so that steady state is not reached. First, new growth is always required to replace tissues as they turnover. Secondly, the requirement for roots (in terms of anchorage, nutrient and water uptake) may be less than the limit imposed by maintenance costs. A model incorporating tissue turnover and functional roots would be required to evaluate the magnitude and consequences of these effects. Finally, the development of short-lived plants, in particular, ensures that the limits imposed by carbon balance are not reached. Nonetheless, these limits set boundaries beyond which development cannot stray.

The model simulates carbon allocation by using a constant allocation ratio throughout the ontogeny of a plant and therefore has only one predetermined allocation strategy throughout. However, allocation is usually allometric, such that it depends upon size, and the proportion of carbon allocated to leaves or roots varies with time (Weiner, 2004). The choice of a simplified assumption of allocation within the model provides a framework to test distinct strategies of allocation and make predictions on how certain behaviours can influence plant growth. Further analysis with this model could consider an allocation strategy that is variable and dependent upon plant size.

Having the roots solely as sinks within the model underpins the finding that minimal allocation to roots maximises plant growth. However, resources obtained via the roots influence the allocation of carbon to sources and sinks and also overall plant growth (Running and Gower, 1991). Environmental conditions control fluctuations in carbon and nitrogen availability, causing crosstalk between signalling pathways of carbon and nitrogen (White et al., 2016). This crosstalk determines allocation to sources and sinks. When there is ample nitrogen, cytokinins are produced, which increases sink strength (Kuiper, 1993; Ghanem et al., 2011; Thomas, 2013), and this also increases carbon acquisition. When there is high carbon availability, nitrogen sources are up-regulated (Stitt and Krapp, 1999) and sink activity is increased (Klein et al., 2000; Kaiser et al., 2002; Reda, 2015). There is a need for a new type of allocation model which includes the interaction of carbon and nitrogen feedback and feedforward mechanisms defining allocation in order to further understand how allocation can be used to improve growth.

Overall, this quantitative modelling approach has revealed that the maintenance costs of leaves and roots alter the limitations on allocation by increasing the plasticity of final plant size in vegetative growth and restricting the range of optimal developmental stages for reproduction. It has shown that when leaf respiration is higher than root respiration, reallocating carbon away from leaves and toward the roots can improve plant growth. This analysis indicates that crop improvement and management strategies should consider the effects of canopy maintenance costs for improving growth.

Chapter 3

Simulating plant growth with internal feedbacks on the uptake, allocation and consumption of carbon and nitrogen

Abstract

Growth and biomass partitioning are determined by the balance of energy requirements for processes of resource uptake, transport and use. These processes are particularly sensitive to changes in cellular carbon and nitrogen concentrations. There are multiple observed feedback mechanisms which control the inhibition and induction of photosynthesis, nitrogen uptake, resource translocation and the relative growth rates of leaves and roots. However, how these feedbacks should be modelled individually and how they work together to determine growth rate and the overall ratio of above and below ground biomass remains unclear. The aims of this chapter are to determine how to simulate six feedback mechanisms which have been observed experimentally and unify them into a balanced feedback framework. This is done by using a simple model of carbon and nitrogen. The modelling work showed that not all functions are able to simulate a certain feedback for a range of environmental conditions (equal carbon and nitrogen availability, low carbon availability and low nitrogen availability) and functions which act like a “switch" only work when there is a discrepancy between internal carbon and nitrogen concentrations. The strongest feedback in this framework is the reduction in nitrogen uptake rate when nitrogen concentration is high. It is shown that the framework model is able to allocate growth towards the leaves when there is a reduction in carbon availability when compared to nitrogen and conversely, when there is less nitrogen available when compared to carbon, root growth increases. The combination of the six feedbacks increase plant growth overall when compared to the model without any feedbacks.

1 Introduction

Changes to the partitioning of resources to different plant tissues (e.g. leaves, roots, stem and seeds) greatly impact total biomass and crop yield and are brought about when plants react to changes in the environment. Plant genotype can determine the extent at which environmental heterogeneity alters allocation. These environmental conditions alter processes within the plant required for growth (respiration, photosynthesis, nutrient uptake, etc.) to differing extents, meaning that different internal processes become limiting under different environmental conditions. This imbalance necessitates a balancing of energy producing and utilising reactions 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 for gene expression affecting many cellular processes. This leads to crosstalk between the signalling pathways for carbon and nitrogen. However, thousands of genes respond to the change in sugar concentrations (Lastdrager et al., 2014). A simplification of these processes is needed to understand how they interact at a whole plant scale.

The responses of partitioning to the environment are thought to balance the uptake of carbon and nitrogen. Crop yields depend on the coordinated acquisition of carbon and nitrogen by the leaves and roots respectively and the use of these resources within each part of the plant. Carbon and nitrogen assimilation and the use of their products are entirely interdependent upon one another (Moorby, 1977; Paul and Foyer, 2001; Kaschuk et al., 2010). The energy required for nitrogen assimilation is provided via photosynthesis and byproducts of nitrogen assimilation are required for photosynthesis to occur. Gaining a fuller understanding of how carbon and nitrogen behave together in plant metabolism and signalling can therefore elucidate how such responses can be optimised to enhance plant growth.

Nitrogen is often a limiting factor in plant growth and development (Hodges, 2002). Inorganic nitrogen is absorbed from the soil by the roots. This comprises of nitrate (NO_3^-) and ammonium (NH_4^+), which is acquired through the mineralisation of organic matter or via fertilisers (Hodges, 2002). Nitrate is often converted into ammonium via nitrate reductase (NR) in the cytosol and the chloroplast in the leaves but can also occur in the roots of some plant species. NR is synthesised in the presence of nitrate and is degraded when it is absent. Ammonium is toxic for plants and is therefore immediately converted into glutamate and glutamine in the leaves using the glutamine synthetase (GS) and glutamate synthase (GOGAT) pathway (Lam et al., 1996). When nitrogen supply to the roots is limited, the synthesis of ammonia and consequently glutamine and glutamate synthesis ocurs in the plastids of the roots (Raven et al., 1999). Nitrogen assimilation becomes carbon-dependent as energy is required for the synthesis of glutamate and glutamine and is a key stage where carbon metabolism and nitrogen metabolism interact. GS activity requires the use of ATP and the GOGAT pathway uses c-skeletons and reductant in the form of 2-oxoglutarate (2og) and reduced ferredoxin or NADH which are produced from respiration (Hodges, 2002). Respiration therefore incorporates the carbon costs of nitrogen uptake. Hodges (2002) indicates that 2og could be an important signal in the coordination of the carbon and nitrogen metabolism as it reflects cellular C/N status.

Bloom et al. (2010) showed that increased levels of atmospheric CO_2 reduced nitrogen assimilation since this limits the process of photorespiration. NADH is required for nitrogen assimilation and is produced in the process of photorespiration. However, earlier studies did not find the same effect of high carbon (Stitt and Krapp, 1999), and the work of Bloom et al. (2010) is considered a controversial theory. It is thought that the photorespiratory system and the process of nitrogen assimilation use the same enzymes in the chloroplast and, therefore, when resource-limited, must compete for their use. Glucose, sucrose and 2og have been shown to increase nitrogen uptake by increasing the activity of NR in the leaves and roots (Cheng et al., 1992; Klein et al., 2000; Iglesias-Bartolomé et al., 2004; Reda, 2015) (Feedback 7, see Table 1).

Plant sugars can be used in place of light to induce the genes responsible for NR (Reda, 2015) consequently increasing nitrogen uptake rate. Reda (2015) shows the increased presence of NR activity following sugar treatments of 8 hours but there is no further evidence about how this mechanism works. Nitrogen uptake can also be inhibited (Siddiqi et al., 1990; Clarkson and Lüttge, 1991; Muller and Touraine, 1992; King et al., 1993; Rufty et al., 1993; Imsande and Touraine, 1994) by the presence of plant nitrogen and also be induced (Shaner and Boyer, 1976; Wray, 1993; Gojon et al., 1998; Reda, 2015) dependent upon where nitrogen pools are localised within the plant. Not only have sugars and inorganic acids have been found to stimulate multiple stages of nitrogen assimilation but 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 the leaves (Reda, 2015). In the roots, glutamine and glutamate induce NR activity. Nitrate induces genes responsible for NR within 30 minutes but this is only when photosynthesis is active. In summary, experimental evidence indicates two feedbacks: firstly, the increase in nitrogen uptake when internal carbon concentration is high (Feedback 6, see Table 1) and secondly, the reduction in nitrogen uptake when nitrogen concentration is high (Feedback 2, see Table 1).

When carbon assimilate availability is high, new sinks can be formed (Paul and Foyer, 2001). It has been found that sucrose is important in the regulation of plant growth such that it induces auxin production and transport (hormone responsible for growth), therefore increasing sink activity (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 the activity of root meristems. This represents a positive feedback on root growth when intermediate carbon concentration is high (Feedback 4, see Table 1). Sugars in the leaves can also act as an “off switch" to growth, when sugars reach high concentrations they can stop meristem growth (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). This implies that there are internal constraints to stop sugars reaching overwhelming proportions (Feedback 8, see Table 1). The allocation of carbon within the plant is affected by nitrogen availability (Nunes-Nesi et al., 2010), such that high plant nitrogen content increases shoot:root growth (Stitt and Krapp, 1999). Scheible et al. (1997) show that the presence of nitrogen in the roots increases protein synthesis and root absolute growth rate but shoot growth rate is higher, leading to a stronger allocation of growth towards the leaves. This identifies a further feedback to increase leaf growth when nitrogen concentrations are high (Feedback 3, see Table 1). Accumulation of sugars can also have a negative effect on phloem transport (Chiou and Bush, 1998) (Feedback 9, see Table 1).

Increasing CO_2 typically enables the build up of starch and other carbohydrates in the leaves in a matter of hours. 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). There is debate as to whether high starch concentrations weaken chloroplast function or not (Paul and Foyer, 2001). However, a large body of evidence shows that high carbon concentrations have a negative feedback on carbon uptake (Feedback 1, see Table 1). CO_2 assimilation is entirely dependent upon nitrogen since nitrogen is used to form the amino acids essential for the production of the proteins responsible for photosynthesis (i.e photosynthetic enzymes). 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. This implies that high nitrogen status in the roots promotes an increase in photosynthesis (Feedback 5, see Table 1) (Paul and Foyer, 2001).

Feedback	Signal	Location	Process	Type
1	Carbon(starch)	Leaf	C uptake	Negative
2	Nitrogen (glutmamine & glutamate)	Leaf	N uptake	Negative
3	Nitrogen	Leaf	Leaf growth	Positive
4	Carbon (glucose)	Root	Root growth	Positive
5	Nitrogen	Root	C uptake	Positive
6	Carbon (sucrose & 2-OG)	Root	N uptake	Positive
7	Carbon (sucrose)	Leaf	N uptake	Positive
8	Carbon(glucose & sucrose)	Leaf	Plant growth	Negative
9	Carbon (sucrose)	Leaf	Transport	Negative

Table 1 Summary of feedback responses to carbon and nitrogen concentrations observed experimentally. It shows the signalling molecule, where it is sensed, which process is regulated and whether it is up-regulated or down-regulated.

In many cases, it remains unclear on a molecular level how these feedback processes work. Most papers 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). Xiong et al. (2013) show that meristem activation occurs within 24 hours of treating seedlings with glucose. There is little information on how long it takes from the accumulation or depletion of carbon or nitrogen and the expression of genes, following on to the increased enzyme activity which represents the full feedback process. Without this information, it is difficult to compare the rates at which the various feedbacks operate.

Many models simulate growth by considering the dependence of growth on carbon and nitrogen supply but do not necessarily include the signalling feedbacks on uptake and use. Thornley (1972) enables carbon and nitrogen to become interdependent by using a two substrate Michaelis-

Menten equation, along with a transport mechanism which balances the pools of carbon and nitrogen in leaves and roots. Hunt et al. (1998) and Bartelink (1998) simulate the allocation of biomass to above and below ground compartments based on a functional balance of carbon, nitrogen and water. This assumes that plants make a decision on allocation based on a ratio of resources whereas there are multiple known mechanisms involved in the control of partitioning. Yang and Midmore (2005) assume that nutrients are allocated to the closest compartments first by associating transport resistance costs and growth is allocated based on the most limiting nutrient using Liebig's law of the minimum. Some models simulate the plasticity of growth to nutrient availability but only focus on above or below ground biomass (leaf canopy or root system) (Dunbabin et al., 2002; Pao et al., 2018).

Ågren et al. (2012) investigate the relationship between carbon, nitrogen and phosphorus on plant growth. The interdependence of these resources are considered such as: nitrogen and phosphorus uptake has a carbon cost, photosynthesis is dependent upon labile nitrogen content and the production of structural material is simulated using Michaelis-Menten kinetics such that all resources are required for growth to occur. It accounts for the cost of carbon for growth and maintenance respiration. This model is used to investigate the effect of varying nitrogen and phosphorus availability on plant relative growth rate and argues the importance of phosphorus for nitrogen uptake. This model considers the dynamics between three resources but recommend a mechanism is needed to restrict uptake rates in order to prevent resources becoming too high. Only structural and non-structural components are simulated and the allocation of resources between above and below ground material is not considered.

Other models investigate the effect of resource dependence on growth much more mechanistically. Shaw and Cheung (2018) optimise the allocation of resources by simulating 11,320 reactions and 10,664 metabolites in Arabidopsis. Although there is a high level of detail in the metabolite production for growth, the model still relies on functional balances to allocate resources for growth and does not specifically look at internal feedbacks.

Experimental results show that the fundamental processes involved in plant growth are sensitive to internal carbon and nitrogen concentrations and act as signals to increase or decrease such processes in response to nutrient status. However, the current generation of plant growth models do not consider these, instead focusing on the internal availability, flows and interdependence of carbon and mineral sources. Carbon- and nitrogen-derived signals have been observed in plant growth, but how these signals cooperate together with changes in nutrient availability is still unknown. This brings about 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? 4. Can all of the known feedbacks operate simultaneously to generate a stable system?

Though some previous models have simulated the dependency of source activity on carbon and nitrogen concentrations, no previous models have attempted to simulate feedbacks on source and sink activity. It is unknown how the unification of multiple feedbacks alters growth allocation individually and collectively. Here, a framework of feedback mechanisms is developed using a four compartment carbon and nitrogen model for plant growth using the simplifying assumption that carbon and nitrogen are used equally for growth. Specifically, the work investigates how six individual feedbacks should be modelled and what effect they have on growth under three environmental conditions (equal carbon and nitrogen uptake, low light and low nitrogen availability). The feedback with the strongest effect on growth is identified and how all feedbacks work together within the model is investigated. This chapter shows that a model with internal feedback mechanisms improves growth by balancing internal carbon and nitrogen concentrations.

2 The Model

Plant growth is simulated as the accumulation of leaf and root mass. Carbon and nitrogen is taken up by the leaves and roots respectively and accumulates in intermediate pools, which are then used to produce leaf and root mass.

2.1 Assumptions

Some simplifying assumptions are chosen to aid the comparison of different internal feedbacks on growth.

Continuous growth

Plant growth is measured as the accumulation of leaf and root mass over time, where leaf and root mass are determined by their respective relative growth rates (RGR). There are no loss terms within the model therefore there is no respiration or senescence of plant compartments. This simplification ensures that the plant requires carbon and nitrogen equally to grow, enabling a clearer understanding of how internal feedbacks influence growth and the concentrations of intermediates. As a simplifying assumption, the rates of use of carbon and nitrogen for growth are equal and with no loss terms. The model represents a phase of vegetative growth which is continuous, where plant mass is increasing linearly with a constant growth rate, whilst relative growth rate tends towards zero. Relative growth rate is assumed to be the product of the rate of use of carbon and the rate of use of nitrogen. These can be individually simulated by Michaelis-Menten functions:

$$g_l(C,N) = \frac{V_c C}{k_1 + C} \times \frac{V_n N}{k_2 + N}, \tag{1}$$

$$g_r(C,N) = \frac{V_c C}{k_1 + C} \times \frac{V_n N}{k_2 + N}, \tag{2}$$

where g_l is leaf RGR, V_c and V_n are maximum rate of use of carbon and nitrogen respectively, k_1 and k_2 are Michelis-Menten constants in each case and C and N represent the concentration of total intermediate carbon and nitrogen in solitary pools. This means that, within this framework, growth is a push mechanism, where high levels of carbon and nitrogen drive RGR. When nitrogen and carbon reach excess, RGR tends towards a maximal rate determined by V_c and V_n. Growth of leaves and roots can only occur when there is carbon and nitrogen available. This means that if nitrogen or carbon becomes zero at any point, growth will be zero regardless of the other nutrient concentration.

Resource acquisition

Carbon is acquired by the plant via photosynthesis in the leaves and similarly, nitrogen is imported via the roots. Carbon uptake rate (K_c) represents carbon source activity and is simulated as the average canopy gross photosynthetic rate which is an increasing function of leaf area. As leaf mass increases, the overall photosynthetic efficiency per leaf decreases due to self shading:

$$K_c(l) = \frac{A_c l}{\theta_c + l}, \tag{3}$$

$$K_n(r) = \frac{A_n r}{\theta_n + r} \tag{4}$$

where A_c is maximum rate of photosynthesis and θ_c is a shading coefficient for carbon uptake rate. Nitrogen uptake rate is modelled in the same way. Nitrogen uptake rate (K_n) is assumed to encompass the uptake of nitrogen from the soil and nitrogen assimilation (conversion of inorganic nitrogen to organic nitrogen). Simulating self-shading for carbon uptake rate and self-limitation for nitrogen implements a slow feedback of size onto growth, such that once the plant becomes larger, uptake rates reach a plateau (Farquhar et al., 1980; Waisel et al., 2002). These self-limiting uptake rates are chosen to ensure that intermediate carbon and nitrogen concentrations can reach a steady state, caused by the balance between uptake and use for growth. Simulating equal carbon and nitrogen uptake further ensures that the plant requires the same levels of carbon and nitrogen to grow. It is also assumed that there is no carbon cost of nitrogen uptake and there is no nitrogen cost of carbon uptake.

Resource allocation

Plant mass is compartmentalised into two tissue types: leaves and roots. Along with a leaf and root compartment, the framework has two compartments for intermediate carbon (i.e. sugars, starch) and nitrogen (i.e. nitrate, ammonium, amino acids). These pools of intermediates increase in size when the amounts of carbon or nitrogen taken up into the plant are higher than the amount required for growth:

$$\frac{dC}{dt} = K_c(l) - g_l(C,N)l - g_r(C,N)r, \tag{5}$$

where $K_c(l)$ is the rate of carbon uptake, l is leaf mass, r is root mass and $g_l(C,N)$ and $g_r(C,N)$ are leaf and root RGR respectively. So the amount of carbon in excess accumulates in an intermediate pool. This applies to nitrogen in the same way. The choice of RGR function ensures that equal proportions of carbon and nitrogen are required for the growth of leaves and roots. Leaves and roots have individual RGRs. This ensures that any feedbacks applied to leaf and / or root RGR alter the ratio of leaf and root mass. There is no cost of transport between compartments, which is assumed to happen instantly. It is assumed that without any internal feedbacks on growth, leaf and root RGR is equal, therefore growth is equally allocated to the leaves and roots.

Environmental conditions

Different environmental conditions can be simulated by altering carbon and nitrogen uptake rates and RGRs. Within this framework a plant experiences sunny, wet, warm, fertile soil conditions when maximum carbon and nitrogen uptake are equal (Case 1: $A_c = A_n$). Conditions in which carbon uptake rate is hindered (i.e. cloudy days or a low CO_2 atmosphere) are represented by halving maximum carbon uptake in relation to nitrogen uptake (Case 2: $A_c = A_n/2$). Conditions in which nitrogen uptake rate is hindered (i.e infertile soil) are represented by halving maximum nitrogen uptake in relation to carbon uptake (Case 3: $A_n = A_c/2$).

Internal feedback mechanisms

Internal feedbacks on growth are simulated by making key processes (nutrient uptake rates, RGRs) dependent upon the internal concentrations which are known to cause such feedbacks via the physiological mechanisms described earlier. Within this framework, plants are responsive to changes in internal carbon and nitrogen concentrations. The types of feedback mechanisms were chosen to balance each other such that, if a feedback is applied to nitrogen, the same type of feedback is implemented to carbon to maintain a balance.

The model describes plant growth with the following four equations:

$$\frac{dC}{dt} = K_c(l) - g_l(C,N)l - g_r(C,N)r; \tag{6}$$

$$\frac{dN}{dt} = K_n(r) - g_l(C,N)l - g_r(C,N)r; \tag{7}$$

$$\frac{dl}{dt} = 2g_l(C,N)l; \tag{8}$$

$$\frac{dr}{dt} = 2g_r(C,N)r; \tag{9}$$

where C is carbon concentration, N is nitrogen concentration, l is leaf mass, r is root mass, t is time, $K_c(l)$ is carbon uptake rate (Eq. (4)), $K_n(r)$ is nitrogen uptake rate (Eq. (5)), g_l is leaf growth rate

(Eq. (1)) and g_r is root growth rate (Eq. (2)). The 2 in front of the growth functions in Equations (8) and (9) arise from the assumption that equal carbon and nitrogen is required for growth. For the purposes of behaviour illustration, the following default parameters: $A_c = 20$, $A_n = 20$, $\theta_c = 0.2$, $\theta_n = 0.2$, $V_c = V_n = 1$, $k_1 = k_2 = 400$ with initial conditions of $C = 0.001$, $N = 0.001$, $l = 0.1$ and $r = 0.1$ and a timespan of $t = 0$ to $t = 1000$ by 1 will be used. All values within this chapter are dimensionless in order to develop the feedback model. Although these values are dimensionless, they can provide a useful comparison for determining the effects of environmental conditions and feedback mechanisms on growth. The model is solved using Ode15s in MATLAB, which is a multistep numerical solver for stiff systems.

2.2 Model description

Figure 1 shows the effects of three different environmental conditions on the model. With equal uptake rates (case 1), carbon and nitrogen uptake rate reach close to the maximum value (19.99) by $t = 56.5$ (dimensionless) (Fig. 1a). Carbon and nitrogen concentrations increase until uptake rates reach their maximum due to size restrictions (Fig. 1b). At this point, the plant becomes source limited such that growth potential is much larger than maximum uptake rates. Once the plant becomes source limited, the accumulated concentrations are used for growth and therefore the intermediate pools reduce in size. As pool size declines, RGR tends towards zero (Fig. 1c). This continues until uptake and growth become balanced and linear growth occurs (Fig. 1d–e). Reducing carbon uptake rate in relation to nitrogen uptake rate (Case 2) is the equivalent to simulating an environmental condition in which light availability is limited. This reduction in carbon uptake rate naturally reduces carbon concentration and therefore makes the plant even more source limited than in optimal growth conditions. This creates a lower leaf and root RGR and consequently total plant mass, which is reflected in growth rate over time.

Imposing a source limitation on carbon in comparison to case 1 leads to a reduction in carbon concentration and continual increase in nitrogen. The converse occurs for case 3, where carbon is high and nitrogen is low compared to case 1. Similarly, when imposing a limited nitrogen availability (Case 3), RGR is also reduced and total plant mass behaves in the exact same way as for case 2 except for nitrogen instead of carbon. Varying each environmental case (changing source availability) creates an imbalance between carbon and nitrogen concentrations. Within this framework, the surplus carbon or nitrogen depending on environmental case will continually build and the disparity between carbon and nitrogen becomes more and more uncommon within real plants. To prevent this divergence, feedback mechanisms are needed in the model.

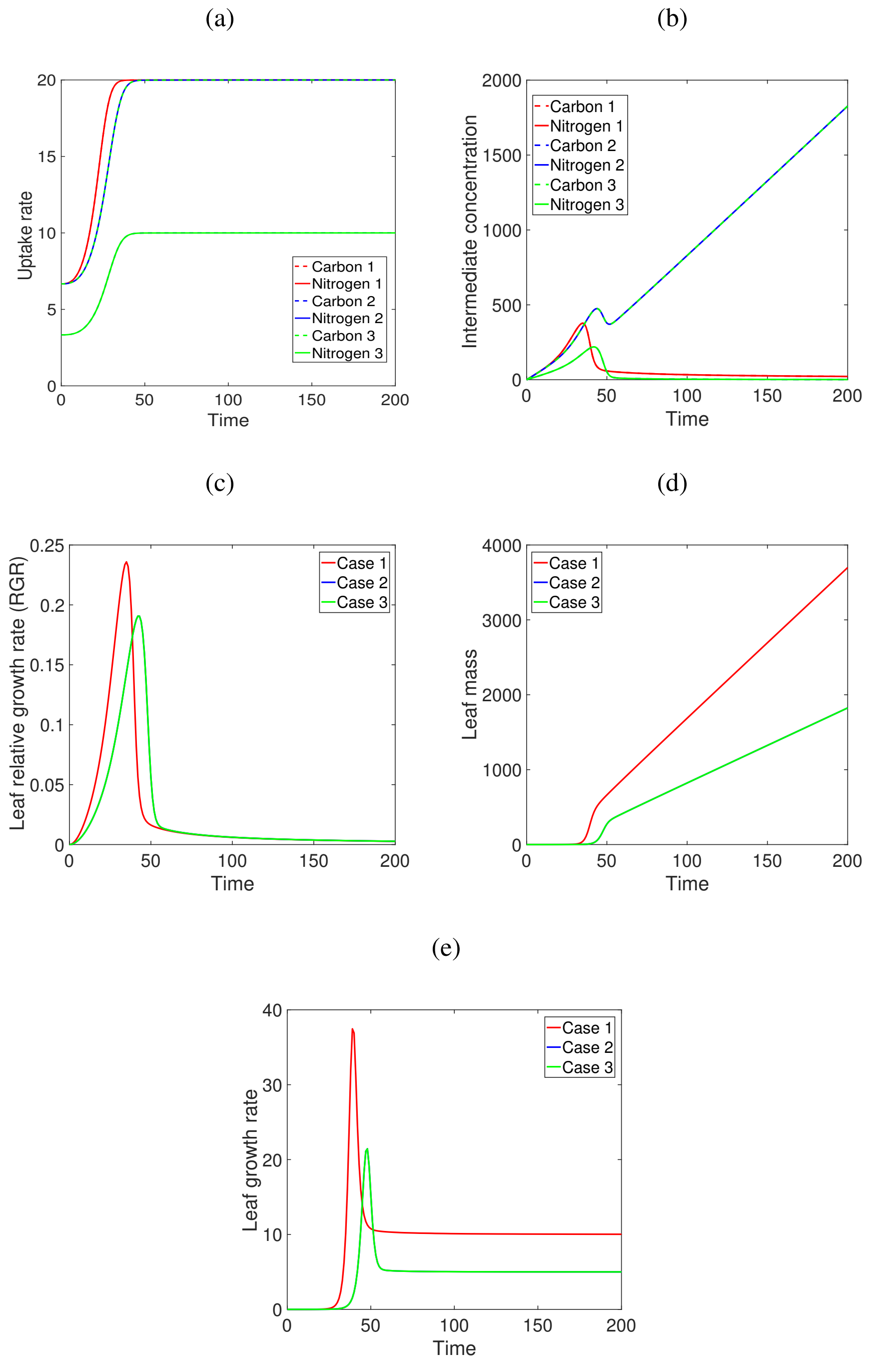


Fig. 1 Varying environmental cases (case 1- equal carbon and nitrogen uptake rates; case 2- low carbon uptake rate; case 3- low nitrogen uptake rate) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, nitrogen uptake rate (Case 3) overlaps carbon uptake rate (Case 2), carbon uptake rate (Case 3) overlaps nitrogen uptake rate (Case 2) and carbon and nitrogen uptake rate (Case 1) overlap b) intermediate carbon (dashed line) and nitrogen (solid line), carbon and nitrogen (Case 1) overlap, carbon (Case 3) overlaps nitrogen (Case 2) and carbon (Case 2) overlaps nitrogen (Case 3) c) leaf RGR, root RGR is the same as leaf RGR and case 2 and 3 overlap d) leaf mass over time, root mass is the same as leaf mass and case 2 and 3 overlap e) leaf growth rate, root mass is the same as leaf mass and case 2 and 3 overlap. The model was run with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0$ for a timespan of 0 to 1000 (Dimensionless). $1-3$ represent environmental case.

3 Simulating feedbacks

As outlined in the introduction, there is strong evidence for a variety of feedback mechanisms within plants and in particular, the negative feedback on carbon uptake rate with high carbon concentrations. The types of feedbacks simulated were chosen to keep the model balanced. For example a reduction in carbon uptake rate with high carbon concentrations was paired with a reduction in nitrogen uptake rate with high nitrogen concentrations. Additionally feedbacks were chosen to ensure that all processes within the model become dependent upon internal concentrations and to simulate different feedback mechanisms such as: reducing uptake rates, increasing uptake rates and increasing growth rates. The feedbacks simulated are:

1. High concentrations of carbon intermediates reduce carbon uptake rate (K_c)
2. High concentrations of nitrogen intermediates reduce nitrogen uptake rate (K_n)
3. High concentrations of nitrogen intermediates increase leaf growth rate (g_l)
4. High concentrations of carbon intermediates increase root growth rate (g_r)
5. High concentrations of nitrogen intermediates increase carbon uptake rate (K_c)
6. High concentrations of carbon intermediates increase nitrogen uptake rate (K_n)

These six feedbacks enable a plant to: increase growth towards sinks when source strength is high for both carbon and nitrogen (Feedbacks 3 & 4); reduce source activity when source strength is high (Feedbacks 1 & 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). By simulating self-shading and root inefficiency, the model also has a slow feedback of size on source strength (Fig. 2).

These feedbacks are internal responses which occur with fluctuations in carbon and nitrogen concentration and affect the processes defining growth (carbon uptake; nitrogen uptake; leaf growth; root growth). Each feedback can be implemented mathematically by making the affected process dependent upon the carbon or nitrogen concentration responsible for such feedback. For instance, feedback 1 alters carbon uptake rate when carbon concentration is high, this means that carbon uptake rate must become dependent upon carbon concentration. Currently, without any internal

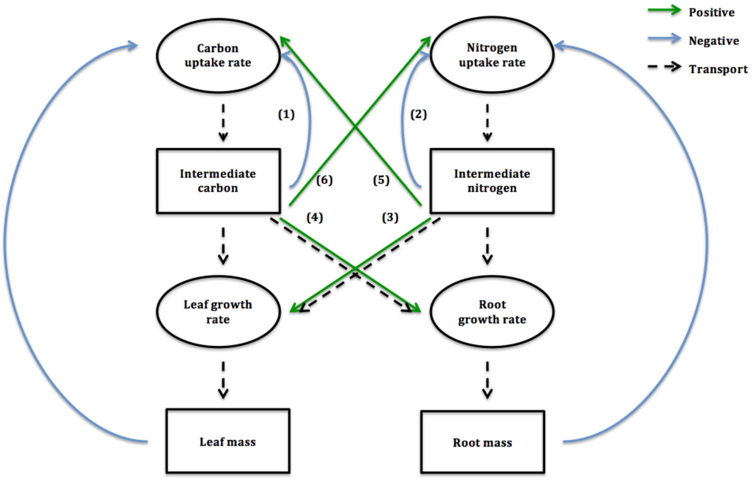


Fig. 2 Feedback mechanisms altering the model. Rectangles represent compartments of the model and ovals represent the internal processes such as resource uptake and growth. Dashed lines represent the transport of resources between compartments. Blue arrows are negative feedbacks and green are positive feedbacks.

feedbacks, carbon uptake rate is assumed to be solely dependent upon leaf mass (Eq. (3)). Carbon uptake rate would become:

$$K_c(l,C) = \frac{A_c l}{\theta_c + l} \times F(C), \tag{10}$$

when incorporating a carbon dependence, where F(C) is a function of carbon concentration. These feedbacks can be simulated using variety of mathematical functions since it is unclear how these feedbacks work biologically. Therefore five different mathematical functions will be tested to simulate each feedback and compared against the model output with no internal feedbacks (Fig. 1). This is done for each type of internal feedback (1-6) listed above and for all three environmental cases to determine which feedback function has the strongest effect on growth and performs the internal feedback the most reasonably when varying environmental conditions.

Scalar feedback

A scalar function (Function 1) of concentration can be used to simulate either a negative or positive feedback. This is simply the process multiplied or divided by the concentration for a positive or negative feedback respectively. For instance, for feedback 1 (high carbon reduces carbon uptake rate) carbon uptake rate becomes:

$$K_c(l,C) = \frac{A_c l}{\theta_c + l} \div C \tag{11}$$

This makes the process directly proportional to carbon concentration. An additional parameter could be used to scale the sensitivity of the feedback to concentration, but is omitted here for simplicity.

Linear feedback

Similarly, a linear term (Function 2) can be used to simulate a positive or negative feedback on a process. Feedback 1 becomes:

$$K_c(l,C) = \frac{A_c l}{\theta_c + l} \pm zC, \tag{12}$$

where z is a constant which determines the strength of the feedback. Both linear and scalar feedback functions are continuous and therefore occur incrementally over time.

Stepwise feedback

A stepwise function can be used to simulate a feedback that works more like a switch. For instance if a feedback only occurs when concentrations become too high, the feedback function will be nonzero when concentrations become larger than some threshold value and zero when lower than this value. For instance, feedback 1 becomes:

$$K_c(l,C) = \frac{A_c l}{\theta_c + l} \pm \frac{Q(l,C)}{1 + 100000e^{-100(C-w)}}, \tag{13}$$

where $Q(l,C)$ is a function of leaf mass and carbon determining the step change incurred by the feedback and w is some threshold value. This means that if carbon is higher than some threshold value ($C > w$), carbon uptake rate is reduced or increased by $Q(l,C)$ if it is a negative or positive feedback respectively. This type of function ensures that a feedback will only occur for the particular time period when the condition holds.

These three types of feedback function vary in level of simplicity. The scalar feedback function is the simplest as it has no additional parameter values, followed by the linear feedback function which has one additional parameter value. The stepwise function is much more complex as the rate at which the feedback is “switched" on or off can be varied, the increment of change to the process can be altered by $Q(l,c)$ and the threshold value can be varied. Three types of stepwise function will be tested with different $Q(l,c)$:

- A *constant stepwise* feedback function (Function 3) will be simulated by $Q(l,c) = 1$, this reduces the process by 1 at each time step until the concentration becomes lower than the threshold value
- A *fractional stepwise* feedback function (Function 4), where the process is reduced by a fraction of itself. For feedback one, $Q(l,c) = \frac{1}{4}\frac{A_c l}{\theta_c + l}$. This ensures that the process cannot become negative.
- A *variable stepwise* feedback function (Function 5) creates an incremental loss or gain that is dependent on the difference between the concentration and threshold value (for feedback 1, $Q(l,c) = (C - w)/4$)).

For each environmental case and feedback type, the five feedback functions will be tested and the “best" function will be selected for each. If there are multiple functions that correctly simulate the feedback, the simplest function (the least number of parameters) will be chosen. A feedback function is considered reasonable if:

- The function successfully produces the behaviour of the feedback;
- RGR tends towards zero as time increases and is always positive;
- Growth rate tends towards a constant steady state value, therefore representing linear growth;
- RGR is within an order of magnitude compared to 0.3 (maximum RGR without feedbacks);
- Carbon and nitrogen uptake rates are within an order of magnitude compared to their maximum rates ($A_c = A_n = 20$).

Parameter values were initially calibrated to attain a maximum RGR of 0.3 and a maximum carbon and nitrogen uptake rate of 20 but these are dimensionless values and therefore cannot be explicitly compared to their experimental values. A threshold value for the stepwise functions is set to $w = 400$ for both carbon and nitrogen for all feedbacks and environmental cases. For the fractional stepwise function, the dependent process is scaled by $1/4$ for all feedbacks and cases and, for the variable stepwise function, the difference between intermediate carbon or nitrogen and the threshold value is divided by 4 for all feedbacks and cases. These steps were taken to ensure that the stepwise functions reduce or increase the process gradually and not instantly. These values are kept the same throughout to aid the comparison of function type. The criteria regarding RGR and resource uptake rates ensure that these rates remain within a similar magnitude in relation to their default values to prevent extreme changes to the processes.

3.1 Case 1: equal carbon and nitrogen uptake rates

Feedback 1: a linear function best simulates a negative feedback on carbon uptake rate with high carbon concentrations

When carbon and nitrogen uptake rates are equal, only scalar and linear feedback functions alter carbon and nitrogen uptake rate (Fig. 3a). A scalar feedback function causes carbon uptake rate to reach a maximum of 60000. Initial carbon concentration is 0.01, therefore initially, when concentrations are low, as a consequence of multiplying uptake rate by concentration, carbon uptake rate becomes very large. As concentrations increase, this then decreases rapidly. This is unrealistic behaviour and therefore can be discounted as a viable option to simulate a negative feedback on carbon uptake rate. The linear feedback function reduces carbon uptake rate, becoming close to zero until leaf mass becomes large enough and carbon concentration low enough that carbon uptake rate increases until it reaches 20. This feedback consequently slows down the rate at which nitrogen uptake rate reaches its maximum of 20. All other feedback functions behave in the exact same way as the model output without any feedbacks (Fig. 3a-e). The stepwise functions do not alter carbon uptake rate and therefore plant growth since intermediate carbon doesn't exceed the threshold value of $C = 400$ for the functions. This threshold value is kept the same for all three cases. This implies that for an equal carbon and nitrogen uptake rate, the feedback is not needed. Both linear and scalar feedback functions produce a lower RGR and consequently a lower total plant mass than without any feedbacks (Fig. 3c-d). The linear feedback function produces the lowest RGR and plant mass. Growth rate converges to 10 for the linear feedback, all stepwise functions and no feedback output (Fig. 3e). RGR is equal for leaves and roots and therefore growth rate and mass are also equal since there is no feedback altering RGR. Implementing a feedback of high carbon on carbon uptake rate produces higher nitrogen and lower carbon concentrations than without any feedback, therefore the implementation of this feedback further imbalances carbon and nitrogen concentrations (Fig. 3b). This shows that a linear function has the strongest effect on plant growth and is the only function to simulate a negative feedback on carbon uptake rate effectively.

Feedback 2: a linear function best simulates a negative feedback on nitrogen uptake rate with high nitrogen concentrations

The exact same behaviour occurs when implementing a negative feedback on nitrogen uptake rate when nitrogen is high as for when implementing feedback 1 for high carbon. Both linear and scalar feedback functions replicate feedback behaviour of reducing nitrogen uptake rate, RGR and mass, whilst the stepwise functions (3-5) have no effect on growth. The scalar function increases nitrogen uptake rate initially, therefore the best function for feedback 2 is a linear term.

Feedback 3: a scalar function best simulates a positive feedback on leaf growth with high nitrogen concentrations

Similarly to feedbacks 1 and 2, the only viable functions are linear and scalar since the stepwise functions (3-5) do not alter plant growth. This is because carbon concentration does not reach the threshold value. Both functions increase shoot to root ratio but a linear function has a higher allocation towards leaf growth (Fig. 4e). This is reflected in the RGR plot, where a linear feedback produces a maximum leaf RGR of 1.3642, a scalar feedback produces a maximum leaf RGR of 0.9632 with the corresponding root RGR with each function close to zero (Fig. 4c). The maximum RGR with zero feedbacks is 0.2359 for both leaves and roots, as it is for all of the other stepwise feedbacks. Although maximum RGRs close to 1.4 are not typically observed in nature, these values are dimensionless and therefore cannot be directly compared. Both of these values are within one order of magnitude different to the default maximum RGR (0.3) and can therefore be considered reasonable. All stepwise functions and zero feedbacks produce a total plant mass of 39422, a scalar feedback produces a total plant mass of 32574, whilst a linear feedback produces a total plant mass of 13392. This reflects the difference in RGRs such that the linear function reduces total plant mass drastically. Additionally, the allocation of growth towards leaves is so high for a linear feedback that root mass never increases enough to increase the rate of nitrogen uptake (Fig. 4a), thus carbon concentration is much higher than nitrogen for linear and scalar feedbacks (Fig. 4b). All functions allow for linear growth to occur and therefore all growth rates reach a steady state (Fig. 4f). The overall effect of allocation between leaves and roots on uptake rate and total plant mass implies that the best function to simulate a positive feedback on leaf growth when nitrogen is high is a scalar function.

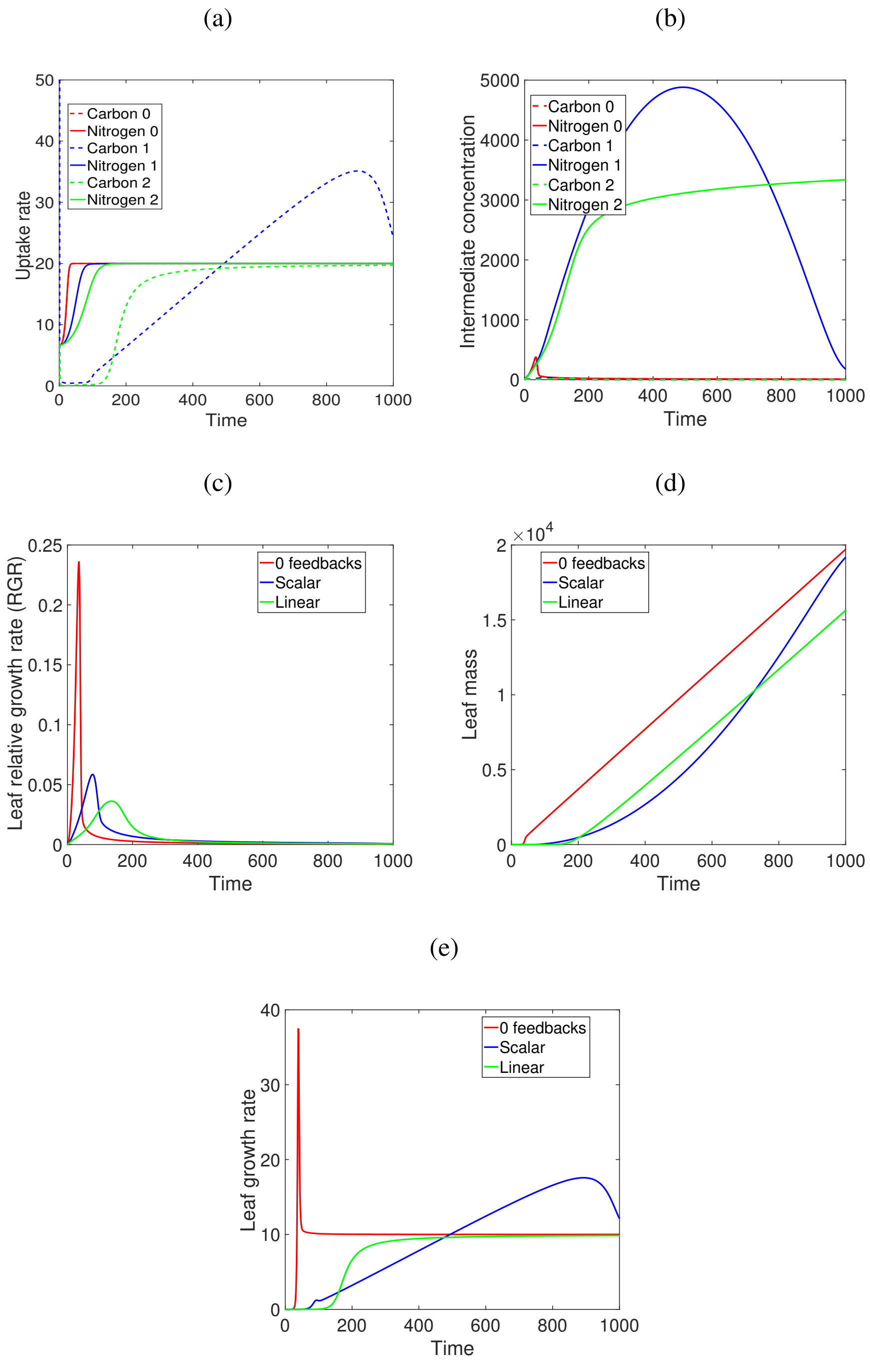


Fig. 3 Model output for equal carbon and nitrogen uptake rate (Case 1), implementing a variety of functions ($0-2$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a negative feedback on carbon uptake rate when carbon is high (Feedback 1) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, carbon and nitrogen uptake rate without feedback (red lines) overlap b) intermediate carbon (dashed line) and nitrogen (solid line), carbon and nitrogen without feedback (red lines) overlap c) leaf RGR, root RGR is the same as leaf for all functions d) leaf mass, root mass is the same as leaf e) leaf growth rate, root growth rate is the same as leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

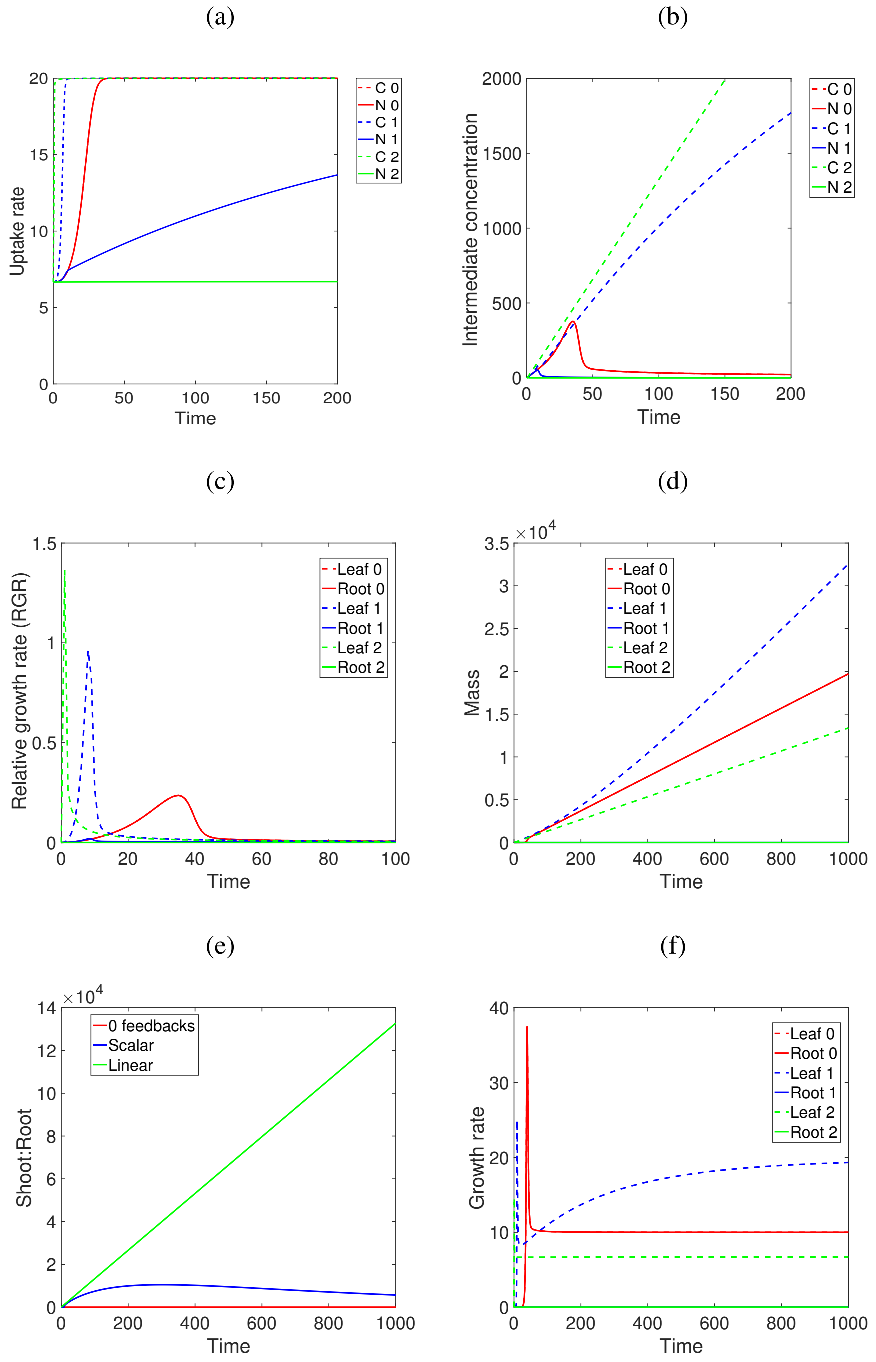

(a) (b) (c) (d) (e) (f)

Fig. 4 Model output for equal carbon and nitrogen uptake rate (Case 1) implementing a variety of functions ($0-2$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a positive feedback on leaf growth when nitrogen is high (Feedback 3) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, carbon and nitrogen uptake rate without feedback (red lines) overlap. b) intermediate carbon (dashed line) and nitrogen (solid line), carbon and nitrogen without feedback (red lines) overlap c) leaf (dashed line) and root (solid line) RGR, leaf and root RGR without feedback (red lines) overlap d) leaf (dashed line) and root (solid line) mass, leaf and root mass without feedback (red lines) overlap, root mass (Case 2) overlaps root mass (Case 3) e) leaf (dashed line) and root (solid line) growth rates, leaf and root mass without feedback (red lines) overlap, root growth rate (Case 2) overlaps root growth rate (Case 3) . The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Feedback 4: a scalar function best simulates a positive feedback on root growth with high carbon concentrations

A positive feedback on root growth with high carbon concentration behaves in the same way as feedback 3 for high carbon on leaf growth such that only linear and scalar feedbacks alter growth. Therefore, the best function for feedback 4 is a scalar function.

Feedback 5: a linear function best simulates a positive feedback on carbon uptake rate with high nitrogen concentrations

Only linear and scalar feedback functions increase carbon uptake rate when nitrogen increases. A scalar feedback function creates a maximum carbon uptake rate of 4097.3 but as time increases, it tends towards 4.0744 (Fig. 5a). This creates a maximum which is two orders of magnitude larger than the maximum uptake rate with default values, implying that the feedback is too strong. Once nitrogen concentration reduces in size, this also allows carbon uptake rate to be smaller than default values. A linear feedback function produces a maximum carbon uptake rate of 259.8 and tends to 20.2. This is one order of magnitude larger than without feedbacks and is therefore considered reasonable. Both linear and scalar feedback functions produce a similar RGR that is larger than without any feedbacks and the stepwise functions (Fig. 5c). Scalar has an RGR of 0.3352, whilst linear has an RGR of 0.3253 but all functions tend towards a growth rate of 10 (Fig. 5e). Both scalar and linear produce a higher total plant mass than without any feedbacks but scalar has the highest total plant mass (Fig. 5d). A scalar feedback function has the highest carbon concentration, followed by a linear feedback (Fig. 5b). The linear function is the only one to meet all criteria for this feedback when carbon and nitrogen uptake rates are equal.

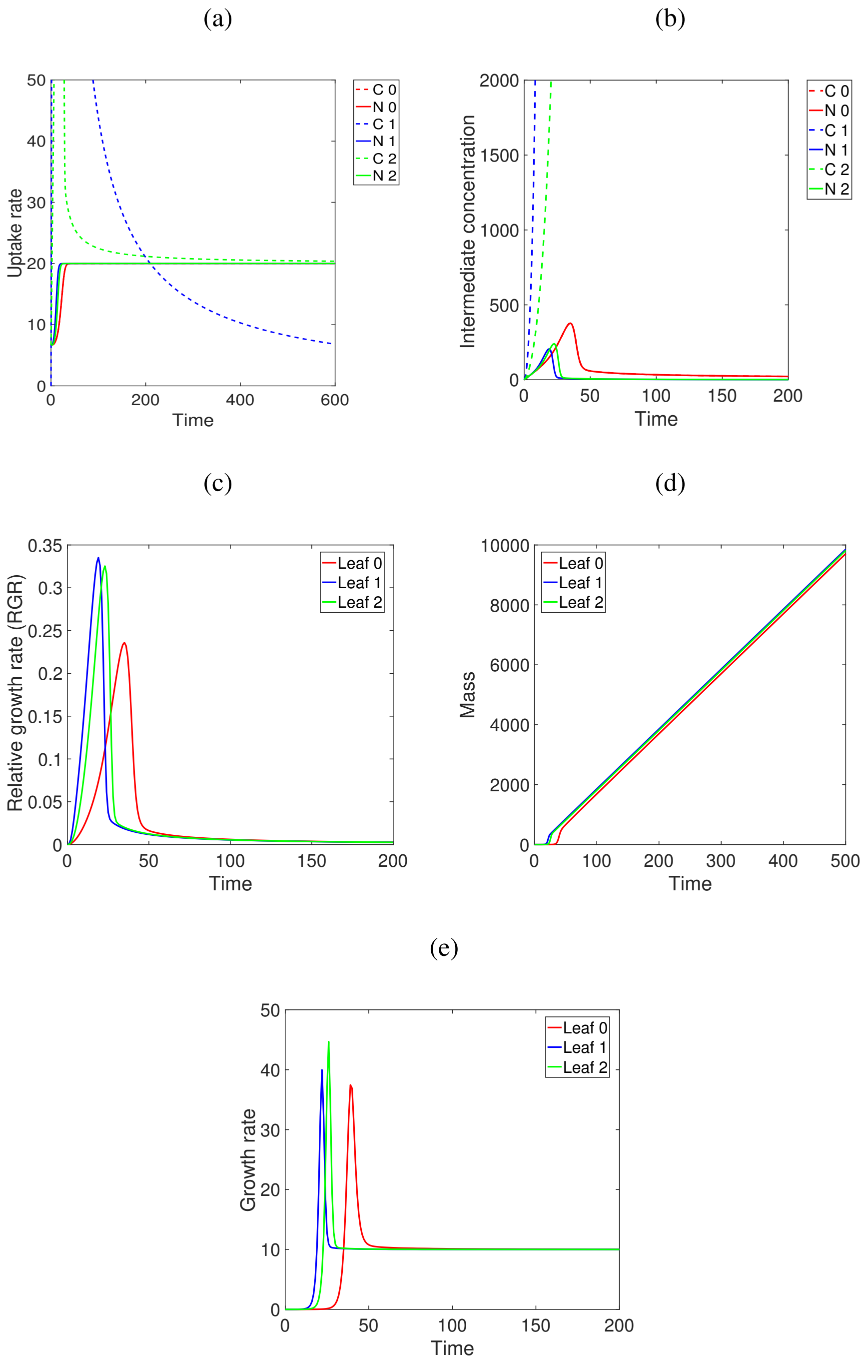


Fig. 5 Model output for equal carbon and nitrogen uptake rate (Case 1) implementing a variety of functions ($0-2$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a positive feedback on carbon uptake rate when nitrogen is high (Feedback 5) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, carbon and nitrogen uptake rates without feedback (red lines) overlap b) intermediate carbon (dashed line) and nitrogen (solid line), carbon and nitrogen without feedback (red lines) overlap c) leaf RGR, root mass is the same as leaf d) leaf mass over time, root mass is the same as leaf e) leaf growth rate over time, root growth rate is the same as for leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Feedback 6: a linear function

Similarly, only linear and scalar functions implement a positive feedback on nitrogen uptake rate when carbon concentration is high. This feedback alters plant growth in the exact same way as feedback 5, therefore the best choice of function is a linear feedback.

Separately, each feedback further increases the disparity between carbon and nitrogen concentration, implying that multiple feedbacks are needed to work together to balance concentrations.

3.2 Case 2: low carbon uptake rate

Feedback 1: a linear function best simulates a negative feedback on carbon uptake rate with high carbon concentrations

Like case 1, only linear and scalar functions implement a negative feedback on carbon uptake rate when carbon is high. As with case 1, a linear function best reflects a negative feedback on carbon uptake rate since a scalar function initially increases carbon uptake rate (Fig. 6a). The linear function has a lower total plant mass than without any feedback and the stepwise functions whereas the scalar function produces a higher total plant mass (Fig. 6d). Figure 6c shows that a scalar function reduces maximum leaf and root RGR from 0.1906 to 0.0468 and the linear function has an even lower maximum RGR of 0.02, implying that the linear function has the strongest feedback on growth. Growth rate reaches a steady state of 4.84 for the linear function, smaller than the steady state of 5 for no feedbacks and the stepwise functions (Fig. 6e). By $t = 1000$, growth rate has not reached steady state for the scalar function. Implementing a linear function creates a slightly lower nitrogen concentration than nitrogen without any feedbacks, whereas scalar increases the concentration of nitrogen when compared to no feedbacks and stepwise functions. Although these functions are reducing carbon uptake rate, they in fact increase final carbon concentration (when $t = 1000$), such that $C = 0.4498$ for scalar, $C = 0.3119$ for linear and for no feedbacks and stepwise, $C = 0.2119$. The only function which simulates a negative feedback on carbon uptake rate with high carbon concentrations whilst meeting all criteria is a linear term.

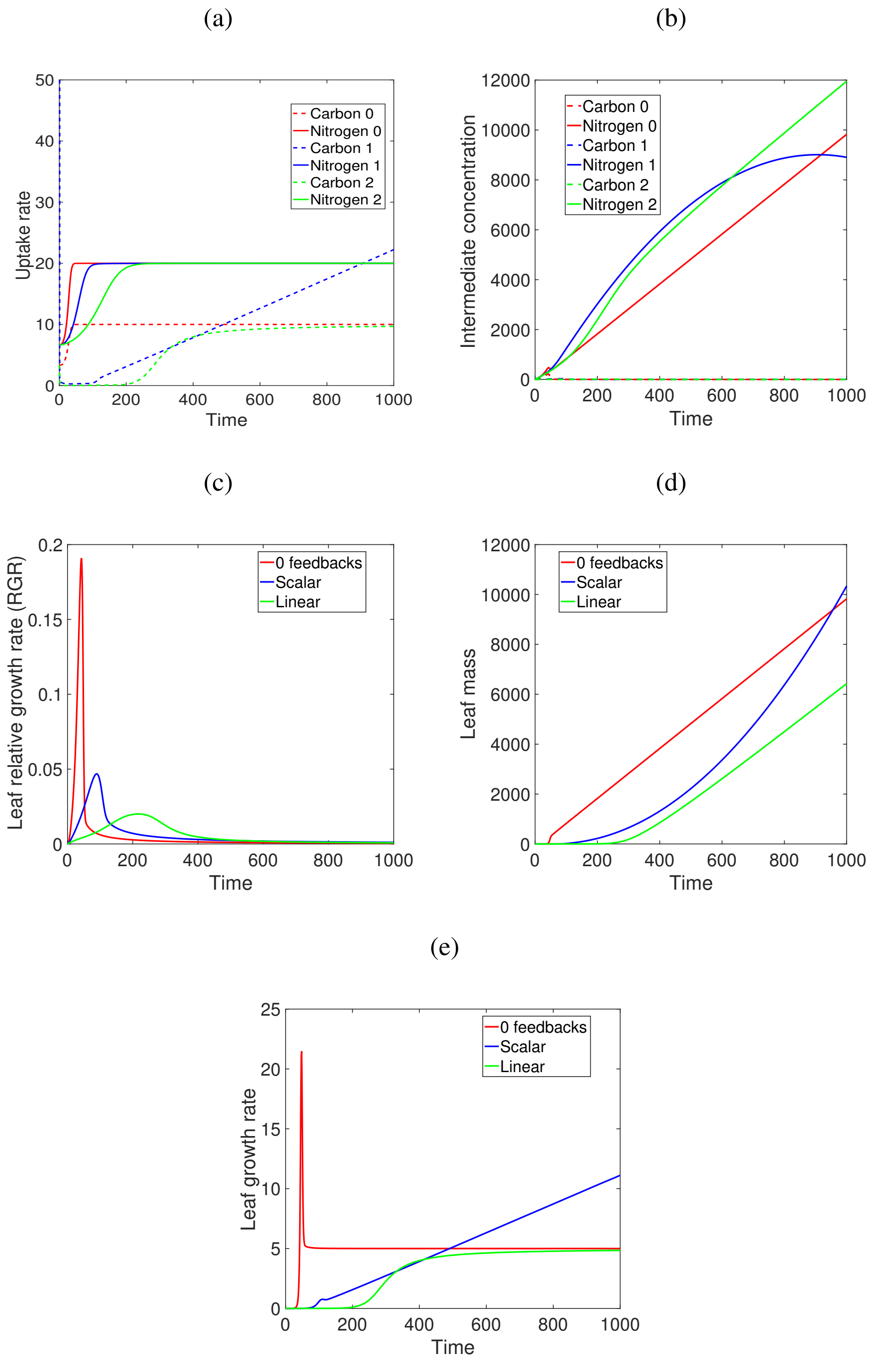
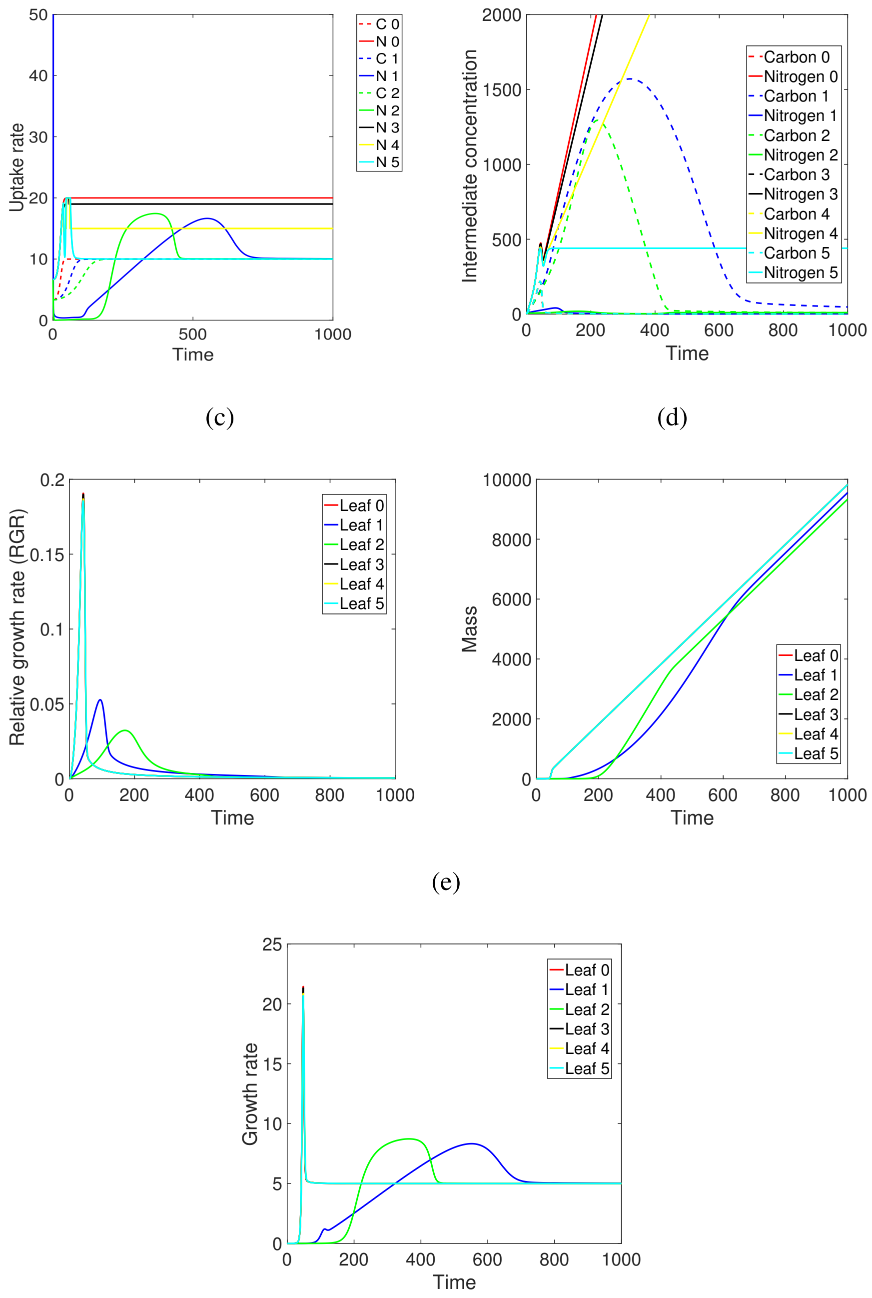


Fig. 6 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-2$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a negative feedback on carbon uptake rate when carbon is high (Feedback 1) for a) carbon (dashed line) and nitrogen (solid line) uptake rate b) intermediate carbon (dashed line) and nitrogen (solid line) , all carbon concentrations are close to zero. c) leaf RGR, root RGR is equal to leaf d) leaf mass, root mass is equal to leaf e) leaf growth rate, root growth rate is equal to leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Feedback 2: a linear function best simulates a negative feedback on nitrogen uptake rate with high nitrogen concentrations

When carbon uptake rate is lower than nitrogen, all functions alter nitrogen uptake rate. A scalar function produces an initial increase in nitrogen uptake rate and therefore does not reflect a negative feedback on nitrogen uptake rate when nitrogen is high. Figure 7a shows that a constant stepwise function (black lines) reduces final nitrogen uptake rate to 19, whilst a fractional (yellow lines) and variable stepwise (cyan lines) function reduces final nitrogen uptake to 15 and 10 respectively. Final nitrogen uptake rate is also reduced to 10 with a linear feedback. This suggests that the linear and variable stepwise functions are the strongest feedbacks. Only scalar and linear functions additionally alter carbon uptake rate in comparison to no feedbacks by delaying the effect of shading. Scalar has the lowest RGR and therefore has the strongest impact on growth but has been discounted due to its unreasonable effect on uptake (Fig. 7c). Following this, linear has the second strongest effect on growth followed by variable stepwise, this is also reflected in the plot of growth rate (Fig 7e). Although the stepwise functions do slightly alter RGR, it is not enough to alter plant mass, therefore only scalar and linear functions decrease total plant mass (Fig. 7d). The strength of functions is reflected in intermediate concentrations, constant stepwise has the least effect on nitrogen concentration, followed by fractional stepwise. It is clear that linear and scalar have the strongest impacts on nitrogen concentration. This shows that all functions except scalar are able to simulate a negative feedback on nitrogen uptake rate with high nitrogen concentration. As linear is the simplest feedback, it is the most reasonable function to simulate a negative feedback.



(a) (b) (c) (d) (e)

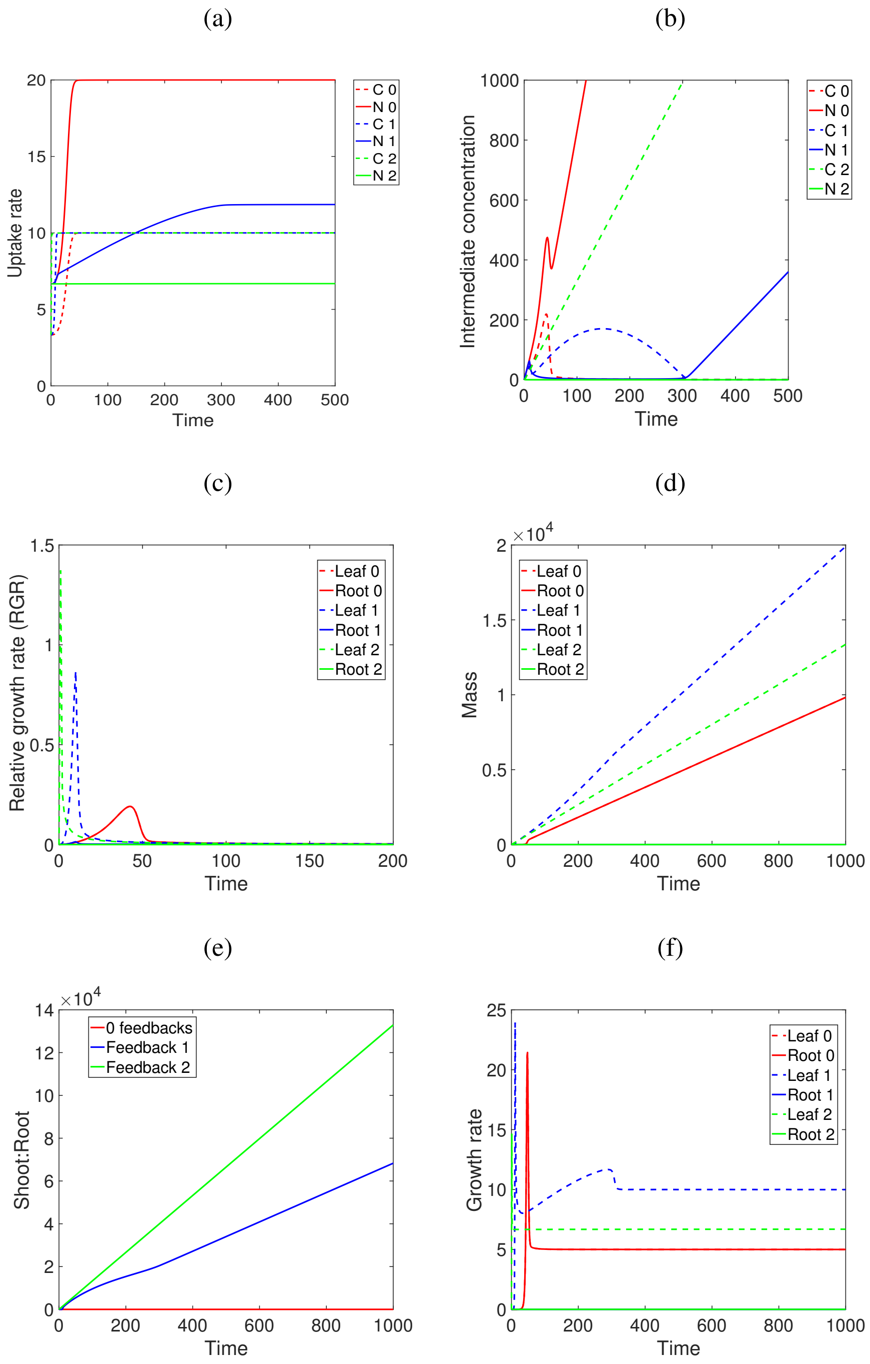
Fig. 7 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-6$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines), 3 - constant stepwise (black lines), 4 - fractional stepwise (yellow lines), 5 - variable stepwise (cyan lines)) to test a negative feedback on nitrogen uptake rate when nitrogen is high (Feedback 2) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, nitrogen uptake using function 5 overlaps carbon uptake without any feedbacks, nitrogen uptake function 3 and function 5. b) intermediate carbon (dashed line) and nitrogen (solid line), carbon without feedbacks, nitrogen with function 1 and function 2, and carbon with function 3, 4 and 5 all overlap each other c) leaf RGR, root RGR is the same as for leaves, leaf RGR using function 5 overlaps that of functions 3 and 4 and without feedback (0). d) leaf mass, root mass is the same as for leaves, leaf mass with function 5 overlaps that of functions 3 and 4 and without feedback (0). e) leaf growth rate, root growth rate is the same as for leaves, leaf growth rate with function 5 overlaps that of functions 3 and 4 and without feedback (0). The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Feedback 3: a scalar function best simulates a positive feedback on leaf growth with high nitrogen concentrations

Relative growth rate becomes negative when implementing constant, fractional and variable stepwise functions, therefore for a positive feedback on leaf growth with high nitrogen, only a scalar or linear feedback function is viable, since in this situation, it is not realistic for a plant to have negative RGR (Fig. 8c). The linear function has a maximum leaf RGR of 1.373, whilst the scalar function produces a maximum leaf RGR of 0.8688. Like feedback 3 when carbon and nitrogen uptake rate are equal, using a linear function creates such a high allocation towards leaf growth that there is no root growth whatsoever (Fig. 8d-e). The effect of a scalar function is not so extreme and is therefore the most viable option to simulate a positive feedback on leaf growth with high nitrogen. A further consequence of this feedback is to reduce nitrogen uptake rate. This is because root mass is not increasing at the same rate as leaf mass and therefore cannot reach its full potential (Fig. 8a). This type of feedback increases the differences in carbon and nitrogen concentration (Fig. 8b). Therefore, a scalar function best simulates a positive feedback on leaf growth with high nitrogen concentration.

Feedback 4: a scalar function best simulates a positive feedback on root growth with high carbon concentrations

Only a linear and scalar function implement a positive feedback on root growth with high carbon, this is reflected in shoot:root mass (Fig. 9d & 9e). Similarly to feedback 3, a linear function has a much stronger feedback onto root growth, such that root RGR has a maximum of 2.3927, whilst scalar produces a maximum root RGR of 0.6830 and leaf and root maximum RGR of 0.1906 for no feedbacks and stepwise functions (Fig. 9c). The linear function has such a strong feedback on root growth that leaf growth is nonexistent. This can be seen in figure 9d, leaf mass is close to zero. Linear and scalar functions have a higher root mass than without any feedbacks and stepwise functions but have almost half the total mass as no feedbacks due to the allocation towards the roots. This high allocation to root growth when using a linear function, leads to a very low carbon uptake rate for linear (Fig. 9a), therefore for a positive feedback on root growth with high nitrogen, a scalar feedback is the most viable function, since it has a weaker feedback on growth. The scalar function still allows carbon uptake rate to increase towards a maximum of 10. This positive feedback on leaf growth creates a much faster effect of shading for nitrogen uptake rate when implementing both linear and scalar functions in comparison to zero feedbacks and stepwise functions. Scalar tends towards the largest steady state root growth rate of 10, followed by no feedbacks and stepwise functions (5), and linear root growth rate reaches 3.35 (Fig. 9f). Although linear has a stronger feedback on root growth with the highest RGR, this feedback becomes weaker with time and has a lower growth rate than all other feedbacks. All feedbacks and zero feedbacks produce high nitrogen and low carbon concentrations. A linear function produces the highest nitrogen and carbon concentration (Fig. 9b). This clearly shows that the most suitable function to simulate a positive feedback on root growth is scalar.



(a) (b) (c) (d) (e) (f)

Fig. 8 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-6$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a positive feedback on leaf growth when nitrogen is high (Feedback 3) for a) carbon (dashed line) and nitrogen (solid line) uptake rate. b) intermediate carbon (dashed line) and nitrogen (solid line), c) leaf (dashed line) and root (solid line) RGR, leaf and root RGR without feedback (red lines) overlap and root RGR for function 1 and 2 are close to zero. d) leaf (dashed line) and root (solid line) mass, leaf and root mass without feedback (red lines) overlap and root mass for function 1 and 2 are close to zero. e) Shoot:root mass over time. f) leaf (dashed line) and root (solid line) growth rate, leaf and root growth rates without feedback (red lines) overlap and root growth rate for function 1 and 2 are close to zero. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

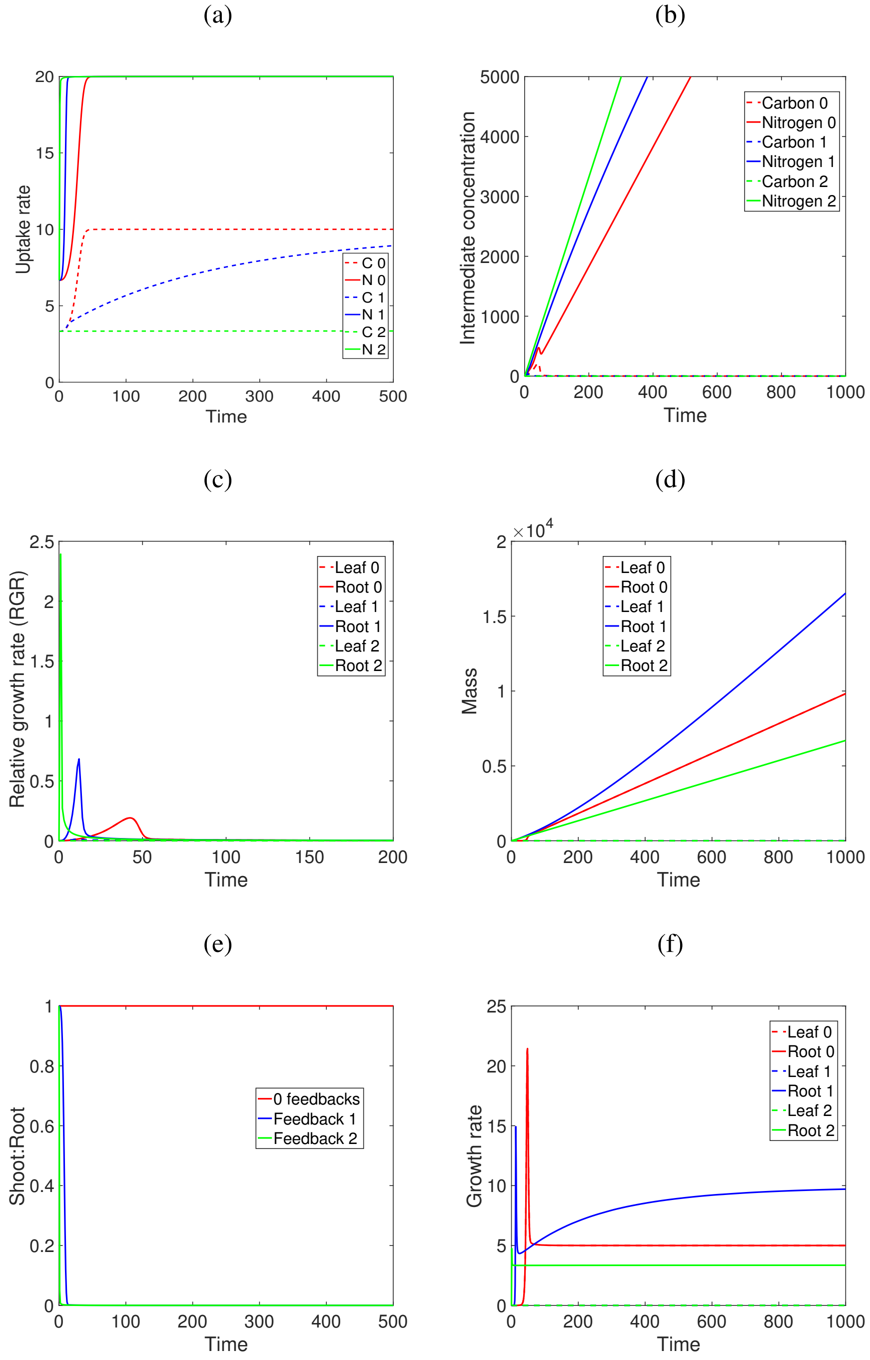

(a) (b) (c) (d) (e) (f)

Fig. 9 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-6$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a positive feedback on root growth when carbon is high (Feedback 4) for a) carbon (dashed line) and nitrogen (solid line) uptake rate b) intermediate carbon (dashed line) and nitrogen (solid line), carbon without feedback overlaps carbon using function 1 and 2 c) leaf (dashed line) and root (solid line) RGR, leaf RGR for $1-2$ overlap and leaf and root RGR without feedback (red lines) overlap d) leaf (dashed line) and root (solid line) mass, leaf and root mass overlap without feedback (red lines) and leaf mass $(1-2)$ overlap. e) Shoot:root mass f) leaf (dashed line) and root (solid line) growth rate, leaf and root growth rate overlap without feedback (red lines) and leaf growth rate $(1-2)$ overlap. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Feedback 5: a fractional stepwise function best simulates a positive feedback on carbon uptake rate with high nitrogen concentrations

All feedback functions have distinct effects on growth when carbon uptake rate is lower than nitrogen uptake rate. The scalar function has the strongest feedback on carbon uptake rate, increasing it to a maximum of 2107 (Fig. 10a). Although this system is dimensionless and therefore the maximum imposed as 20 cannot be linked to experimental results for carbon uptake, a feedback which causes an increase of multiple orders of magnitude larger can be deemed unreasonable and therefore the scalar function is considered too strong. A linear function also creates a very high carbon uptake rate of 252 but quickly reduces in magnitude and fluctuates in size until it reaches a constant uptake of 20. Since, carbon uptake rate is lower than nitrogen uptake rate, without any feedbacks, it is constrained to a maximum of 10. A fractional stepwise function increases this upper bound to 15, a constant stepwise function has an upper bound of 11, a variable stepwise function increases carbon uptake rate to a maximum of 27, where it then decreases to a constant rate of 20. The effects of a positive feedback of high nitrogen on carbon uptake rate is reflected in their RGRs (Fig. 10c). Linear and scalar functions have the highest maximum leaf and root RGRs. They are followed by variable and fractional stepwise functions at 0.2173 and 0.2032 respectively. No feedback function gives the lowest RGR of 0.1906. Similarly, linear and scalar feedbacks produce the largest plants, and zero feedbacks produces the smallest plant mass, whilst fractional stepwise function has the largest plant mass out of the stepwise functions (Fig. 10d). Apart from the scalar function, all other feedbacks increase carbon concentration but nitrogen is still much higher (Fig. 10b). Although the linear function has the strongest effect on uptake rate and growth, the stepwise functions simulate a positive feedback on carbon uptake rate for a longer timespan, therefore a fractional stepwise function best simulates feedback 5.

Feedback 6: a linear function best simulates a positive feedback on nitrogen uptake rate with high carbon concentrations

Similar to case 1 (equal carbon and nitrogen uptake rate), only linear and scalar functions reflect the behaviour of a positive feedback on nitrogen uptake rate when carbon concentration is high. All three types of stepwise function have no effect on growth. The scalar function produces a maximum nitrogen uptake rate of 2623 and decreases towards 4 with time (Fig. 11a). A linear function has a smaller maximum nitrogen uptake rate of 174.5 and tends towards 20 as time increases. Not only does a scalar feedback have too strong a feedback initially on nitrogen uptake rate, increasing it to two orders of magnitude larger than its default maximum, it then becomes much lower than its potential maximum uptake rate (10), therefore a linear function is the only reasonable function to simulate a positive feedback on nitrogen uptake rate when carbon is high. A scalar feedback has the highest maximum RGR of 0.2446, closely followed by the linear feedback (Fig. 11c). They both produce a plant which grows faster than without any feedbacks. This is reflected in total plant mass, where a scalar function produces the largest plant, followed by linear function and then no feedbacks (Fig. 11d). All growth rates tend towards 5 but the scalar function has a growth rate which is slightly lower, with a change of 0.0004 (Fig. 11e). Since, this environmental case has a higher nitrogen uptake rate compared to carbon, nitrogen concentrations are higher than carbon and incorporating a feedback on nitrogen uptake rate only increases this difference between carbon and nitrogen concentration (Fig. 11b). The scalar function produces the largest nitrogen concentration and is 30000 higher than without any feedbacks. A linear function increases nitrogen concentration but not to that extreme. Thus, a positive feedback on nitrogen uptake rate with high carbon concentrations is best simulated using a linear function.

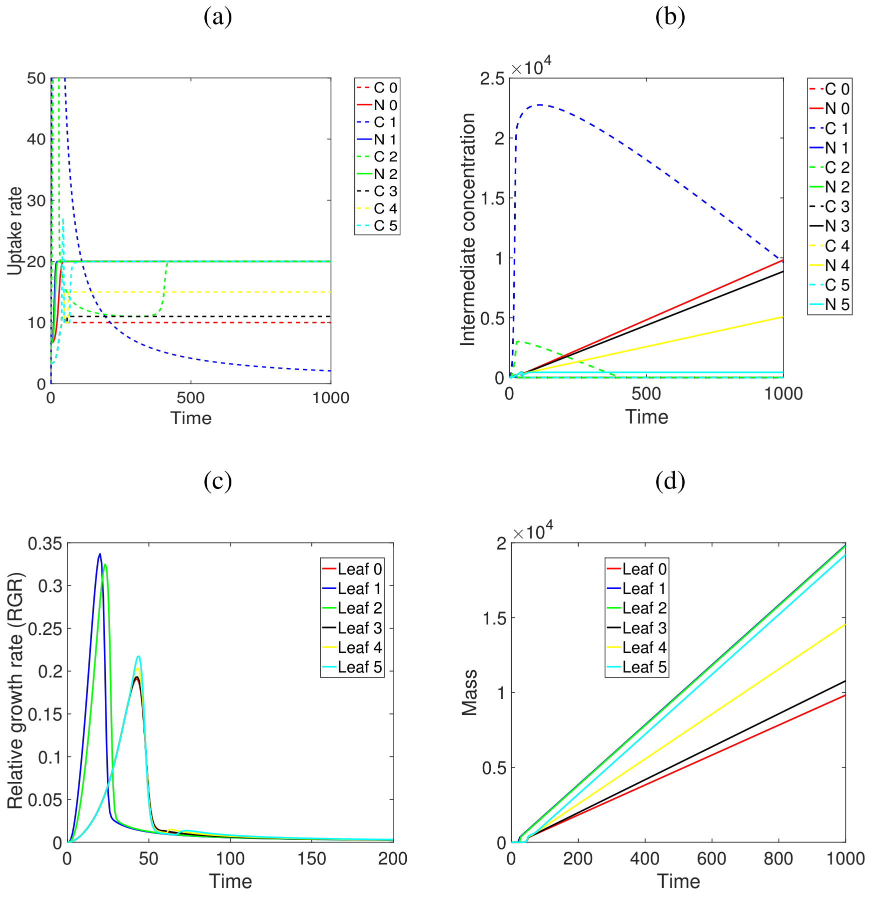

(a) (b) (c) (d)

Fig. 10 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-6$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines), 3 - constant stepwise (black lines), 4 - fractional stepwise (yellow lines), 5 - variable stepwise (cyan lines)) to test a positive feedback on carbon uptake rate when nitrogen is high (Feedback 5) for a) carbon (dashed line) and nitrogen (solid line) uptake rate, nitrogen uptake rate without feedback (red line) overlaps nitrogen uptake rate ($3-5$) b) intermediate carbon (dashed line) and nitrogen (solid line), carbon ($0,3-5$) and nitrogen ($1-2$) overlap c) leaf RGR, root RGR is the same as leaf d) leaf mass, root mass is the same as leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

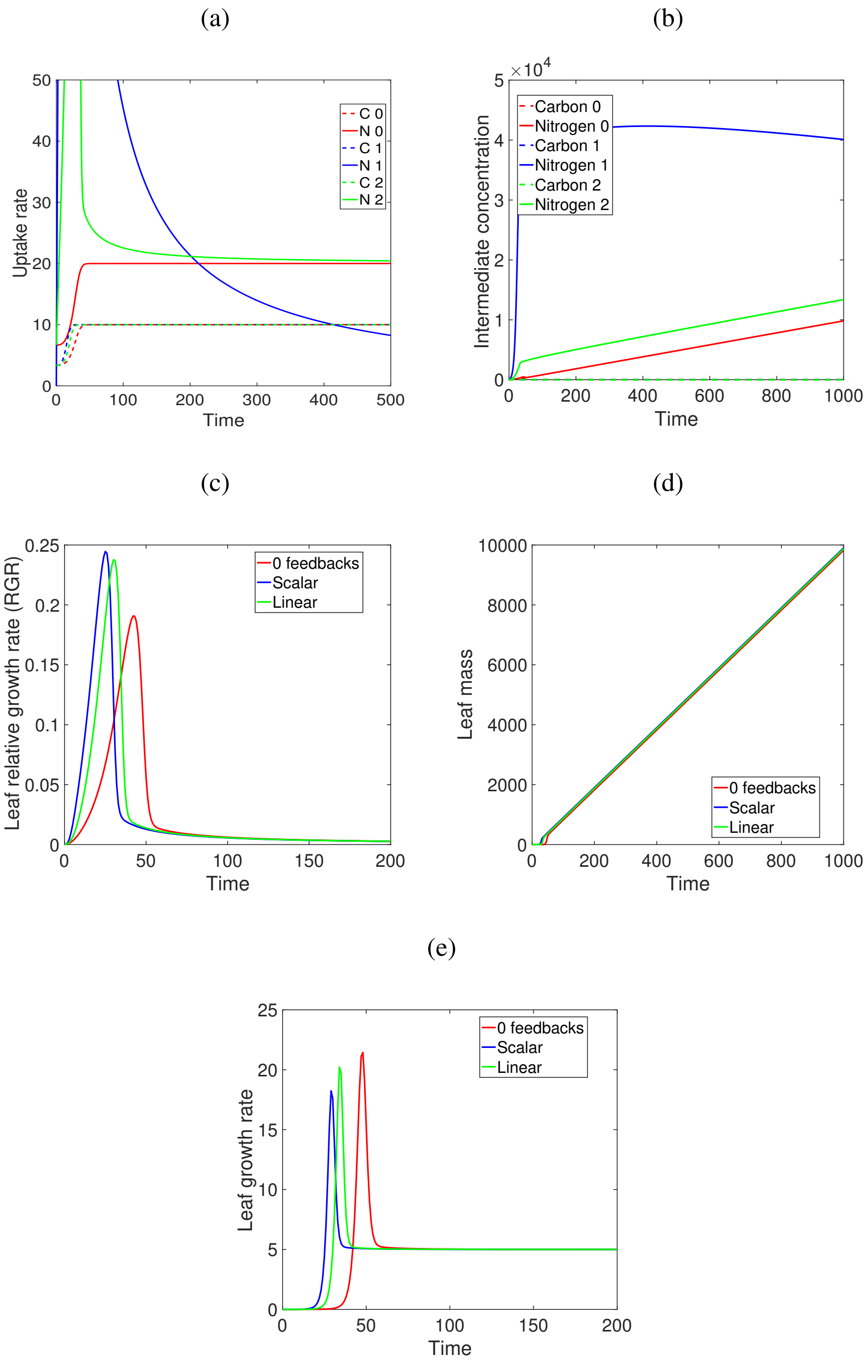


Fig. 11 Model output for a low carbon uptake rate when compared to nitrogen (Case 2) implementing a variety of functions ($0-6$ represent the function type: 0 - without any feedbacks (red lines), 1 - scalar (blue lines), 2 - linear (green lines)) to test a positive feedback on nitrogen uptake rate when carbon is high (Feedback 6) for a) carbon (dashed line) and nitrogen (solid line) uptake rate b) intermediate carbon (dashed line) and nitrogen (solid line), carbon concentrations ($0-2$) are close to zero. c) leaf RGR, root RGR is the same as leaf d) leaf mass, root mass is the same as leaf e) leaf growth rate, root growth rate is the same as leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

3.3 Case 3: low nitrogen uptake rate

Feedback 1: a linear function best simulates a negative feedback on carbon uptake rate with high carbon concentrations

All functions have a different effect on growth when nitrogen uptake rate is lower than carbon uptake rate. Implementing the five functions to simulate feedback 1 has the same effect on growth as feedback 2 when carbon uptake rate is lower than nitrogen uptake rate (case 2). Therefore scalar and linear functions have the strongest impact on leaf and roof RGRs which is reflected in total plant mass, such that the linear feedback has the lowest total plant mass, followed by the scalar function and then zero feedbacks. Since the linear function has the strongest feedback on carbon uptake rate whilst having reasonable behaviour, it is the best option for this feedback.

Feedback 2: a linear function best simulates a negative feedback on nitrogen uptake rate with high nitrogen concentrations

Testing the five functions for feedback 2 produces the exact same results as for a negative feedback on carbon uptake rate when carbon is high (feedback 1) for case 2. This produces the exact same total plant mass, RGR, growth rate, concentrations and uptake rates for each function. Therefore a linear function is the best option.

Feedback 3: a scalar function best simulates a positive feedback on leaf growth with high nitrogen concentrations

Testing the five functions for feedback 3 produces the exact same results as for a positive feedback on root growth when carbon is high (feedback 4) for case 2. Therefore a scalar function is the best option.

Feedback 4: a scalar function best simulates a positive feedback on root growth with high carbon concentrations

Testing the five functions for feedback 4 produces the exact same results as for a positive feedback on leaf growth when nitrogen is high (feedback 3) for case 2. All stepwise functions produce negative growth and a linear function has too strong of an effect on growth, therefore a scalar function is best suited to simulate a positive feedback on root growth when carbon is high.

Feedback 5: a linear function best simulates a positive feedback on carbon uptake rate with high nitrogen concentrations

Testing the five functions for feedback 5 produces the exact same results as for a positive feedback on nitrogen uptake rate when carbon is high (feedback 6) for case 2.Therefore a linear function is the best option.

Feedback 6: a fractional stepwise function best simulates a positive feedback on nitrogen uptake rate with high carbon concentrations

Running the five functions for feedback 6 produces the same results for a positive feedback on carbon uptake rate when nitrogen is high (feedback 5) for case 2. Therefore a fractional stepwise function is the best option since the strongest functions (linear and scalar) produce very high maximum uptake rates.

Each feedback has a function that suits all requirements for growth and some have multiple suitable functions

Tables 2 - 4 show a summary of which functions best simulate each feedback based on which criteria they meet. These are conditions based on growth rate, RGR and uptake rates. A linear function is the most suitable to simulate a negative feedback on carbon uptake rate with high carbon concentrations (Feedback 1) when carbon uptake rate and nitrogen uptake rate is equal (Case 1). For this environmental case and feedback, two functions can simulate the feedback but the linear function is the only one which meets all requirements since, the scalar function initially increases carbon uptake rate drastically. Similarly for feedback 2, case 1, the only function to suitably simulate the feedback is a linear one. Two functions simulate a positive feedback on leaf growth with high carbon (Feedback 3) and therefore the feedback which is less extreme is selected (scalar). This same result occurs for feedback 4. For the rest of the feedbacks in case 1, one function is suitable (linear).

For case 2, a linear function is the only one suitable to simulate a negative feedback on carbon uptake when carbon is high (Feedback 1). This is also the case for feedback 6 (Table 3). For feedback 2, (nitrogen uptake rate decreases with high nitrogen) all functions except scalar meet the requirements on growth. Both scalar and linear functions simulate feedbacks 3 and 4 (root growth increases with high carbon). All functions except scalar are suitable to simulate a positive feedback on carbon uptake rate when nitrogen is high (Feedback 5) and therefore the function which best simulates the feedback and is the strongest is selected (fractional stepwise). When carbon uptake rate is lower than nitrogen, the only instance when stepwise functions are suitable are for feedbacks reducing nitrogen uptake rate and increasing carbon uptake rate. For each environmental case, there are some instances where a feedback isn't necessary. For example, for feedback 1, whilst carbon uptake rate is lower than nitrogen, a further reduction in carbon uptake rate with carbon concentration is only going to increase the differences in carbon and nitrogen. The stepwise functions only alter growth when the conditions of the function are met and therefore the fact that they only alter growth for two out of the six feedback types implies that only feedback 2 and 5 are required to rectify the lack of carbon.

When nitrogen uptake rate is lower than carbon (Case 3), all functions except scalar are suitable to simulate a reduction of carbon uptake rate when carbon is high (Feedback 1). The strongest of these is the linear function (Table 4). For a reduction of nitrogen uptake rate (feedback 2), only a linear function is suitable. Both scalar and linear functions are suitable to simulate a feedback on leaf and root growth (Feedback 3 & 4) where, a scalar function is the best. Similarly, for a positive feedback on carbon uptake rate with high nitrogen concentrations (Feedback 5), a linear function is the only one which meets all criteria. For an increase in nitrogen uptake rate with high carbon (Feedback 6), all functions except scalar simulate the feedback correctly, the best chosen is a fractional stepwise function. When nitrogen uptake rate is lower than carbon, the only feedbacks which stepwise functions are suitable for are reducing carbon uptake rate when carbon is high and increasing nitrogen uptake when carbon is high. As with case 2, these are logical instances to implement these feedbacks onto growth with a lack of nitrogen, suggesting that only these two feedbacks are required for carbon and nitrogen use to become equal.

For all three environmental cases a linear function best simulates a negative feedback on carbon uptake rate when carbon concentrations are high (Feedback 1) and also a negative feedback on nitrogen uptake rate when nitrogen is high (Feedback 2). A scalar function best simulates feedbacks on leaf and root growth when nitrogen and carbon concentrations are high (Feedbacks 3 & 4) for all three environmental cases. For a positive feedback on carbon uptake rate when nitrogen is high (Feedback 5), a linear function is best when uptake rates are equal and when nitrogen uptake is low compared to carbon (Case 3), although for case 2 (low carbon uptake rate), a fractional stepwise function is best. Additionally, for a positive feedback on nitrogen uptake rate when carbon is high (Feedback 6), a linear function is considered best to simulate the feedback when uptake rates are equal (Case 1) and when carbon uptake is low compared to nitrogen (Case 2) but when nitrogen uptake is low in comparison to carbon (Case 3), the fractional stepwise function is best. Although a linear function best simulates feedbacks 5 and 6 for two out of the three environmental cases, a fractional stepwise function is chosen to represent all environments. As the stepwise functions are only active when there is a disparity between carbon and nitrogen concentrations, this can explain why fractional stepwise function is best for some cases and not others. They are simply “switching on and off" when the environment causes major differences between carbon and nitrogen.

Case	**Feedback**	**Function**	**Does it work?**	**Positive RGR**	**RGR close to** 0.3	**constant growth rate**	**Uptake rate close to** 20
1	1	Scalar	Yes	Yes	Yes	No	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	2	Scalar	Yes	Yes	Yes	No	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	3	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	4	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	5	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	6	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-

Table 2 Summary of which five functions meet the following criteria on uptake rates and RGRs and growth rates for all environmental cases for each feedback when carbon and nitrogen uptake rates are equal. These include: if the function simulates the feedback for uptake rates, RGRs and growth rates; RGR is greater than zero; RGR is within the same order of magnitude as 0.3; growth rate reaches steady state and uptake rates are within the same order of magnitude as 20. Bold text identifies which functions meet all criteria. The rest of the entries are empty when a feedback is not simulated or a negative RGR is produced.

Case	Feedback	Function	Does it work?	Positive RGR	RGR close to 0.3	constant growth rate	Uptake rate close to 20
2	1	Scalar	Yes	Yes	Yes	No	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	2	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Fractional stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Variable stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	3	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	Yes	No	-	-	-
		Fractional stepwise	Yes	No	-	-	-
		Variable stepwise	Yes	No	-	-	-
	4	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	5	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Fractional stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Variable stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	6	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-

Table 3 Summary of which five functions meet the following criteria on uptake rates and RGRs and growth rates for all environmental cases for each feedback when carbon uptake rate is low. These include: if the function simulates the feedback for uptake rates, RGRs and growth rates; RGR is greater than zero; RGR is within the same order of magnitude as 0.3; growth rate reaches steady state and uptake rates are within the same order of magnitude as 20. Bold text identifies which functions meet all criteria. The rest of the entries are empty when a feedback is not simulated or a negative RGR is produced.

Case	Feedback	Function	Does it work?	Positive RGR	RGR close to 0.3	constant growth rate	Uptake rate close to 20
3	1	Scalar	No	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Fractional stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Variable stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	2	Scalar	Yes	Yes	Yes	No	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	3	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	4	**Scalar**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	Yes	No	-	-	-
		Fractional stepwise	Yes	No	-	-	-
		Variable stepwise	Yes	No	-	-	-
	5	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	No	-	-	-	-
		Fractional stepwise	No	-	-	-	-
		Variable stepwise	No	-	-	-	-
	6	Scalar	Yes	Yes	Yes	Yes	No
		Linear	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Constant stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Fractional stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
		Variable stepwise	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**

Table 4 Summary of which five functions meet the following criteria on uptake rates and RGRs and growth rates for all environmental cases to simulate each feedback when nitrogen uptake rate is low. These include: if the function simulates the feedback for uptake rates, RGRs and growth rates; RGR is greater than zero; RGR is within the same order of magnitude as 0.3; growth rate reaches steady state and uptake rates are within the same order of magnitude as 20. Bold text identifies which functions meet all criteria. The rest of the entries are empty when a feedback is not simulated or a negative RGR is produced.

4 Multiple feedbacks

Now that the best function to simulate each feedback has been found for all three environmental conditions, all six feedbacks can be implemented at once and compared against the model output with no feedbacks to determine their overall effect on growth.

4.1 Case 1

The inclusion of all feedbacks increases the rate of self-limitation for carbon and nitrogen uptake rate, reaching a total maximum rate of uptake sooner than without any feedbacks (Fig. 12a). Incorporating feedbacks into the model increases maximum RGR from 0.2359 to 1.0742, which is reflected in total plant mass and growth rate (Fig. 12c-e). Carbon and nitrogen concentrations are also much lower with feedbacks than without (Fig. 12b). This implies that these feedbacks improve growth and intermediate concentrations are depleted from growth. This can be expected since there are no deficiencies within this environmental case, carbon and nitrogen are imported at the same rate and are required in the same quantity for growth and therefore these feedbacks are simply enhancing an already suitable environment for growth.

4.2 Case 2

For an environmental condition where there is a lack of light and therefore a reduction in carbon uptake rate (Case 2), carbon uptake rate reaches a maximum of 10, this leads to a smaller RGR than case 1 with no feedbacks and therefore a lower total plant mass. Additionally, the imbalance of carbon and nitrogen uptake rate leads to a surplus nitrogen concentration. Incorporating all feedbacks modifies both uptake rates to become similar to one another over time (Fig. 13a). Both carbon and nitrogen uptake rates are reduced initially and then slowly increase towards a maximum of 10. They both decline at similar rates but initially, nitrogen uptake rate is slightly higher. Carbon and nitrogen uptake rate behave in the same way since all feedbacks on growth happen continuously except for feedback 5 (leaf growth increases when nitrogen is high).

With all six feedbacks, carbon and nitrogen concentrations tend towards 0.0112 and 1.0516 respectively (Fig. 13b). This implies that supply and demand of resources are becoming close to equal as time increases. The incorporation of feedbacks reduces both leaf and root maximum RGR with root

RGR being higher than leaf RGR (Fig. 13c). This is reflected by the plots of plant mass, growth rate and shoot:root ratio (Fig. 13d-f). With feedbacks, the model is rectifying the deficiency of carbon by increasing allocation towards the leaves. Figure 13e shows that shoot:root ratio reaches 2500 which is an extreme difference in leaves and roots. This is a consequence of the type of feedbacks which simulate the increase in leaf growth. Both feedbacks which alter shoot:root ratio are continuous. Carbon concentration is always higher than nitrogen given the environmental case and parameter set, therefore shoot:root ratio will continue to increase until nitrogen concentration overtakes carbon. This increase in root growth, in turn speeds up the effect of self-limitation for nitrogen uptake rate as root mass increases at a faster rate and also reduces carbon uptake rate as leaf mass increases as a slower rate. This strong allocation towards leaf growth produces a smaller plant overall when compared to the situation without feedbacks. This implies that the feedbacks altering leaf and root RGR are too strong, as such a high allocation to one compartment hinders the uptake of one resource.

4.3 Case 3

Since the best functions for each feedback are the same for case 2, the model behaves in the same way. As there is a deficiency in nitrogen, root growth is promoted and therefore producing a higher root RGR than leaf, a large shoot:root ratio and a larger root growth rate.

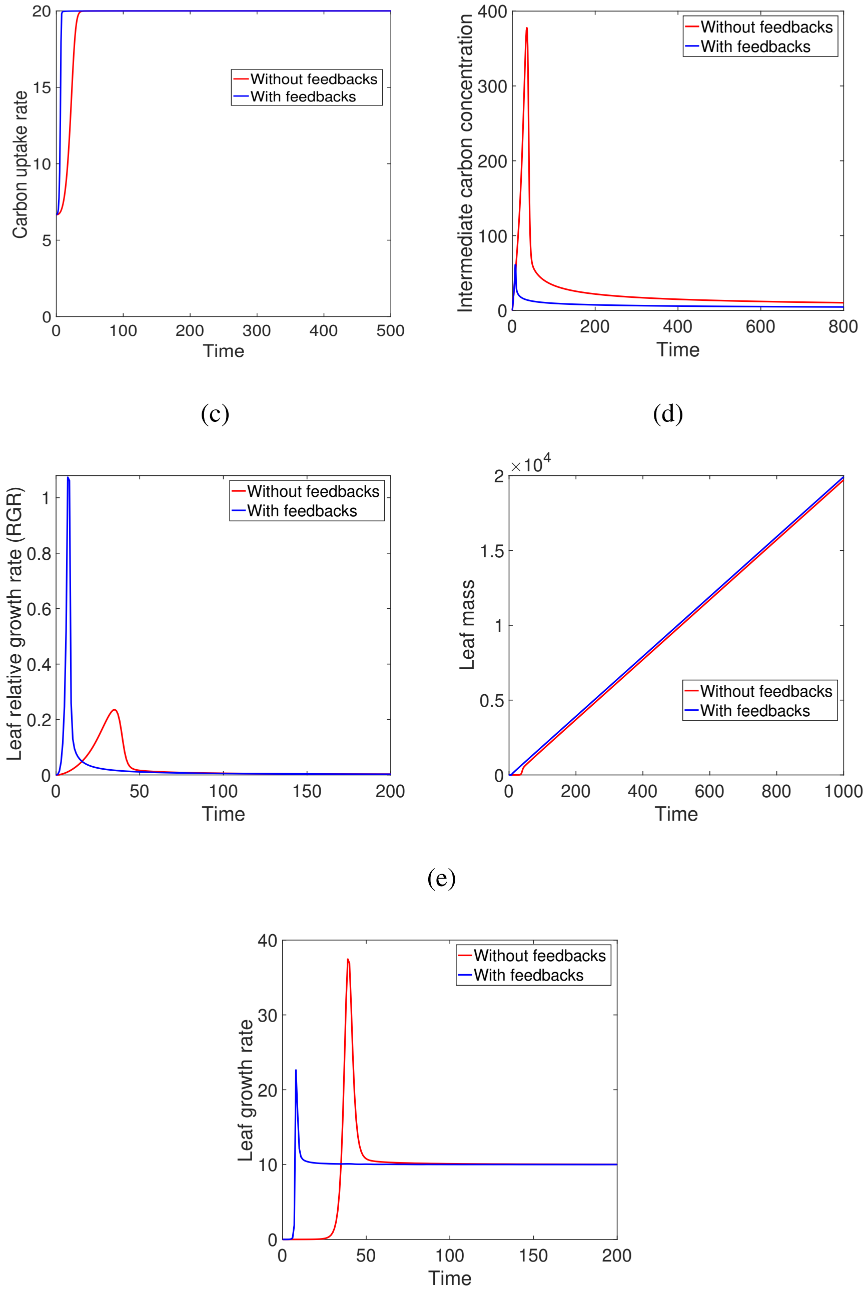

(a) (b) (c) (d) (e)

Fig. 12 Model output when carbon and nitrogen uptake rate are equal (case 1) implementing the best function for all six internal feedbacks for a) carbon uptake rate, nitrogen uptake rate is the same as carbon b) intermediate carbon, nitrogen is the same as carbon. c) leaf RGR, root RGR is the same as leaf d) leaf mass, root mass is the same as leaf e) leaf growth rate, root growth rate is the same as leaf. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

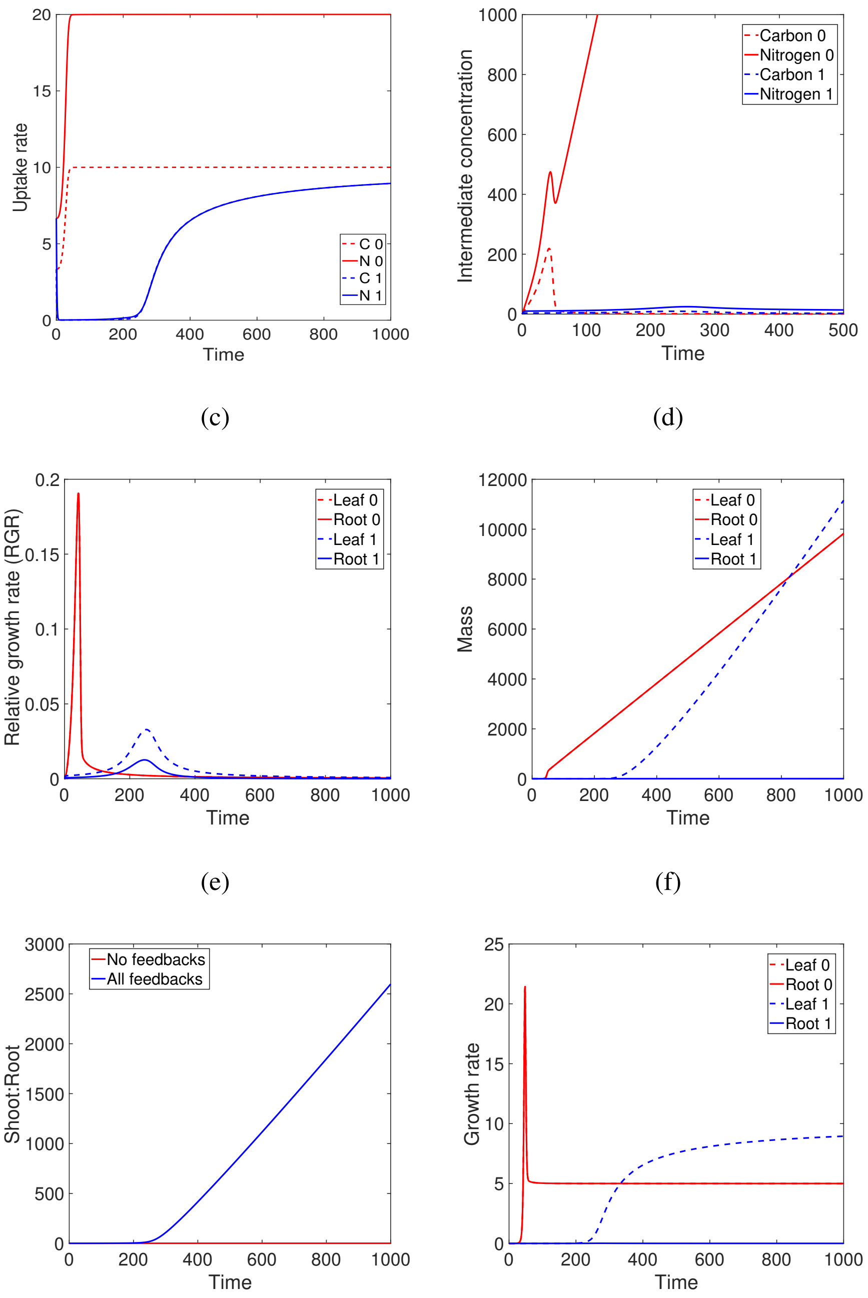

(a) (b) (c) (d) (e) (f)

Fig. 13 Model output when carbon uptake rate is lower than nitrogen uptake rate (case 2) implementing the best function for all six internal feedbacks for a) carbon (dashed line) and nitrogen (solid line) uptake rate, carbon and nitrogen uptake rate with feedbacks (blue lines) overlap b) intermediate carbon (dashed line) and nitrogen (solid line) c) leaf (dashed line) and root (solid line) RGR, leaf and root RGR without feedbacks (red lines) overlap d) leaf (dashed line) and root (solid line) mass, leaf and root mass without feedbacks (red lines) overlap e) Shoot:root mass f) leaf (dashed line) and root (solid line) growth rate, leaf and root growth rates without feedbacks (red lines) overlap. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless). 0 represents model output without any feedbacks and 1 represents model output with feedbacks.

5 Which feedbacks have the strongest effect on growth?

It is clear that implementing all six feedbacks to the model enables the plant to respond to changes in environment but are all six needed for this response? The viability of stepwise functions for only certain feedbacks (Table 2) could indicate that only a couple of feedbacks are needed to rectify environmental changes. The effect of each feedback on growth is compared to determine which feedbacks are the strongest for each environmental case.

5.1 Case 1: equal carbon and nitrogen uptake rates

Figure 14 compares the effect of each feedback on growth against the situation where there are no feedbacks when carbon and nitrogen uptake rates are equal (Case 1). Feedback 6 has the largest effect on nitrogen uptake rate and similarly for maximum carbon uptake rate, feedback 5 is the highest as both reach a maximum of 259.834, and they then decrease until they reach 20. This increase in uptake speeds up the effect of self-limitation for the uptake rate that is not being increased. Feedbacks 3 and 4 have the same effect on nitrogen and carbon uptake rate respectively. Feedback 3 increases the effect of self-shading for carbon uptake rate and slows it down for nitrogen uptake rate. This is the same for feedback 4 but speeds up nitrogen uptake rate and slows down carbon. Carbon uptake rate is slowed down with a feedback on nitrogen uptake rate (Feedback 2) whilst nitrogen uptake rate is greatly altered. This behaviour occurs for feedback 1 but for carbon and nitrogen respectively.

Feedbacks 3 and 4 equally have the strongest effect on leaf and root RGR respectively reaching 0.9632 with very low allocation to the other compartment. The joint second highest RGR is produced by feedbacks 5 and 6 and feedbacks 1 and 2 reduce RGR when compared to zero feedbacks. This behaviour is reflected in the plot for growth rate (Fig. 14e). Implementing negative feedbacks on carbon and nitrogen uptake rates (Feedback 1 & 2) has the strongest effect on total plant mass by reducing mass the most. Increasing carbon and nitrogen uptake rates increases total plant mass by 210, since total plant mass is two orders of magnitude higher, this doesn't visibly alter mass in comparison to without any feedbacks in figure 15d. Since feedbacks 3 and 4 invest almost entirely into leaf and root growth respectively, leaf and root mass represent the majority of total plant mass. This reduction in growth in the alternate compartment dampens its respective uptake rate and therefore reduces growth slightly, such that they create the second smallest plants.

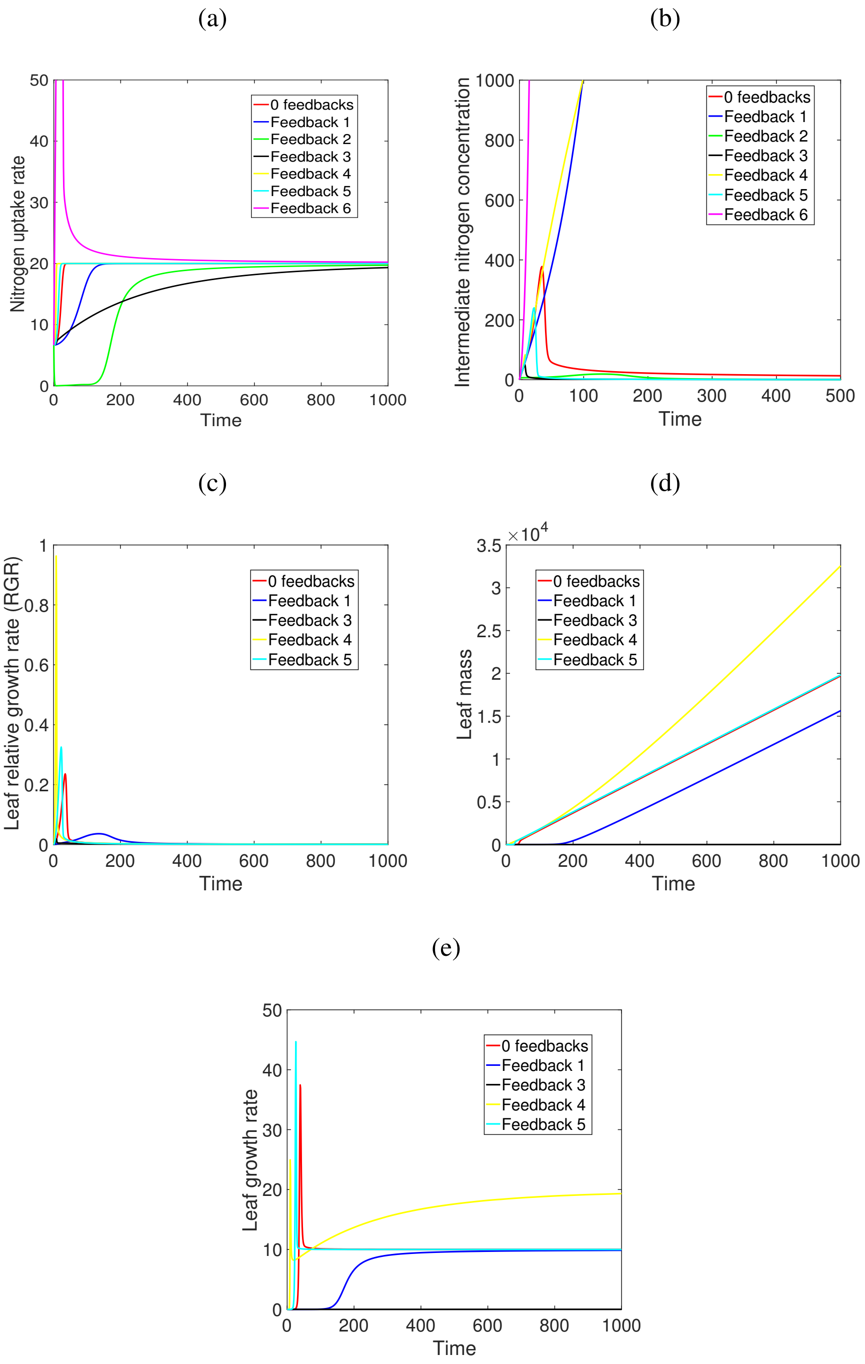

(a) (b) (c) (d) (e)

Fig. 14 Model output when carbon and nitrogen uptake rates are equal (case 1) comparing the effect of each feedback for a) nitrogen uptake rate, carbon uptake is the same as nitrogen for 0 feedbacks, . b) intermediate nitrogen c) leaf RGR d) leaf mass e) leaf growth rate . The model was run with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless). $0-6$ represents feedback type (see Fig. 2). For nitrogen uptake rate and intermediate nitrogen, feedback 1 overlaps carbon (Feedback 2), feedback 2 overlaps carbon (Feedback 1), feedback 3 overlaps carbon (Feedback 4), feedback 4 overlaps (Feedback 3), feedback 5 overlaps (Feedback 6) and feedback 6 overlaps (Feedback 5). For leaf RGR, growth rate and mass, feedback 1 overlaps leaf (feedback 1 & 2) and root (feedback 2), feedback 3 overlaps leaf (feedback 4), feedback 4 overlaps leaf (feedback 3), feedback 5 overlaps leaf (feedback 5 & 6) and root (feedback 6).

When there are no feedbacks, carbon and nitrogen concentration are equal and reach a maximum of 377.8 and decline until they reach 9.2. Each feedback creates a vast difference in concentration between carbon and nitrogen, such that one has a high concentration and the other is very low. Which resource is larger is related to the type of feedback. The feedback which creates the largest difference in concentration between carbon and nitrogen is feedbacks 5 and 6 equally, with a difference of 4020. The second largest difference in concentration is created from feedbacks 3 and 4, therefore feedbacks 1 and 2 have the weakest effect on concentration. Overall, feedbacks which reduce carbon and nitrogen uptake equally have the strongest effect on growth by reducing total plant mass the most, since they are both modelled using the same function. Feedbacks which increase carbon and nitrogen uptake rates have the least effect on growth by only marginally increasing total plant mass.

5.2 Case 2: Low carbon uptake rate

Feedback 6 increases nitrogen uptake rate to a maximum of 174.5154 and then decreases to 20 (Fig. 15a). This makes carbon uptake rate reach a maximum faster than without any feedback. Feedback 5 increases maximum carbon uptake rate from 10 to 12.5 and does not affect nitrogen uptake rate at all. Feedback 2 reduces nitrogen uptake rate to a final value of 10 and slows down the rate at which maximum carbon uptake rate is met. Feedback 4 delays the effect of self-shading substantially such that 10 is still not fully reached by $t = 1000$ whilst nitrogen uptake rate meets a maximum of 20 faster than without any feedback. Carbon uptake rate is reduced and self shading is reduced when implementing feedback 2. Feedback 3 reduces nitrogen uptake rate to 11.85 whilst carbon uptake reaches its maximum faster. This shows that each feedback has very different effects on carbon and nitrogen uptake rates when carbon uptake rate is lower than nitrogen (Case 2).

Feedback 3 increases leaf RGR to 0.8688 whilst root RGR is reduced to 0.014, compared to a leaf and root RGR of 0.1906 without any feedbacks (Fig. 15c). Feedback 3 has the strongest positive effect on growth, followed by feedback 4. Feedback 6 also increases both leaf and root RGR. Feedback 5 only increases RGR marginally. Conversely, feedback 2 reduces RGR to 0.032 and feedback 1 reduces RGR to 0.02, therefore a negative feedback on carbon uptake rate has the strongest negative effect on growth. This is reflected in the plot of growth rate (Fig. 15e) and total plant mass (Fig. 15.d). Feedback 5 produces the largest total plant mass at 24406, followed by feedback 6 and then the model output without any feedbacks (19650). Feedback 1 has the lowest total plant mass at 12852 and therefore has the strongest impact on plant growth. Feedback 3 increases plant mass by 250.

Without any feedbacks, nitrogen concentration is much higher than carbon. The same holds for all feedbacks except for feedback 2, where carbon concentration is higher initially and then tend towards similar values.This implies that feedback 2 has the strongest effect on carbon and nitrogen concentrations. Feedbacks 1, 4 and 6 increase the difference between carbon and nitrogen concentration. Feedback 6 has the largest difference, followed by feedback 1 and then feedback 4. Overall, the feedback which reduces carbon uptake rate (Feedback 1) has the strongest effect on plant growth by reducing total plant mass the most, whilst feedback 3 (increasing leaf growth) has the weakest effect on growth.

5.3 Case 3: low nitrogen uptake rate

The relationship between each feedback and plant growth for case 3 is the same as for case 2 but carbon and nitrogen and leaf and root are swapped. Therefore feedback 2 has the strongest effect on plant growth by reducing total plant mass the most, whilst feedback 4 has the weakest effect on plant growth, and feedback 1 has the strongest effect on internal carbon and nitrogen concentrations.

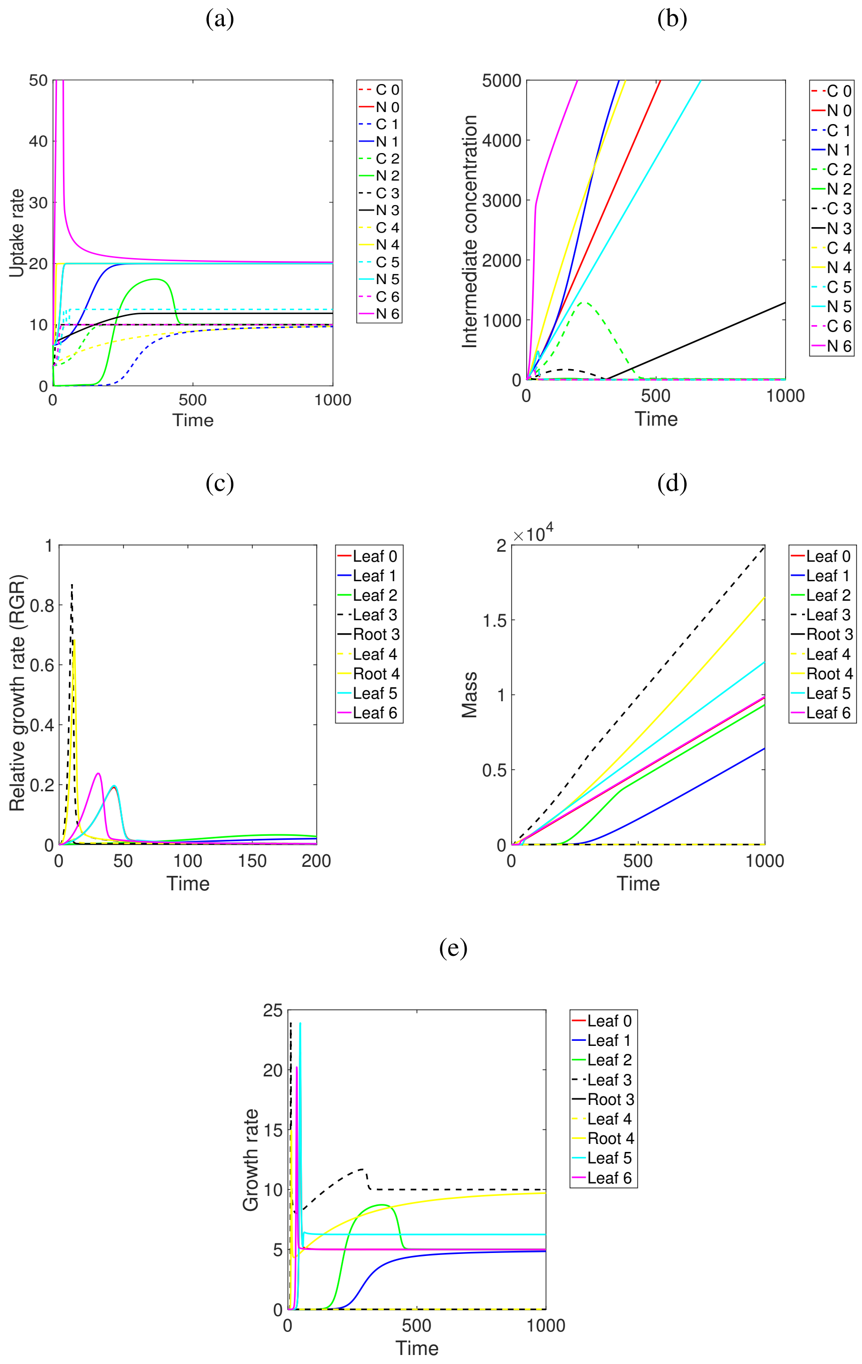


Fig. 15 Model output when carbon uptake rate is lower than nitrogen uptake rate (case 2) comparing the effect of each feedback for a) carbon (dashed line) and nitrogen (solid line) uptake rate, nitrogen uptake rate (feedback 5) overlaps nitrogen without feedback (red line) b) intermediate carbon and nitrogen c) leaf and root RGR d) leaf and root mass e) leaf and root growth rate. For RGR, growth rate and mass, leaf and root is equal for feedbacks $0-2$ and $5-6$ and feedback 5 (cyan lines) overlaps without any feedbacks (red lines). The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).0 represents without feedbacks and $1-6$ represents feedback type (see Fig. 2).

6 Respiration

Choosing to not include respiratory costs of leaves and roots is clearly a simplifying assumption to determine the effect of internal feedbacks on growth, but what happens to the model with respiratory costs? Respiration requires carbon to maintain living leaf and root tissue such that additional loss terms can be added to the equation for carbon. The model becomes:

$$\frac{dC}{dt} = K_c(l) - g_l(C,N)l - g_r(C,N)r - R_1 l - R_2 r; \tag{14}$$

$$\frac{dN}{dt} = K_n(r) - g_l(C,N)l - g_r(C,N)r; \tag{15}$$

$$\frac{dl}{dt} = 2g_l(C,N)l; \tag{16}$$

$$\frac{dr}{dt} = 2g_r(C,N)r; \tag{17}$$

where R_1 and R_2 are the respiratory cost of maintaining leaves and roots respectively. The inclusion of respiration reduces the amount of carbon available for growth. This in turn, reduces maximum RGR and therefore a smaller plant is produced. Figure 16 shows that growth oscillates before reaching a steady state. This is caused by the carbon loss terms. At $t = 38$, the amount of carbon imported via photosynthesis is smaller than the amount required for growth and maintenance:

$$g_l(C,N)l + g_r(C,N)r + R_1 l + R_2 r > K_c(l). \tag{18}$$

Therefore carbon becomes negative and so RGR also becomes negative since growth is dependent upon carbon and nitrogen. Carbon and nitrogen uptake rates reduce in size as plant mass is declining due to negative RGR. This occurs until leaf and root mass become small enough that carbon uptake is greater than what is needed for growth and maintenance. This behaviour repeats until carbon gain and use become balanced. Clearly there is now an imbalance of carbon and nitrogen since, within this framework, the plant uses them both equally except for this additional cost for respiration. This means that nitrogen now accumulates because respiration is taking a proportion of the carbon and there are no internal feedbacks to balance nitrogen uptake with demand. Which internal feedbacks enable the plant to grow?

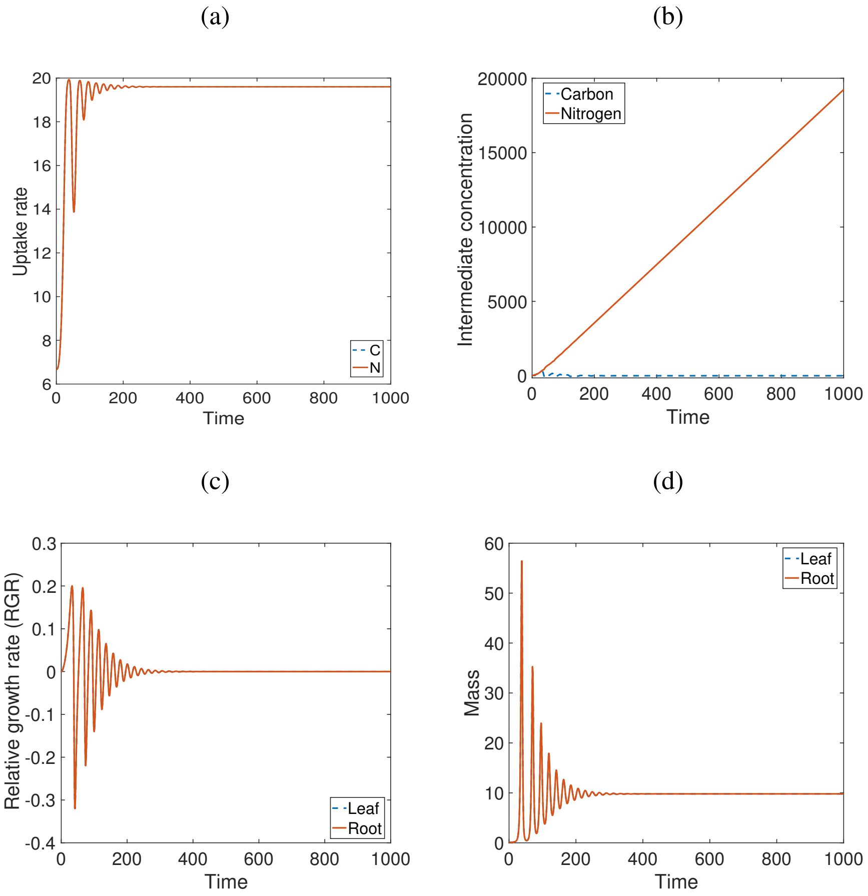


Fig. 16 a) Model output when carbon and nitrogen uptake rates are equal (case 1) with respiration a) carbon (blue dashed line) and nitrogen (red solid line) uptake rate, both lines overlap b) intermediate carbon (blue dashed line) and nitrogen (red solid line) c) leaf (blue dashed line) and root (red solid line) RGR, both lines overlap d) leaf (blue dashed line) and root (red solid line) mass, both lines overlap. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

A negative feedback on carbon uptake rate with high carbon is the only feedback which can stop growth becoming negative (Fig. 17b). Although reducing carbon uptake rate further reduces the amount of available carbon for growth and maintenance costs, the cost of carbon for leaves and roots for growth ($g_l(C,N)l$ and $g_r(C,N)r$) and maintenance ($R_1 l$ and $R_2 r$) are indirectly dependent upon carbon uptake rate. Carbon levels are lower with a reduced carbon uptake rate, which reduces growth rates for leaves and roots. This leads to fewer leaves and roots and in turn leads to a reduction in maintenance costs. The reduction of carbon, dampens the loss terms responsible for causing negative concentrations and therefore simulates growth reasonably. Feedback 1 is a linear loss term on carbon uptake rate such that carbon uptake rate becomes:

$$Kc(l,C) = \frac{A_c l}{\theta_c + l} - \alpha C, \tag{19}$$

where C is carbon concentration and $\alpha = 1$. Feedback 1 produces positive growth when $\alpha \geq 0.48$. A positive feedback on root growth with high carbon (feedback 4) does not stop growth from oscillating but it does make plant growth reach steady state a lot sooner than without any feedbacks. This also happens for feedback 3 (leaf growth increases with nitrogen) and 5 (carbon uptake rate increases with nitrogen) but reach steady state slower than feedback 4 (Fig. 17a). Conversely, a negative feedback on nitrogen uptake rate further increases the frequency of oscillations.

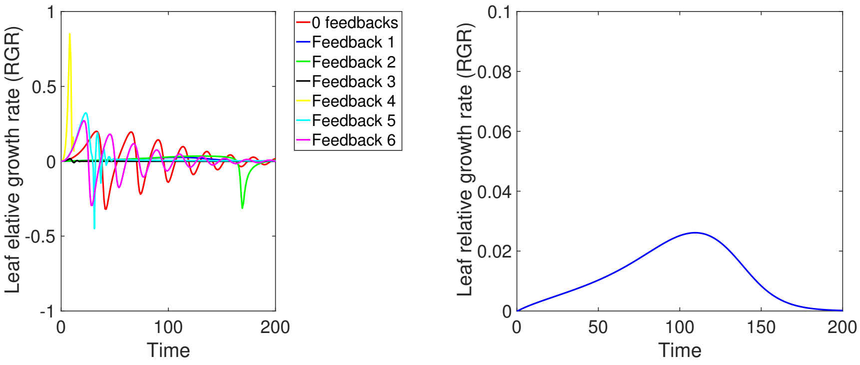


Fig. 17 a) The effect of each feedback on leaf relative growth rate (RGR) for the respiration version of the model when carbon and nitrogen uptake rates are equal (case 1). b) The effect of feedback 1 (high carbon reduces carbon uptake rate) on leaf RGR. The model was run with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

Figure 18 shows the model output with feedback 1. Leaf and root RGR reach a maximum of 0.025 and then decrease towards zero. Maximum carbon and nitrogen uptake rate tend towards the same value (19.6) as without an internal feedback on carbon uptake rate but no longer oscillate. With the inclusion of respiration, plant mass reaches steady state when carbon uptake rate is equal to growth and maintenance; given this parameter set steady state plant mass is 9.8 for leaves and roots. As carbon concentration tends towards zero, so does RGR for leaves and roots. Therefore growth stops when carbon uptake rate and respiration become equal:

$$\frac{A_c l}{\theta_c + l} = R_1 l + R_2 r. \tag{20}$$

Since respiratory costs of maintaining leaves and roots are considered to be proportional to leaf and root mass ($R_1 = R_2 = 1$), total plant mass is equivalent to maximum uptake rate.

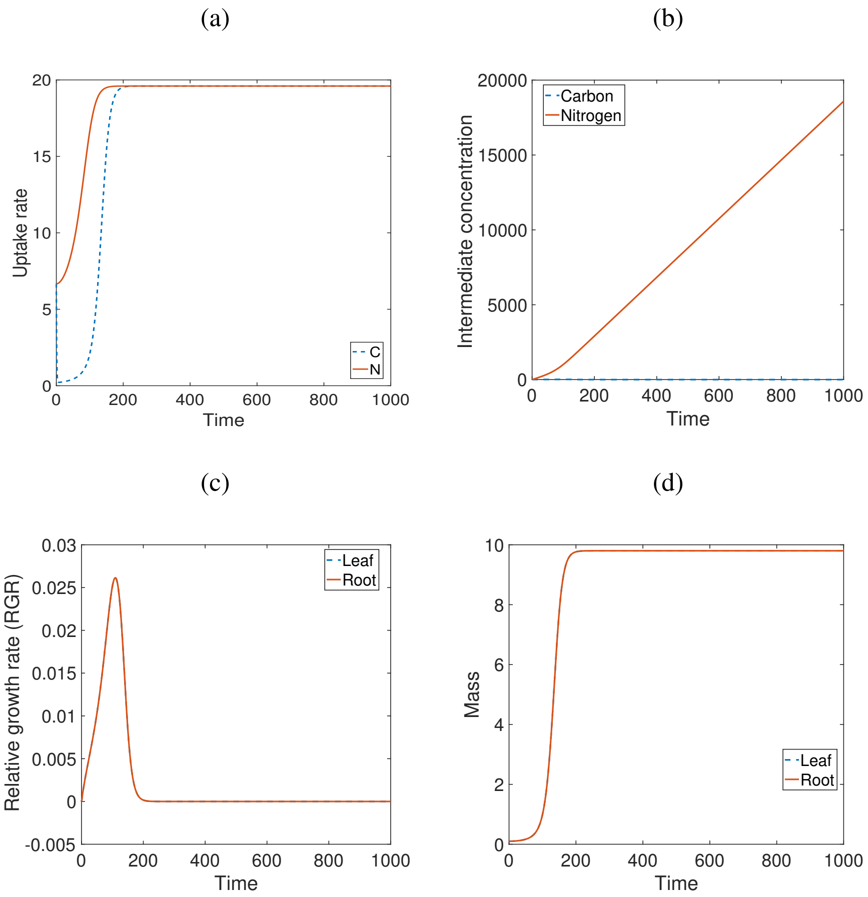

(a) (b) (c) (d)

Fig. 18 Model output when carbon and nitrogen uptake rates are equal (case 1) with respiration and feedback 1 (high carbon reduces carbon uptake rate) a) carbon (blue dashed line) and nitrogen (red solid line) uptake rate b) intermediate carbon (blue dashed line) and nitrogen (red solid line) c) leaf (blue dashed line) and root (red solid line) RGR, leaf and root RGR overlap d) leaf (blue dashed line) and root (red solid line) mass, leaf and root mass overlap. The model was run first using Equation (2) with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

As in the situation without any feedbacks (Figure 16b), carbon concentration remains low whereas nitrogen is continually increasing with time when incorporating feedback 1. Another feedback can be implemented to rectify this excess of nitrogen. There are three types of feedbacks out of the six discussed that respond to high nitrogen concentration:

1. Negative feedback on nitrogen uptake rate with high nitrogen;
2. Positive feedback on leaf growth with high nitrogen;
3. Positive feedback on nitrogen uptake rate with high nitrogen.

Clearly increasing nitrogen uptake rate will only further increase the difference in carbon and nitrogen concentration and increasing leaf growth would attempt to balance the carbon and nitrogen but there would be no active reduction in nitrogen concentration. Figure 19 shows that implementing a negative feedback on carbon uptake rate and a negative feedback on nitrogen uptake rate with high concentrations of carbon and nitrogen respectively stops nitrogen concentration increasing without bounds. Feedback 2 has a strong impact on nitrogen uptake rate as it becomes close to zero instantly. Nitrogen concentration reaches a maximum of 19.6, the same as maximum carbon uptake rate, whilst carbon tends to zero. This reduction in nitrogen uptake rate heavily reduces leaf and root RGR but total plant mass remains the same. This indicates that only two internal feedbacks are required to balance carbon and nitrogen for growth when incorporating respiration into the model. This is specifically for an environmental condition in which carbon and nitrogen uptake rates are equal. Similarly, for environments which create a low carbon uptake rate (case 2) and a low nitrogen uptake rate (Case 3), feedbacks 1 and 2 are all that are needed to balance growth and stop negative plant growth. Total plant mass is halved for both cases whilst carbon is halved for case 2 and nitrogen is halved for case 3.

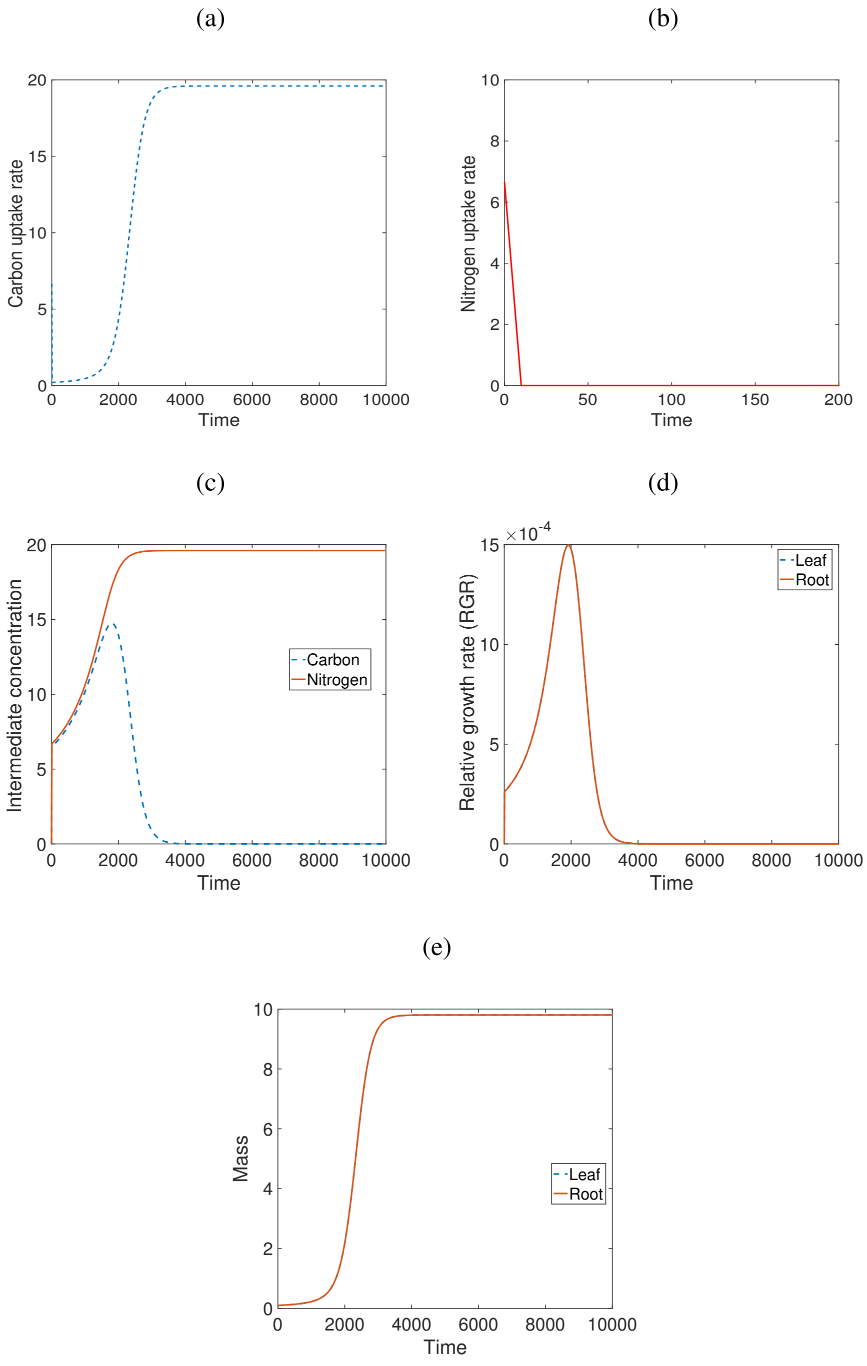


Fig. 19 Model output when carbon and nitrogen uptake rates are equal (case 1) with respiration and feedback 1(high carbon reduces carbon uptake rate) and feedback 2 (high nitrogen reduces nitrogen uptake rate) a) carbon uptake rate b) nitrogen uptake rate c) intermediate carbon (blue dashed line) and nitrogen (red solid line) d) leaf (blue dashed line) and root (red solid line) RGR, leaf and root RGR overlap e) leaf (blue dashed line) and root (red solid line) mass, both lines overlap. The model was run with $A_c = 20$, $\theta_c = 0.2$, $A_n = 20$, $\theta_n = 0.2$, $k = 400$, $v = 1$ with initial conditions $l = 0.1$, $r = 0.1$, $C = N = 0.001$ (dimensionless).

7 Discussion and conclusions

The aims of this chapter were to gain a better understanding of how internal feedbacks on carbon and nitrogen work by determining how to simulate them and which feedbacks have the strongest effect on growth. This was done by using a simple four compartment model of leaf mass, root mass and intermediate carbon and nitrogen. This model provides a framework to further understand how internal feedbacks on growth processes work together to determine the allocation of growth to above and below ground biomass. The results of this chapter show that these feedbacks react to resource deficiencies in ways that are observed experimentally and that incorporating the framework optimises growth by producing a larger plant size. Additionally this framework reduces the disparity between internal carbon and nitrogen concentrations in a model with and without maintenance respiration.

Many models simulate the dependence of growth on two nutrients or more (Ågren et al., 2012; Cheeseman, 1993; Siddiqi and Glass, 1986) and their response to environmental heterogeneity (Yang and Midmore, 2005; Pao et al., 2018) by incorporating dependencies upon nutrient status but do not necessarily address their specific effects on growth by comparing feedback types or including multiple feedback mechanisms. The models which do simulate resource dependencies of uptake rates or growth are limited by either not considering above and below ground material (Ågren et al., 2012), using a functional balance assumption (Bartelink, 1998; Hunt et al., 1998; Shaw and Cheung, 2018) or only focusing on only leaf or root canopies (Dunbabin et al., 2002; Pao et al., 2018). The work of this chapter extends this by allowing the pool sizes of intermediates to feed back on growth and uptake, actively changing source and sink capacities.

Models which do not fully represent whole plant behaviours have been used to investigate how specific feedback mechanisms should be modelled. Klausmeier et al. (2007) simulate an increase in nitrogen and phosphorus uptake rates when internal nitrogen and phosphorus is low respectively. They use stepwise functions and specifically investigate the speed of the transition of these functions. These rates are specific to bacteria.

Stepwise functions only alter growth when simulating a feedback which can rectify a deficiency caused by environmental conditions, therefore these functions are only needed in low carbon and low nitrogen environments. This implies that the stepwise functions accurately simulate a “switch" type feedback since they only alter growth when carbon and nitrogen concentrations can be equalised.

Whether a function can reasonably simulate a feedback is determined by the criteria set. For the model presented here, this limits functions to ones which simulate uptake rates within one order of magnitude to their initial maximum rates and for leaf and root RGRs within one order of magnitude to 0.3, to ensure that feedbacks are not too extreme. These values are assumed to be the maximum rates of carbon and nitrogen uptake rate and maximum RGR without any feedbacks. Altering the criteria determining which functions are reasonable could alter the number of feasible functions for each feedback. Regardless of the criteria set, some functions for feedbacks and environmental cases simply do not reflect the feedback behaviour intended. The types of functions simulating the feedback can alter growth significantly. All functions make the process dependent upon a concentration but the type of function alters when the feedback occurs. Linear and scalar feedbacks continuously alter the process whereas stepwise functions only alter the process for a discrete timespan if certain conditions are met. If both types of function simulate a feedback in a similar way, then the simplest function was chosen. These results show which functions best simulate the overall behaviour of a feedback mechanism but it isn’t necessarily known how these feedbacks work. The results of this chapter show that a linear function best simulate negative feedbacks on carbon and nitrogen uptake rates, a scalar function best simulate positive feedbacks on leaf and root RGRs and a fractional stepwise function best simulates positive feedbacks on carbon and nitrogen uptake rates.

Nunes et al. (2013) show that when imposing sink limitation by reducing temperature or low nitrogen and supplying arabidopsis with sucrose, T6P increases which increases the use of carbon for growth. T6P inhibits the expression of SnRK1 in order to increase growth processes. They present a starvation threshold for sucrose of $3 \mu mol g^{-1}$ (fresh weight) and T6P of $0.3 - 0.5 nmol \mathrm{T6P} g^{-1}$ (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. This finding is specific to Arabidopsis when it is under sink limiting conditions and growth rate is not directly linked to T6P levels. Most experiments do not focus on the time it takes for these feedbacks to affect plant growth or the threshold values or sensitivities of signal and response to pool size, they simply address the time scale of their measurements. 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. More information is needed on these mechanisms and the sensitivity of the responses before their combined effects can be understood.

Incorporating all feedbacks in the model whilst carbon and nitrogen uptake rates are equal increases growth. With this environmental condition, the functions which best simulate each feedback are continuous and therefore “switched on" at all times. This creates a plant which is continuously working slightly harder than without any dependence upon intermediate concentrations. A positive of the scalar feedback function is that when concentrations are high, RGR is increased and if concentrations become very small, this in turn reduces growth. However, if concentrations become very small, this can create an unrealistic change in the process affected. This can enable a two way feedback dependent upon internal concentrations. This increase in growth indicates that a plant which is more responsive to its internal carbon:nitrogen status is better at growing.

Incorporating all feedbacks into the model ensures that carbon and nitrogen uptake rate become equal. When there is a deficiency in carbon uptake, leaf growth is increased. Similarly, when there is a deficiency in nitrogen uptake, root growth is increased. This result confirms the model behaves reasonably to known resource deficiencies (Priestley and Catlin, 1974; Bongarten and Teskey, 1987; Weinstein et al., 1991; Cannell, 1994; Maillard et al., 1999). The positive effect of the feedbacks on RGRs produce high shoot:root or root:shoot ratios depending on the environmental case. Such extremes observed in these results would not occur in nature. This can be avoided by using an additional parameter to dampen their respective strengths.

The use of carbon and nitrogen is assumed to be equal within this framework to determine the effects of feedbacks on carbon and nitrogen. This means that carbon and nitrogen is required equally for growth and this is also the same for leaves and roots. This is an oversimplification since leaves and roots are known to contain different proportions of carbon and nitrogen (Garnier and Laurent, 1994; Yang and Luo, 2011). It is also assumed that carbon and nitrogen uptake rate behave in the same way such that maximum carbon and nitrogen uptake are equal and the rate of shading / inefficiency are equal. For carbon uptake rate, as the number of leaves increase, self shading increases and therefore photosynthesis becomes less efficient over time until reaching a plateau (Farquhar et al., 1980). Nitrogen uptake rate is assumed to be the same, so that the root system becomes less efficient as it is larger. The latter situation could arise either because the roots become less efficient as they age or a larger root system becomes less efficient at exploiting the soil volume (Waisel et al., 2002). For carbon and nitrogen use to be equal, respiration is not included as this would only alter carbon concentration.

When carbon and nitrogen uptake rates are equal, negative feedbacks on carbon and nitrogen uptake rates had equally the strongest impact on growth by reducing total plant mass substantially. When imposing a lack of light, further reducing carbon uptake rate has the strongest impact on growth and similarly, when imposing low nitrogen availability, reducing nitrogen uptake rate impacted growth the most. For the two altered environmental conditions, imposing a feedback onto an environmentally limited process significantly reduced growth. For all environmental cases, increasing resource uptake had the least effect on growth. The feedback functions were simulated using the fewest parameters as possible to ensure direct comparisons between feedbacks. These results shown on feedback strengths therefore could be altered by including additional parameters to alter their respective strengths.

Carbon and nitrogen concentrations become balanced by incorporating only a negative feedback on carbon uptake rate in the situation where there is high carbon, and a negative feedback on nitrogen uptake rate in the situation where there is high nitrogen, especially when including respiration in the model. Including respiration creates a large concentration of nitrogen compared to carbon and therefore a negative feedback on nitrogen uptake is required to balance the two. Including a higher ratio of carbon to nitrogen (as observed in plants) needed to produce leaf and root tissue would only increase this difference between carbon and nitrogen. This argues that there is a need for a negative feedback on nitrogen uptake rate. Plants are not often in situations where there is an excess of nitrogen in the soil and are typically in nitrogen limited situations. Reda (2015) suggests that there is such a negative feedback on nitrogen uptake imposed by products of nitrogen assimilation (glutamine and glutamate). Others found that nitrogen uptake rate is sensitive to internal nitrogen, which can be inhibited via the inhibition of nitrate reductase activity (Siddiqi et al., 1990; Clarkson and Lüttge, 1991; Muller and Touraine, 1992; King et al., 1993; Rufty et al., 1993; Imsande and Touraine, 1994) and induced (Shaner and Boyer, 1976; Wray, 1993; Gojon et al., 1998).

The simplifying assumption that carbon and nitrogen are stored in solitary pools enabled an easy comparison to develop the feedback framework. Internal carbon and nitrogen concentrations have been shown to act as signals to promote these feedbacks in the leaves (Chiou and Bush, 1998; Klein et al., 2000; Reda, 2015) and the roots (Scheible et al., 1997; Paul and Foyer, 2001; Xiong et al., 2013) and therefore this framework should be extended to a model which simulates individual pools of resources for leaves and roots respectively. This work can be extended by incorporating different types of feedback mechanisms, since this model only addresses feedbacks which are induced by high concentrations. A more detailed analysis of which feedbacks are more essential than others is needed to begin to understand how allocation works. This framework needs to be calibrated to experimental data to determine how the feedbacks respond to changes in resource availability.

Overall, this chapter produces a framework of feedback mechanisms observed in nature which can be used to investigate the dynamics of allocation of carbon and nitrogen between above and below ground biomass.

Chapter 4

How does a plant growth model incorporating internal carbon and nitrogen feedbacks respond to environmental heterogeneity?

Abstract

The differential allocation of resources to sources and sinks can greatly alter total plant mass. Allocation is thought to be the consequence of growth processes (i.e uptake rates, transport rates, growth rates) and the communication between them via signalling mechanisms. Feedbacks that alter growth processes are induced in nature by changes in the internal pools of carbon and nitrogen, but how these function together to define allocation remains unclear. This chapter aims to understand how internal feedback mechanisms work together to improve growth by implementing the framework developed in Chapter 3 to a model that has been validated experimentally. How well the framework model responds to changes in carbon and nitrogen availability is determined by simulating external environmental perturbations that influence the uptake of resources, including a combination of two atmospheric CO_2 and two soil nitrogen concentrations along with an additional test of defoliation on leaf mass. Results show that high soil nitrogen increases the positive effect of atmospheric CO2 on total plant mass in the model. High levels of CO_2 and nitrogen increase carbon and nitrogen uptake rate and total nitrogen content. Additionally, high CO_2 increases root:shoot ratio whilst high nitrogen reduces root:shoot ratio. Defoliation increases the positive effect of high CO_2 on growth, specifically it imposes a higher leaf nitrogen content and an increased carbon uptake rate whilst reducing leaf carbon concentration. Overall this shows that a combination of known signalling mechanisms are sufficient to reproduce experimentally observed responses to external resource availability.

1 Introduction

Plant mass is greatly affected by above and below ground resource allocation. Plant growth is sensitive to changes in temperature, light, CO_2, water and nutrient availability. In particular, internal allocation responds to changes in the external environment by tending to alleviate resource limitations.For example, when phosphorus, nitrogen, water is depleted and CO_2 is high, plants invest more into root growth; alternately, when the rate of photosynthesis is reduced due to low levels of irradiance and CO_2, plants will invest more into leaf growth (Priestley and Catlin, 1974; Bloom et al., 1985; Weinstein et al., 1991; Cannell, 1994; Maillard et al., 1999; Drouet and Pagès, 2007).

Plant growth is limited by resource uptake via the roots and influences the allocation of growth to sources and sinks (Running and Gower, 1991). The explicit inclusion of nitrogen allows models to respond to environmental conditions in a more balanced way. Sources and sinks are linked by a series of rules stemming from feedback mechanisms between different processes and the spatial distribution of sources and sinks (Génard et al., 2008). Therefore, understanding the coordination between sources and sinks can provide insight into how to model the allocation growth.

Most plant growth models assume that the allocation of resources is prioritised to sink growth (Davidson, 1969; Hogsett et al., 1985; Ford and Keister, 1990; Ewel and Gholz, 1991; Luxmoore, 1991) and therefore allocation is not often an emergent property within these models. No models attempt to simulate the internal crosstalk between source and sink capacities that balance the uptake, transport and use of resources. To simulate these internal feedbacks, the processes which they alter need to be considered in a model. Here, growth processes which are sensitive to changes in internal carbon and nitrogen are identified; the types of carbon and nitrogen dependencies that are simulated in plant growth models are discussed and the types of feedbacks which are not simulated are pinpointed.

1.1 Resource uptake

Carbon and nitrogen concentrations in the leaves and roots alter the capacities for photosynthesis and nitrogen uptake. High levels of nitrogen in the leaves reduces the capacity for nitrogen uptake rate, whilst high levels of carbon in the leaves and the roots both increase nitrogen uptake rate capacity (Reda, 2015). Additionally, carbon uptake capacity is reduced when leaf carbohydrate concentrations are high and is increased when root nitrogen status is high (Paul and Foyer, 2001). Therefore, to simulate crosstalk between carbon and nitrogen statuses within the plant, photosynthesis and nitrogen uptake rates must be dependent upon internal carbon and nitrogen concentrations.

One model which combines plant architecture and physiological details of plant growth is GRAAL-CN (Drouet and Pagès, 2007). This functional structural plant model of carbon and nitrogen simulates similar feedbacks addressed in Chapter 3: an increase in nitrogen uptake rate with carbon availability; a decrease in nitrogen uptake rate with nitrogen availability; an increase in nitrogen uptake rate with carbon and a decrease in nitrogen uptake rate with nitrogen. The allocation of growth to plant compartments is defined by a ratio of demand and supply. DESPOT is a teleonomic model which simulates the allocation of growth by maximising carbon gain which enables carbon uptake rate to be proportional to leaf nitrogen content (Buckley and Roberts, 2006). Similarly, Pao et al. (2018) investigate the acclimation of photosynthesis to light saturation whilst varying nitrogen availability. Within this framework they simulate the dependence of photosynthesis on leaf nitrogen content, however the model is limited as it only looks at leaf canopy level and does not include the dependence of root growth on plant carbon or nitrogen status.

Although the models discussed above simulate certain dependencies of carbon and nitrogen on their respective uptake rates (Drouet and Pagès, 2007; Pao et al., 2018), there is still a lack of knowledge on how these feedbacks on processes defining growth work and behave collectively with other known feedbacks. Additionally, these models don't simulate feedbacks on sink activity. This gap in the literature identifies the need for a model that simulates the known feedback mechanisms responsible for changes in allocation which are brought about through environmental heterogeneity.

1.2 Transport

Nitrogen is taken up by the roots in an inorganic form and is converted into amino acids and ureides (nitrogen compounds) in the roots and photosynthetically active leaves (Rentsch et al., 2007). These nitrogen compounds are transported in the xylem to developing leaves and roots (Masclaux-Daubresse et al., 2010) and carbon is transported in the form of sugars in the phloem from leaves to developing leaves and roots, but is there any cost to this transportation?

The Münch pressure flow model for phloem transport (Münch, 1930) is an obvious choice to simulate transport as it is widely accepted (Thornley, 1971, 1972; Minichin et al., 1994; Allen et al., 2005; Gould et al., 2005; De Schepper et al., 2013b). The theory underpinning this model states that the translocation of solute is induced by an osmotic pressure gradient. This pressure gradient is created by the loading and unloading of sugars from source to phloem sieve elements and from sieve elements to sink respectively (Taiz & Zeiger, 2006). This means that phloem flow rate is proportional to the difference between the resource in the source and the sink with some transport cost.

$$\text{Phloem flow rate} = \frac{P_{source} - P_{sink}}{R}, \tag{1}$$

where R is transport resistance of the phloem pathway, P_{source} is source pressure and P_{sink} is sink pressure (Münch, 1930; De Schepper et al., 2013b). Münch's theory represents the unloading and loading mechanisms as a solitary source and sink whereas this also occurs throughout the length of the phloem because sources and sinks are distributed along it. This is possible because the sieve tubes are permeable along the phloem instead of impermeable (assumed by Münch)(De Schepper et al., 2013b). Alternatively, Thompson (2006) hypothesises that phloem pressure gradients should be negligible to regulate the movement of solutes between source and sink. There are many theories about how the phloem behaves:

- Many plant species have the same phloem flow rate (Fisher, 1978; Köckenberger et al., 1997; Jahnke et al., 1998; Windt et al., 2006; De Schepper et al., 2013a);
- The phloem is scaled to keep a slow and constant rate of flow (Thompson, 2006; Windt et al., 2006);
- Phloem transport resistance is dependent upon sieve tube length (Thompson and Holbrook, 2003);
- Phloem pressure doesn't scale with plant size (Turgeon, 2010).

Some argue that Münch pressure flow is more applicable to herbaceous species than trees (Turgeon, 2010; De Schepper et al., 2013b). However, it has been confirmed that all vascular plants use the same mechanism for phloem transport by a universal scaling law. This states that leaf length and stem length are linearly related to the third power of the sieve tube radius (Jensen et al., 2011, 2012; De Schepper et al., 2013b). The Münch transport mechanism has been validated mathematically (Henton et al., 2002) and has been refined to simulate the transport of sugars at a higher level of mechanistic detail (Thorpe et al., 2005; Mullendore et al., 2010; Jensen et al., 2011, 2012).

Inorganic nitrogen is primarily transported in the xylem, however nitrogen can be tranferred from the xylem to the phloem for the immediate supply of sinks (Pate et al., 1975; Bailey and Leegood, 2016; Tegeder and Masclaux-Daubresse, 2018) and once assimilated, nitrogen in the form of amino acids is transported in the phloem. Although the xylem and phloem are structurally different, many models assume that either nitrogen is translocated in the phloem (Sheehy et al., 1995) or assume that the Münch (1930) phloem transport mechanism can be used for both carbon and nitrogen (Thornley, 1972; Rastetter et al., 1991; Thornley, 1995; Thornley et al., 1997; Higgins et al., 2012; Barillot et al., 2016). Alternatively, Dewar (1993) simulates instantaneous transport of nitrogen and water from roots to leaves via the xylem, whilst leaf carbon and nitrogen is transported to the roots via the phloem.

Minchin et al. (1993) also simulate phloem transport by extending the work of Thornley and Johnson (1990). This simulated the transport between a source and multiple sinks in order to investigate the dynamics of sink priority. This model accurately represents observed source-sink behaviour. The later work of Thornley strengthens the idea presented in Minchin et al. (1993) and Minichin et al. (1994) which shows that the use of minimal detail to simulate phloem transport is sufficient to represent observed behaviour in whole plants (Thornley, 1995). Thornley argues that all allocation models should use the Münch (1930) transport framework.

Thornley (1972) simulates the allocation of growth to the leaves and roots by using a model simulating transport resistance. This balanced model simulates the translocation of two substrates (carbon and nitrogen) between the roots and shoots of a plant. The “shoot" refers to parts of the plant carry out photosynthesis. The model compartments are split into shoot structure, shoot carbon and shoot nitrogen, and the root is divided similarly. Thornley assumes that growth depends on two main processes: 1. The transport of resources between leaves and roots and its dependence on the carbon and nitrogen concentrations in each compartment (i.e. a Münch-type transport model); 2. The consumption of resources for growth and the dependence of growth rate upon substrate concentration. This model has been widely used in a range of different contexts, these include: tobacco growth (Wann et al., 1978; Wann and Raper Jr, 1984), forest and ecosystem modelling (Rastetter and Shaver, 1992), water transport (Dewar, 1993) and can effectively reflect multiple source-sink interactions (Minichin et al., 1994).

Reynolds and Thornley (1982) and Higgins et al. (2012) also extended the work of Thornley (1972) by making carbon and nitrogen uptake rates sensitive to changes in atmospheric CO_2 concentration, light flux density, soil nitrogen content and atmospheric and soil temperature. Allocation is typically modelled as a functional balance between carbon and nitrogen status (the levels of carbon and nitrogen within the plant) and is calibrated to maximise relative growth rate or constraining the carbon-nitrogen status (Reynolds and Thornley, 1982; Mäkelä and Sievänen, 1987; Bartelink, 1998; Feller et al., 2015). This approach begins to investigate the effect of environmental change on growth by simulating a functional balance between internal carbon and nitrogen. However, it remains a strongly resource-limited approach, whereby external environmental factors impose constraints on resource availability, and the internal allocation of growth to source organs ensures that resources are taken up in the proportions that they are required. No model has yet taken the next step, which is to simulate feedbacks from the internal availability of resources to balance source supply and sink demand for those resources. In particular, sinks are typically treated as having fixed capacities, with activities limited by the flow of resources from sources.

1.3 Use of resources for growth

Thornley (1972) accounts for the use of resources for growth by using a Michaelis-Menten function which makes growth dependent upon both carbon and nitrogen concentration, however this is the extent to which sink activity is considered in plant growth models. They simulate the dependence of growth on resources but not the direct feedbacks on growth capacity which have been observed experimentally. For instance, leaf growth increases when leaf nitrogen concentration is high (Stitt and Krapp, 1999) and when root carbon concentration is high, root growth increases (Paul and Foyer, 2001; Lilley et al., 2012; Sairanen et al., 2012; Stokes et al., 2013; Xiong et al., 2013).

Chapter 3 formalised a framework of internal feedback mechanisms which make resource uptake and growth rates dependent upon internal carbon and nitrogen concentrations. Although it showed that plant growth responds as expected to changes in maximum uptake rates and consequently changes in environment, it was developed within a non-dimensional framework, and there is a need to test this framework within real values.

This chapter investigates how a parameterised model of internal feedback mechanisms based on internal concentrations of carbon and nitrogen responds to changes in atmospheric CO_2 and soil nitrogen availability and to leaf canopy defoliation. Experiments show that high soil nitrogen increases the positive effect of atmospheric CO_2 on total plant mass (Coleman et al., 1993; Farage et al., 1998; Kirschbaum and Lambie, 2015), whilst high levels of CO_2 reduce overall plant nitrogen content (Cotrufo et al., 1998; Curtis and Wang, 1998; Norby et al., 1999; Jablonski et al., 2002; Ainsworth and Long, 2005; Taub et al., 2008) and net carbon uptake against intercellular CO_2 (Coleman et al., 1993; Ainsworth et al., 2003). The model results also show the positive effect of soil nitrogen and CO_2 on total plant mass, however the model produces higher nitrogen concentrations and an increase in net carbon uptake rate against intercellular CO_2 with elevated atmospheric CO_2. Additionally, reducing carbon source size by defoliating the leaves increases leaf nitrogen and carbon uptake rate, whilst reducing leaf carbon content (Rogers et al., 1998). The model also reflects experimental results such that defoliation increases the positive effect of high CO_2 on growth. The aim of this chapter is to implement the non-dimensional framework model developed in Chapter 3 into a widely used and tested transport resistance model and to evaluate the extent to which the parameterised model can reproduce these experimentally observed behaviours in qualitative terms. This chapter shows that the model mostly reacts to changes in CO_2 and nitrogen availability in the same way as experiments carried out on plants, providing a framework to further investigate the dynamics between internal feedback mechanisms underpinning allocation.

2 Assumptions

The model developed here is a unification of Thornley (1972) and the framework of internal feedbacks developed in Chapter 3, thus it 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. First, Thornley assumes that growth of new plant volume (Eq. (5) & (6)) is dependent upon the use and transport of substrate.

Use of substrate

Thornley (1972) chooses the rate of use of substrate for growth to be derived from bisubstrate enzyme kinetics (Dixon and Webb, 1964 - taken from Thornley (1972)) in which the rate of use of carbon is defined as:

$$F(C,N) = \frac{kCN}{1+\sigma_c C+\sigma_n N+\sigma_{cn} CN}, \tag{2}$$

where k is the Michaelis-Menten constant ($(kgmol)^{-1}m^3s^{-1}$) and C is carbon concentration, N is nitrogen concentration. σ_c, σ_n, and σ_{cn} are rate constants which determine the concentrations at which carbon ($(kgmolm^{-3})^{-1}$), nitrogen ($(kgmolm^{-3})^{-1}$) and both carbon and nitrogen ($(kgmolm^{-3})^{-2}$) start to saturate. This function represents the amount of carbon and or nitrogen used for the growth of new plant tissue. This allows the growth of leaves and roots to be dependent upon both carbon and nitrogen. For simplicity, the Michaelis-Menten constants for carbon and nitrogen are assumed to be equal (See Eq. (23) & (24)).

Continuous growth

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 (Eq. (25) & (26)). Growth respiration is accounted for by an efficiency constant (Y_g), assuming that not all resources available (carbon and nitrogen) for growth are translated directly to plant tissue. 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 for perennial plant species where growth will only stop when relative growth rate (RGR) becomes zero. For this to occur, carbon or nitrogen pool sizes in the leaves must be zero and carbon or nitrogen pool sizes in the roots must be zero.

Environmental dependence

Thornley (1972) assumes that carbon and nitrogen uptake rate is constant per unit shoot or root volume. Here, this assumption is modified so that carbon uptake rate is dependent upon atmospheric CO_2 (Eq. (21)) and nitrogen uptake rate is dependent upon soil nitrogen concentration (Eq. (22)). This allows the plant to be responsive to environmental events which may cause changes to external carbon and nitrogen concentrations.

Transport of substrate

The transport of substrate is assumed to follow a Münch mass flow approach 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, whilst transport resistance is ontogenically scaled. As the plant increases in size, the level of transport resistance increases. Thornley (1972) assumes that it takes approximately one day for intermediates to be transported from source to sink. This is reduced to roughly 3 hours in the modified model to increase growth.

Internal feedback mechanisms

The framework of internal feedback mechanisms developed in Chapter 3 are applied to the Thornley (1972) model. Internal feedbacks on growth are simulated by making key processes (resource uptake rates, relative growth rates) dependent upon the internal concentrations 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. The types of feedback mechanisms were chosen to balance each other such that, if a feedback is applied to nitrogen, the same type of feedback is implemented to carbon. Processes are assumed to be dependent upon local concentrations, for example, 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 teleconnections between the specific resource status of a compartment and a 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 linked.

3 Model description

The Thornley (1972) model is an excellent framework to investigate source-sink dynamics. It is a two compartment model (leaf and root) with two substrates moving between them (carbon and nitrogen). It is a system of 6 first order ODEs, where the first four equations represent the four pools of substrate (intermediate carbon and nitrogen concentrations in the leaves and roots) and the final two equations represent the volume of leaf and root tissue (Fig. 1).

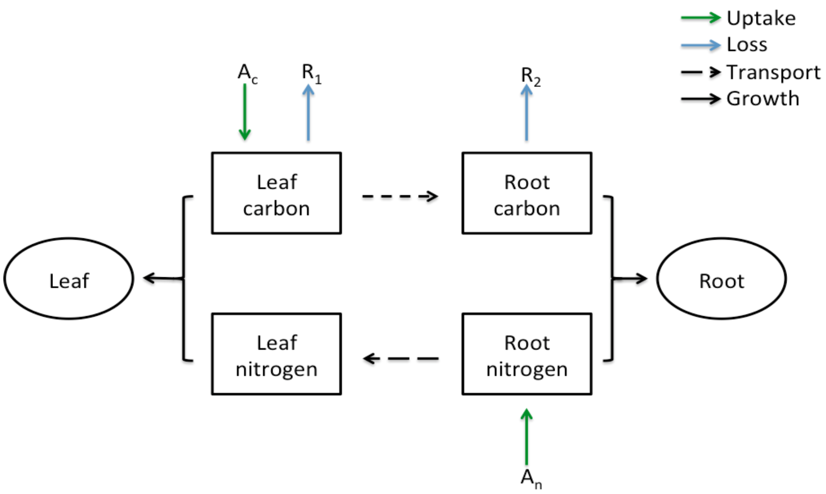


Fig. 1 Diagram of the Thornley (1972) model with maintenance respiration. The boxes represent intermediate carbon and nitrogen concentration per unit leaf or root mass and the circles represent total leaf or root mass. Leaf carbon and root nitrogen concentrations increase via carbon (A_c) and nitrogen (A_n) uptake rates per unit leaf or root volume (green arrows). Leaf carbon and root carbon are reduced 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 volume.

The conversion of substrate to plant tissue incurs an efficiency cost (Y_g). Total leaf carbon concentration depends on the uptake rate of carbon via photosynthesis, the export of carbon to the roots and the cost of producing new plant material. Therefore there is a depletion factor within the model. The concentration of substrate increases via photosynthesis but is decreased via growth based on the amount of substrate already in the pool. The Thornley model is thus:

$$\frac{d(V_lC_l)}{dt} = A_cV_l - \frac{\beta(C_l - C_r)}{r_c} - V_lF(C_l,N_l); \tag{3}$$

$$\frac{d(V_lN_l)}{dt} = \frac{\beta(N_r - N_l)}{r_n} - V_l\lambda F(C_l,N_l); \tag{4}$$

$$\frac{d(V_rC_r)}{dt} = \frac{\beta(C_l - C_r)}{r_c} - V_rF(C_r,N_r); \tag{5}$$

$$\frac{d(V_rN_r)}{dt} = A_nV_r - \frac{\beta(N_r - N_l)}{r_n} - V_r\lambda F(C_r,N_r); \tag{6}$$

$$\frac{dV_l}{dt} = \theta Y_g V_l F(C_l,N_l); \tag{7}$$

$$\frac{dV_r}{dt} = \theta Y_g V_r F(C_r,N_r); \tag{8}$$

where V_l and V_r, are shoot and root volume, respectively (m^3), C_l and N_l are carbon and nitrogen concentration per unit leaf volume ($kgmolm^{-3}$), C_r and N_r are carbon and nitrogen concentration per unit root volume, respectively ($kgmolm^{-3}$), t is time (s), A_c and A_n are carbon and nitrogen uptake rate per unit leaf and root volume respectively ($kgmolm^{-3}s^{-1}$), β is a parameter that describes how transport scales with plant size (m^3), r_c and r_n are carbon and nitrogen transport resistance (s), λ is the N:C ratio of atoms in the plant (dimensionless), θ is the conversion of dry matter to volume ($m^3(kgmol)^{-1}$), Y_g is the efficiency of converting carbon and nitrogen concentrations into plant material (dimensionless) and $F(C_l,N_l)$ and $F(C_r,N_r)$ are functions for the rate of use of substrate (i.e. sink activities $kgmols^{-1}$). In this current form, Equations (3) - (6) represent the total amount of leaf carbon and nitrogen and root carbon and nitrogen within the whole plant.

C_lV_l represents the amount of carbon in all above ground biomass and therefore C_l is the amount of carbon per kg of leaf (i.e. the concentration of carbon intermediates available for growth). For simplicity, the product rule is applied to the differentials in Equations (3) - (6), to obtain size-independent substrate concentration equations. The model becomes:

$$\frac{dC_l}{dt} = A_c - \frac{\beta(C_l - C_r)}{r_cV_l} - (1 + C_l\theta Y_g)F(C_l,N_l); \tag{9}$$

$$\frac{dN_l}{dt} = \frac{\beta(N_r - N_l)}{r_nV_l} - (\lambda + N_l\theta Y_g)F(C_l,N_l); \tag{10}$$

$$\frac{dC_r}{dt} = \frac{\beta(C_l - C_r)}{r_cV_r} - (1 + C_r\theta Y_g)F(C_r,N_r); \tag{11}$$

$$\frac{dN_r}{dt} = A_n - \frac{\beta(N_r - N_l)}{r_nV_r} - (\lambda + N_r\theta Y_g)F(C_r,N_r); \tag{12}$$

$$\frac{dV_s}{dt} = \theta Y_g V_l F(C_l,N_l); \tag{13}$$

$$\frac{dV_r}{dt} = \theta Y_g V_r F(C_r,N_r). \tag{14}$$

Overall plant growth is defined by Equations (13) and (14). This implies that growth is dependent upon a function for the use of carbon and nitrogen per volume, the conversion of dry matter to volume (θ), and a plant mass conversion efficiency (Y_g), which is the equivalent to growth respiration. $F(C,N)$ is a function representing the rate of use of substrate for leaf and root growth (Eq. (2)). The amount of nitrogen used for growth of new tissue, $F(C,N)$ is multiplied by the N:C ratio of atoms in plant tissue (λ). The solutions to the model (Equations (9) - (14)) are found using Ode23s in MATLAB which is a single step solver for stiff systems by calculating the Jacobian matrix of the system at each time step.

$F(C,N)$ represents sink activity and depends on substrate limitation. This is an increasing function of both carbon and nitrogen. The maximum growth rate represents sink capacity (when C and N $\to \infty$), which is given by k/σ_{cn}. For constant N, $N = N_0$, $F(C,N_0)$ is a Michaelis-Menten function of C.

$$F(C,N_0) = \frac{kN_0C}{1+\sigma_n N_0 + (\sigma_c + \sigma_{cn})C}. \tag{15}$$

As $C \to \infty$,

$$F(C,N_0) \to \frac{kN_0}{\sigma_c + \sigma_{cn}}. \tag{16}$$

The rate of change of substrate concentration is dependent upon its import (via uptake if a source, via transport if a sink), the transport to other compartments, and the use of substrate for growth.

The transport term within the model enables a slow feedback on the tissue concentration pools. The transport term is divided by shoot / root volume. In order for dimensions to balance within the model, it is essential for the ontogenic scaling factor for transport to be equivalent to total plant volume ($\beta = V$, where $V = V_l + V_r$). This enables transport resistance to scale with plant volume such that the rate of transport is slower in larger plants. For leaf carbon, the transport term from the leaf to the root is:

$$\text{Carbon transport rate} = \frac{V_l + V_r}{V_s} \times \frac{(C_l - C_r)}{r_c}. \tag{17}$$

This can be written as

$$\text{Carbon transport rate} = \frac{(C_l - C_r)}{r_c} + \frac{V_r}{V_l}\frac{(C_l - C_r)}{r_c}, \tag{18}$$

illustrating the slow feedback on concentrations. The first term in Equation (18) is a general transport term that moves the difference in concentration from the carbon source to the sink. The second term depends on the ratio of root:shoot volume. Hence, if there is much higher root volume than leaf volume, an additional amount at the value of the size difference is transported to the leaves.

This is an internal feedback within the model to counteract any imbalances in the root:shoot (R:S) ratio.

3.1 Thornley default parameter values

Thornley decides to set most of the parameters in $F(C,N)$ equal to zero ($\sigma_c = \sigma_n = \sigma_{cn} = 0$) for simplicity, making the rate of use of carbon and nitrogen a linear function ($F(C,N) = kCN$). However, carbon and nitrogen are limiting factors in plant growth and therefore this function should saturate. Figures 2 and 3 represent the general behaviour of the model using the default parameter values chosen by (Thornley, 1972)(Table 1).

Parameter	Value	Units
A_c - Carbon uptake rate	0.2×10^{-5}	$kgmolm^{-3}s^{-1}$
A_n - Nitrogen uptake rate	0.6×10^{-6}	$kgmolm^{-3}s^{-1}$
β - Ontogenic transport resistance scaling factor	$V = V_s + V_r$	m^3
λ- Ratio of nitrogen to carbon atoms in the plant	0.11	-
r_c - Carbon transport resistance	0.5×10^5	s
r_n - Nitrogen transport resistance	1×10^5	s
k - Michaelis menten constant	9×10^{-4}	$(kgmol)^{-1}m^3s^{-1}$
σ_c - Parameter for use of substrate function	0	$(kgmolm^{-3})^{-1}$
σ_n - Parameter for use of substrate function	0	$(kgmolm^{-3})^{-1}$
σ_{cn} - Parameter for use of substrate function	0	$(kgmolm^{-3})^{-2}$
θ - Conversion of plant volume to plant mass	0.3	$m^3(kgmol)^{-1}$
Y_g - Conversion efficiency of substrate to plant material	0.5	-

Table 1 Default parameter values used in (Thornley, 1972) and definitions.

Since there are no loss terms, plant growth is exponential (Fig. 3a). Total concentration of resource in above or below ground material is the product of concentration and volume, for example for leaf carbon, the total concentration of carbon for all leaves is $C_l V_s$, therefore C_l is the carbon concentration per leaf. Figure 2 shows the pools of nitrogen and carbon concentrations over time. The transport mechanism within the model creates transient oscillatory behaviour of the resources such that leaf and root nitrogen is low when leaf and root carbon is high and vice versa. Resources are being transported from one compartment to the other until concentrations become similar in each pool.

Leaf relative growth rate is:

$$G_l = \frac{1}{V_s}\frac{dV_s}{dt}, \tag{19}$$

therefore substituting (13) gives

$$G_l = F(C_l, N_l)\theta Y_g, \tag{20}$$

which is equivalent to the rate of use of carbon and nitrogen with the cost of conversion of concentration to plant volume. Similarly, root RGR (G_r) depends on root carbon and nitrogen.

The shape of the RGR curve depends entirely upon the Michaelis-Menten function for substrate consumption rate. Initially, leaf and root growth increase equally and quickly reflect the behaviour of the intermediate concentrations such that, when leaf carbon and nitrogen is high, leaf RGR is high, whilst root carbon and nitrogen is low and therefore root RGR is low simultaneously. This can be seen in Figure 3b where leaf and root RGR oscillate until they reach an equal constant relative growth rate given the initial conditions of $l_0 = r_0 = C_{l0} = C_{r0} = N_{l0} = N_{r0} = 0.0001$. Although leaf and root RGR become equal, the ratio of nitrogen to carbon used to form leaf and root tissue and the difference in carbon and nitrogen uptake rates and transport resistances, create larger leaf nutrient pools when compared to root resources and therefore more leaf mass is produced than root mass (Fig. 3c & d).

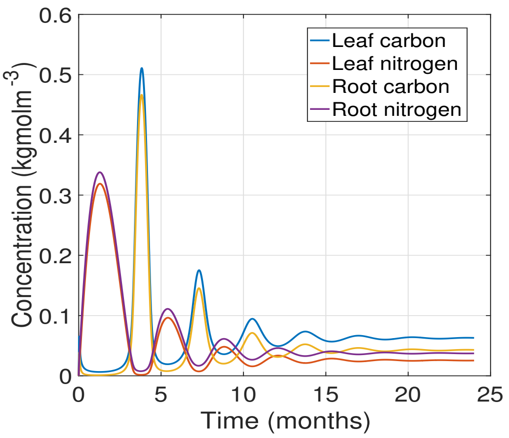


Fig. 2 Carbon and nitrogen concentration per leaf and root respectively. Using default parameter values from (Thornley, 1972)(Table 1). The model was run with initial conditions of $l_0 = r_0 = C_{l0} = C_{r0} = N_{l0} = N_{r0} = 0.0001$.

The model produces a reasonable relative growth rate ($0.02 day^{-1}$, converted from seconds in Fig. 3b) but the other output values of the model appear to be rather large when compared to real plants.

For instance, after 20 months, plant volume reaches $150m^3$ and carbon concentration reaches $0.5kgmolm^{-3}$. This behaviour shows exponential growth without any factors to slow it down.

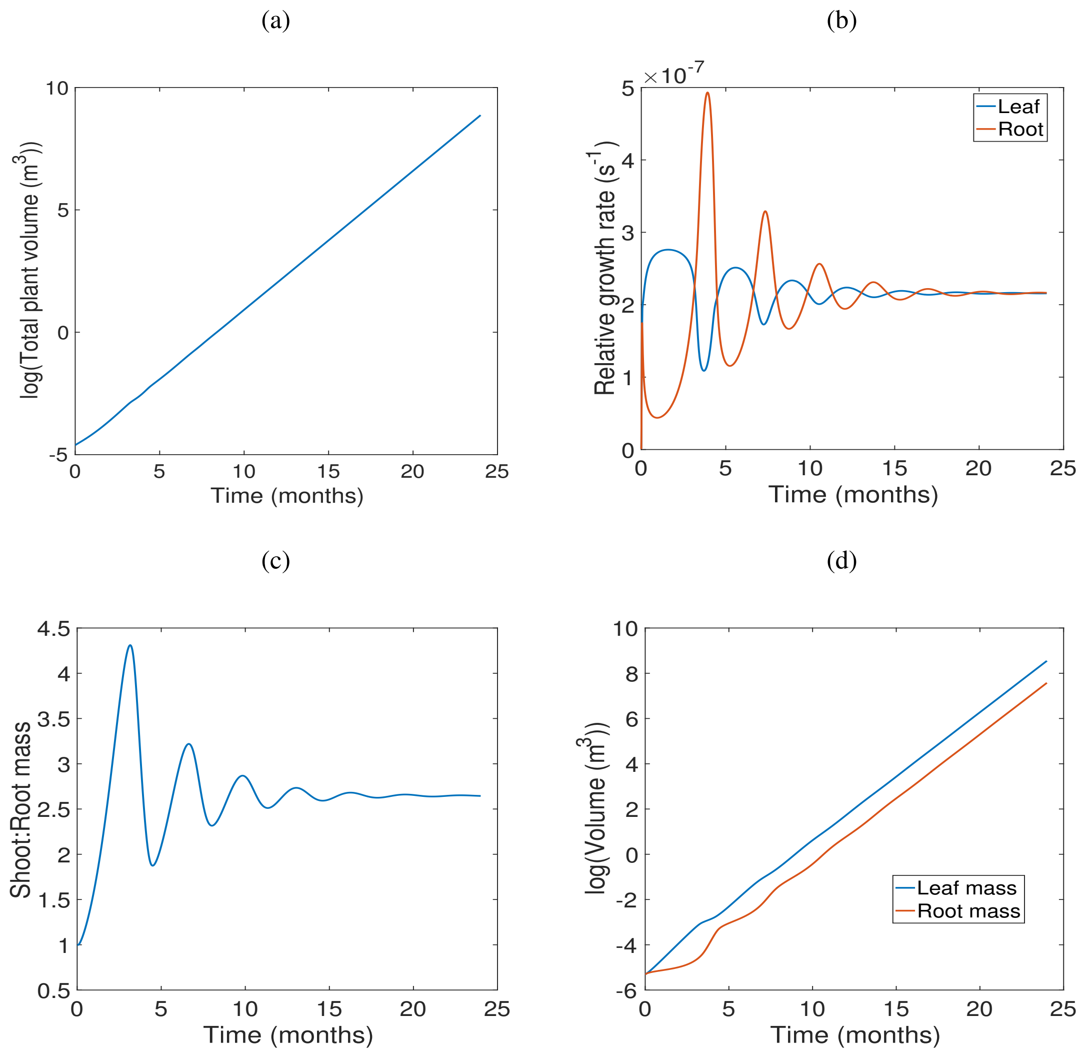

(a) (b) (c) (d)

Fig. 3 Model output (Eq. (9) - (14) & (2)) using default parameter values from (Thornley, 1972)(Table 1). a) Total plant volume. b) Shoot:root ratio. c) Leaf and root relative growth rate. d) Total leaf and root volume. The model was run with initial conditions of $l_0 = r_0 = C_{l0} = C_{r0} = N_{l0} = N_{r0} = 0.0001$.

3.2 Parameterising the model

The aim of this chapter is to implement the feedback mechanisms on growth based on internal carbon and nitrogen concentrations that were developed in Chapter 3 to a mechanistic model of plant growth which simulates transport and use of substrate and to assess how the model responds to changes in environment. Parameter values are chosen based on experimental data using different plant species. 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 specific species but the behaviour of a “generic" plant. Table 2 shows the parameter values used for the modified model throughout this chapter.

Parameter	Value	Units
V_c - Maximum carbon uptake rate	40	$\mu molm^{-2}s^{-1}$
V_n - Maximum nitrogen uptake rate	61	$\mu molkg^{-1}s^{-1}$
k_c - Atmospheric CO_2 concentration at half of V_c	200	$\mu molmol^{-1}$
k_n - Soil nitrogen concentration at half of V_n	103	μmol
ρ - Conversion of atmospheric CO_2 to intercellular CO_2	0.7	$\mu molmol^{-1}m^3$
β - Ontogenic transport resistance scaling factor	V	m^3
r_c - Carbon transport resistance	0.5×10^3	s
r_n - Nitrogen transport resistance	1×10^3	s
θ - Conversion of plant volume to plant mass	0.012	$m^3(kgmol)^{-1}$
Y_g - Conversion efficiency of substrate to plant material	0.66	-
l_0 - Initial leaf mass	0.01	g
r_0 - Initial root mass	0.01	g
C_{l0} - Initial leaf carbon	92.8	$nmolmg^{-1}$
C_{r0} - Initial leaf carbon	63	$nmolmg^{-1}$
N_{l0} - Initial leaf carbon	0.1	$nmolmg^{-1}$
N_{r0} - Initial leaf carbon	7.54	$nmolmg^{-1}$
R_1 - Maintenance leaf respiration	15	$nmolg^{-1}s^{-1}$
R_2 - Maintenance root respiration	10	$nmolg^{-1}s^{-1}$
v - Maximum rate of use of carbon and nitrogen for growth	3600	$(kgmol)^{-1}m^3s^{-1}$
k_1 - Michaelis Menten constant for carbon use	1000	$kgmolm^{-3}$
k_2 - Michaelis Menten constant for nitrogen use	1000	$kgmolm^{-3}$
λ_1 - Ratio of nitrogen to carbon atoms within leaf tissue	0.1	$-$
λ_2 - Ratio of nitrogen to carbon atoms within root tissue	0.2	$-$

Table 2 Default parameter values used in modified Thornley model throughout the chapter and definitions.

Uptake rates

For the model to respond to changes in environment, gross carbon uptake rate is modified to become dependent upon atmospheric CO_2. Originally a constant rate (Thornley, 1972), carbon uptake rate (A_c) becomes:

$$A_c = \frac{V_c \rho c_a}{\rho c_a + k_c}; \tag{21}$$

where V_c is maximum carbon uptake ($40 \mu molm^{-2}s^{-1}$ (Sage, 1994)), ρ is the conversion factor of atmospheric to intercellular carbon (0.7 $\mu molmol^{-1}m^3$), c_a is atmospheric CO_2 ($ppm\ m^{-3}$) and k_c

is the concentration of CO_2 at half of V_c ($200\mu mol mol^{-1}$(Farquhar et al., 1980; Sage, 1994; Katul et al., 2000; Nippert et al., 2007)).

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

$$A_n = \frac{V_n N}{N + k_n}; \tag{22}$$

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

Tissue content

Thornley (1972) uses $\lambda = 0.11$ for the ratio of nitrogen to carbon atoms within plant tissue. In the model here, two separate values are used for leaf ($\lambda_1 = 0.1$) and root tissue ($\lambda_2 = 0.2$). This reflects the different structural nature of the two tissues (He et al., 2006).

Conversion of dry mass to volume

Thornley (1972) uses $\theta = 0.3m^3(kgmol)^{-1}$, whilst the new conversion parameter is $\theta = 0.012m^3(kgmol)^{-1}$ by using a leaf dry density of $10^6 gm^{-3}$(Garnier and Laurent (1994), see appendix A for unit conversion).

Rate of substrate use

The rates of use of carbon and nitrogen for plant growth are simulated in the same way to Thornley (1972) but the number of parameter values is reduced by one:

$$F(C_l, N_l) = v \frac{C_l}{C_l + k_1} \frac{N_l}{N_l + k_2}; \tag{23}$$

and for root growth:

$$F(C_r, N_r) = v \frac{C_r}{C_r + k_1} \frac{N_r}{N_r + k_2}; \tag{24}$$

where $v = k$, $k_1 = 1/\sigma_c$ and $k_2 = 1/\sigma_n$ and assuming that $\sigma_{cn} = \sigma_c\sigma_n$ from Eq. (2). These values are parameterised to achieve leaf and root RGR close to $0.3 day^{-1}$. These values are used throughout this chapter ($v = 3600(kgmol)^{-1}m^3s^{-1}$ and $k_1 = k_2 = 1000 kgmolm^{-3}$). Although this means that the maximum possible RGR is high (using Eq (19)), with the ability to reach $21.6s^{-1}$ (as C and $N \to \infty$) this value is never reached due to source limitations and is typically less than $0.3s^{-1}$. These values were chosen to maximise growth.

Respiration

Additional respiration terms were included in the model to simulate maintenance respiration. Growth respiration is already accounted for in the model by Y_G as a conversion efficiency from concentration to tissue but is changed from 0.5 to 0.66 (dimensionless). The cost of leaf respiration is applied to leaf carbon concentration and root respiration is applied to root carbon concentration. The two equations become:

$$\frac{dC_l}{dt} = A_c - \frac{\beta(C_l - C_r)}{r_c V_l} - (1 + C_l\theta Y_g)G_l - R_1; \tag{25}$$

$$\frac{dC_r}{dt} = \frac{\beta(C_l - C_r)}{r_c V_r} - (1 + C_r\theta Y_g)G_r - R_2; \tag{26}$$

where R_1 is leaf respiration ($15 nmolg^{-1}s^{-1}$) and R_2 is root respiration ($10 nmolg^{-1}s^{-1}$) (Tjoelker et al., 2005). All parameters values taken from experimental data were converted to SI units used in (Thornley, 1972) and the model output was converted back to reasonable units for graphical output (see Appendices A and B for table of parameter values and unit conversions).

Transport resistance

In order to maximise total plant mass, transport resistance values are reduced from 24 hours to 3 hours. Increasing the transport of resources from sources to sinks reduces the limitations on growth brought about by the concentrations of resources in sink tissues (i.e. root carbon & leaf nitrogen).

The red line in Fig. 4a shows the dry weight of the whole plant over 20 days of growth without the cost of maintenance respiration. Incorporating maintenance respiration reduces the total dry mass of the plant by 10.68% after 20 days (blue line). Running the model with parameter values from

Table 2 results in a dry plant mass of $0.0705g$ after 20 days of growth (without respiration) from an initial mass of $0.02g$. With smaller transport resistance values when compared to (Thornley, 1972), leaf and root nitrogen accumulate to much higher levels than carbon in the leaves and roots (Fig. 5a & b). Leaf nitrogen is much larger than root nitrogen and, therefore, leaf growth rate is higher than for roots (Fig. 4d). Leaf RGR reaches a maximum of $0.1day^{-1}$. This value is limited by leaf and root carbon within 20 days as these concentrations are small. Running the model for a longer time span shows variation in maximum leaf and root RGR caused by the translocation of carbon and nitrogen between compartments.

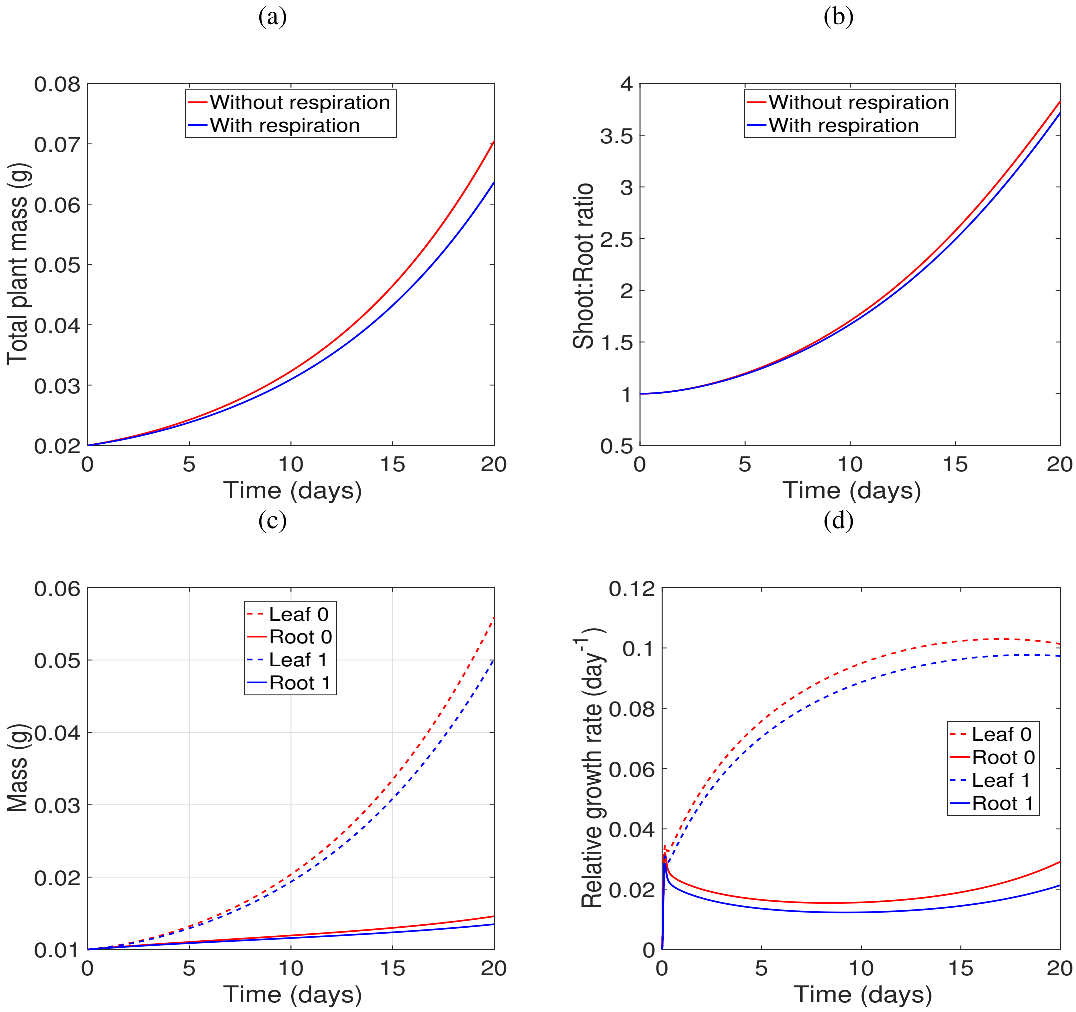


Fig. 4 Parameterised model output using values from Table 2 with (blue lines) and without (red lines) maintenance respiration on a) total dry plant mass. b) shoot:root ratio. c) leaf (dashed lines) and root (solid lines) total dry mass. d) leaf (dashed lines) and root (solid lines) relative growth rate (RGR). When $c_a = 400$, $N = 400$, with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.0 - represents without respiration, 1 - represents with respiration.

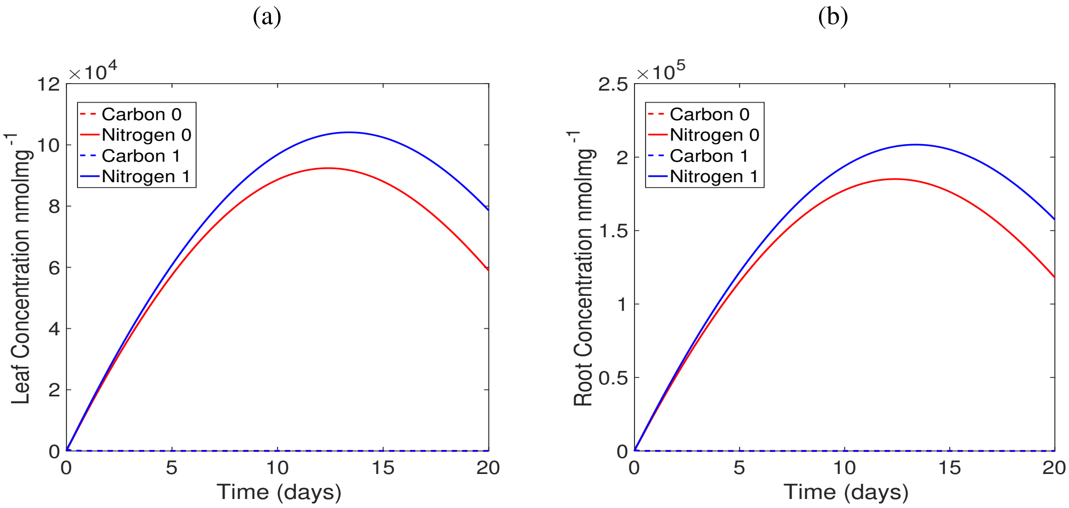


Fig. 5 Parameterised model output using values from Table 2, with (blue lines) and without (red lines) the effect of maintenance respiration on a) leaf carbon (dashed lines) and nitrogen (solid lines) concentration, carbon with respiration (blue dashed line) overlaps carbon without respiration (red dashed line) and are close to zero. b) root carbon (dashed lines) and nitrogen (solid lines), carbon with respiration (blue dashed line) overlaps carbon without respiration (red dashed line) and are close to zero. When $c_a = 400$, $N = 400$, with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8 nmolmg^{-1}$,$C_{r0} = 63 nmolmg^{-1}$,$N_{l0} = 0.1 nmolmg^{-1}$, $N_{r0} = 7.54 nmolmg^{-1}$. 0 - represents without respiration. 1 - represents with respiration.

Increasing maximum nitrogen uptake rate increases total plant mass after 20 days of growth (Fig. 6a). As V_n increases towards 1000 $\mu molkg^{-1}s^{-1}$ total plant mass begins to plateau. This is because nitrogen and carbon are required for growth, as nitrogen uptake tends towards 1000 $\mu molkg^{-1}s^{-1}$, carbon uptake rate becomes limiting. Maximum carbon uptake rate also has a positive relationship with total plant mass and starts to plateau as nitrogen uptake becomes limiting (as V_c tends towards 1000 $\mu molm^{-2}s^{-1}$). The rate at which total dry plant mass reaches a plateau when varying maximum carbon uptake is not as defined as for nitrogen. There are a lot of parameter values which determine the ratio of use of carbon and nitrogen, setting the ratio of nitrogen to carbon atoms within leaf and root tissue to be more equal ($\lambda_1 = \lambda_2$) produces a smoother curve. Although the relationship between carbon uptake rate and total plant mass is similar to nitrogen, carbon uptake rate produces roughly twice the total plant mass when compared to varying total nitrogen uptake rate. This can be explained by the parameterisation of the model. A much larger proportion of plant mass is made up of carbon for leaves and roots than nitrogen (leaves are 90% carbon ($\lambda_1 = 0.1$) and roots are 80% carbon ($\lambda_2 = 0.2$)).

The model results are very sensitive to initial seedling size (which would arise ecologically through variation in seed size (Fig. 6c)). Total dry plant mass after 20 days is proportional to initial seedling mass. This is a product of exponential growth. The range of parameter values for leaf and root maintenance respiration is limited as maintenance respiration has a negative relationship with total plant mass and leaf and root RGR. When maintenance respiration is low, the plant is able to carry out exponential growth. As respiration increases, the amount of available carbon for tissue production is limited until the amount of carbon needed for maintenance becomes larger than the amount imported via photosynthesis. At this point RGR becomes negative. This represents an unrealistic parameter space since plants would alter internal processes to avoid a negative RGR. For the parameters used (see appendix), maintenance respiration for leaf and root tissue cannot exceed $56nmolg^{-1}s^{-1}$ equally (Fig. 6d). The ratio between carbon uptake rate and maintenance respiration must be large enough to sustain growth.

Transport resistance has a negative relationship with total plant mass, therefore increasing resistance reduces total plant mass (Fig. 7). This effect doesn't occur when transport resistance is almost non-existent ($0.01s$), where increasing transport resistance increases total plant mass. This is a consequence of the transport resistance parameter, since it divides the amount of resource being transported. Therefore really small values cause instability within the model. Once transport resistance increases past $1s$, total plant mass decreases. Varying parameter values responsible for the rate of use of substrate for growth (RGR) has little effect on total plant mass since RGR is limited by concentration availability given the default parameter set. This implies that in its current state (without feedbacks), the plant is source limited. Increasing the ratio of nitrogen to carbon atoms that make up leaf and root mass decreases total plant mass. Additionally, increasing the parameters which represent the concentration of carbon and nitrogen, when carbon and nitrogen uptake rate is half of its maximum respectively (k_c and k_n), reduces total plant mass because doing so reduces RGR for given levels of carbon and nitrogen.

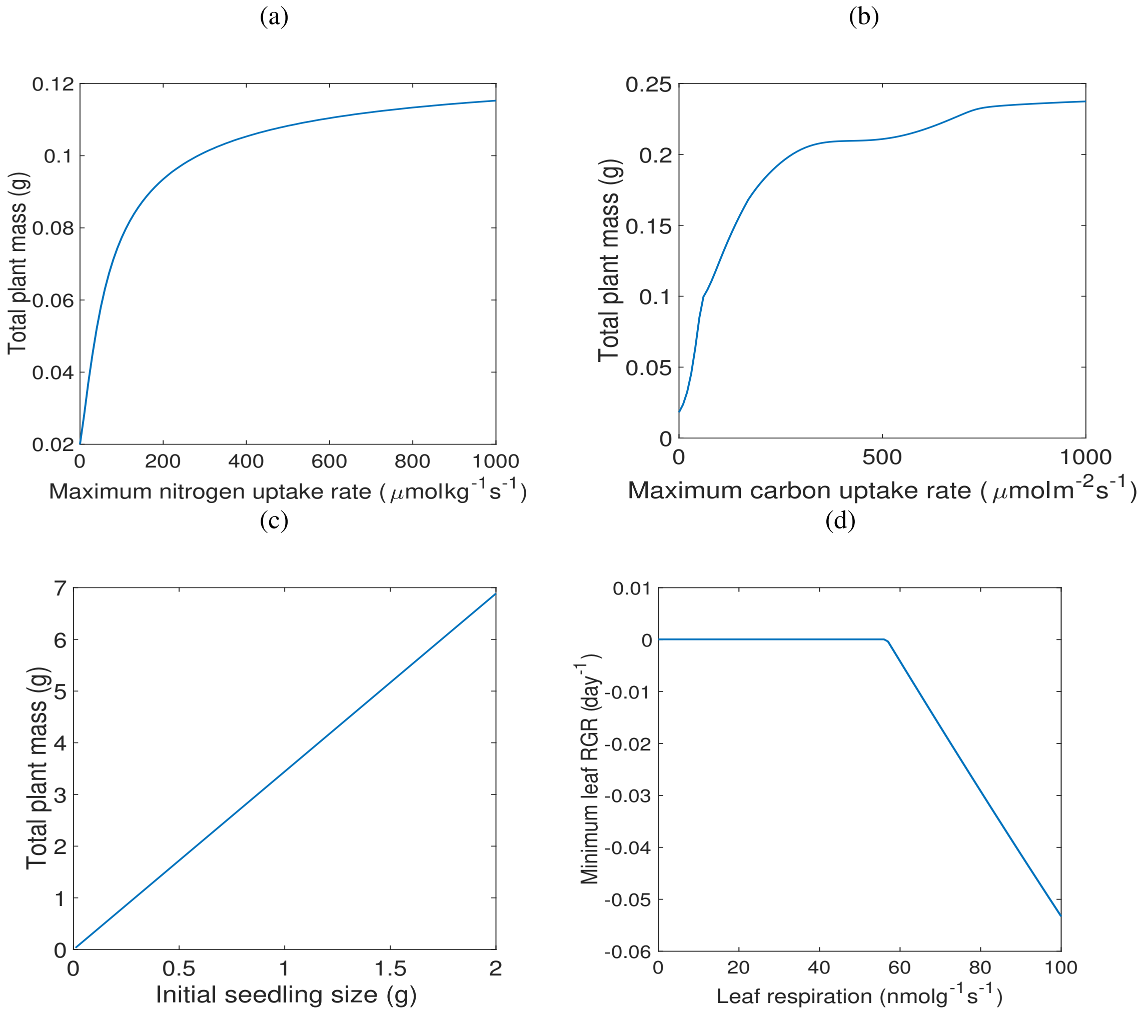


Fig. 6 a) The relationship between maximum nitrogen uptake rate and total plant mass after 20 days of growth. b) The relationship between maximum carbon uptake rate and total plant mass. c) The relationship between initial seedling size (initial leaf mass and initial root mass) and total plant dry mass after 20 days of growth. d) The relationship between leaf maintenance respiration and minimum leaf RGR. Leaf respiration and RGR is equal to root respiration and RGR for this subfigure. All figures produced using default parameter values (see appendix) with initial leaf and root mass of $0.01g$ respectively grown for 20 and initial concentrations $C_{l0} = 92.8 nmolmg^{-1}, C_{r0} = 63 nmolmg^{-1}, N_{l0} = 0.1 nmolmg^{-1}$, $N_{r0} = 7.54 nmolmg^{-1}$.

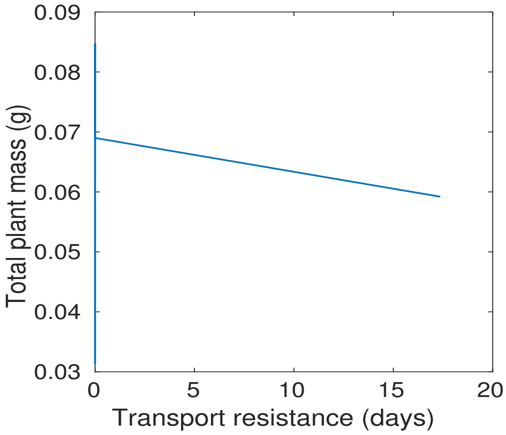


Fig. 7 The relationship between leaf or root transport resistance and total plant mass after growing for 20 days, using default parameter values (see appendix) with initial leaf and root mass of $0.01g$ respectively and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

4 Incorporating feedback mechanisms

Although the Thornley (1972) model allows internal concentrations to be dependent upon each other, and therefore growth rates have a slow feedback on RGR, there are no active feedbacks on uptake rates and growth. The following list represents the six feedback mechanisms developed in Chapter 3 which will be implemented within the Thornley model. Their combined effect on growth; how well they work together and react to changes in environment is investigated.

1. High leaf carbon concentrations decrease carbon uptake rate.
2. High root nitrogen concentrations decrease nitrogen uptake rate.
3. High leaf nitrogen concentrations increases 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.

In Chapter 3, the difference between uptake and use of carbon for growth and maintenance is stored in one pool of resources which can be taken or added to when is needed. Nitrogen is also assumed to have one intermediate pool. Alternatively, the Thornley (1972) model assumes that there are two separate pools of carbon and nitrogen such that there are four intermediate pools of resources (leaf carbon, leaf nitrogen, root carbon and root nitrogen). In Chapter 3, each internal feedback made growth processes such as carbon and nitrogen uptake rates and leaf and root relative growth rates dependent upon total carbon and nitrogen concentrations. For simplicity, the six feedbacks were implemented based on local concentrations. Table 1 in Chapter 3 shows that all feedbacks use local concentrations for signals except for feedbacks 2 and 5, where nitrogen uptake rate is reduced by high leaf nitrogen and carbon uptake rate is increased with high root nitrogen.

It was determined that the best function to simulate negative feedbacks on carbon and nitrogen uptake rates when carbon and nitrogen concentrations are high is a linear one for three environmental cases (equal uptake rates, low carbon uptake and low nitrogen uptake). A scalar function best describes the behaviour of a positive feedback onto leaf and root growth rates when nitrogen and carbon is high. For a positive feedback on uptake rates, a fractional stepwise function best simulates a positive response with high concentrations for all three environmental conditions.

Carbon uptake rate becomes:

$$A_c = \frac{V_c \rho c_a}{\rho c_a + k_c} - \alpha C_l + \frac{\frac{1}{4}\frac{V_c \rho c_a}{\rho c_a + k_c}}{1 + 100000 e^{-100(N_l - w)}}, \tag{27}$$

where αC_l is a negative feedback on carbon uptake rate when leaf carbon is high (feedback 1), $\frac{\frac{1}{4}\frac{V_c \rho c_a}{\rho c_a + k_c}}{1+100000 e^{-100(N_l - w)}}$ is a positive feedback on carbon uptake rate when shoot nitrogen is high (feedback 5), $w = 400 nmol mg^{-1}$ is the threshold value for shoot nitrogen and root carbon. Similarly, nitrogen uptake rate becomes dependent upon root nitrogen (feedback 2: high nitrogen reduces nitrogen uptake rate) and root carbon (feedback 6: high carbon increases nitrogen uptake rate).

Leaf relative growth rate (G_l) is dependent upon carbon and nitrogen concentration such that:

$$G_l = v \frac{C_l}{C_l + k_1} \frac{N_l}{N_l + k_2} \theta Y_g N_l, \tag{28}$$

where v is the maximum rate of use of carbon and nitrogen respectively, C_l is leaf carbon, N_l is leaf nitrogen, k_1 and k_2 are Michaelis-Menten constants for carbon and nitrogen use, θ is a conversion factor from mass to volume and Y_g is a conversion efficiency of concentration to mass. This is equivalent to Eq. (20) but multiplied by efficiency constants for growth and shoot nitrogen to implement a positive feedback on leaf growth rate when nitrogen is high (feedback 3). Similarly, for root growth rate, Eq. (21) is multiplied by Y_g, θ and root carbon to simulate a positive feedback on root growth when carbon concentration is high (feedback 4).

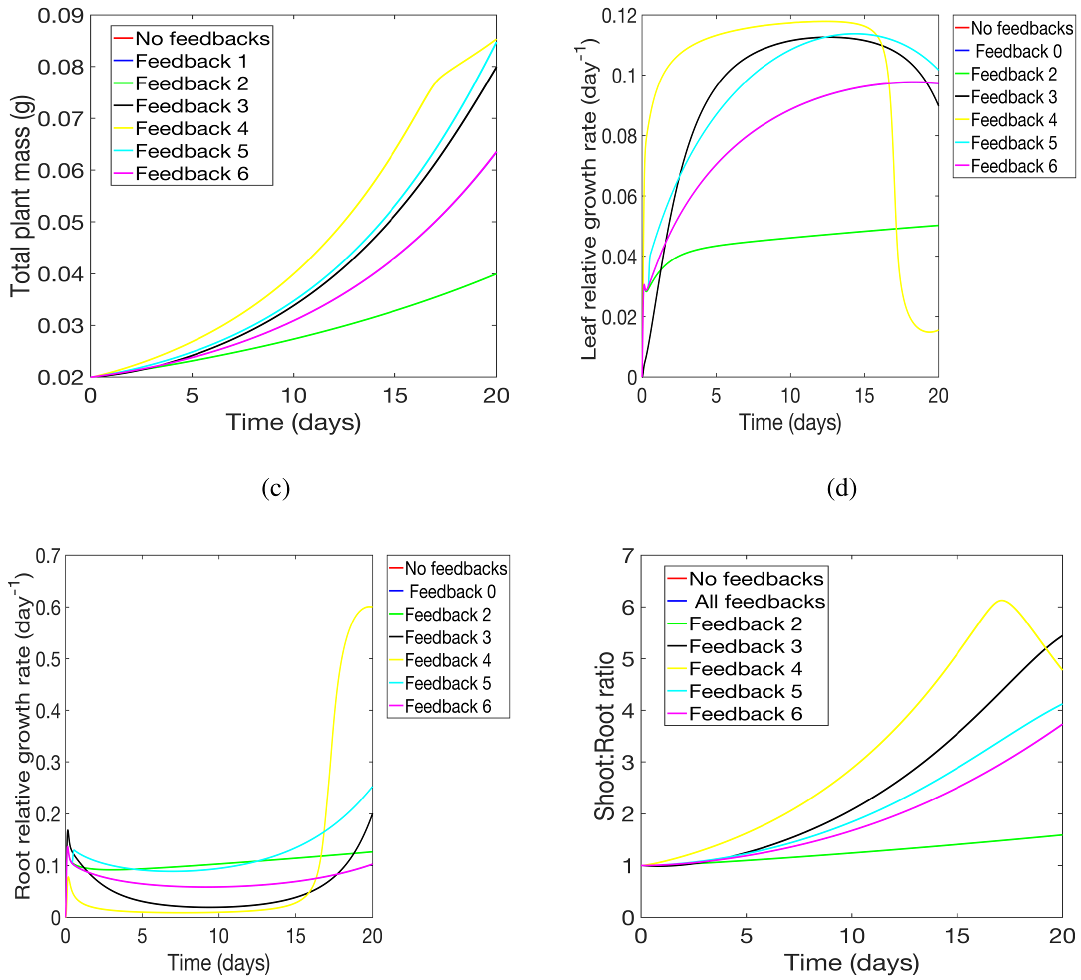
Previously, the feedback functions were implemented in a dimensionless model without any additional parameter values to dampen their effects. For them to behave reasonably with the dimensions imposed throughout this chapter, feedbacks 1 and 2 require a dampening factor of $\alpha = 10^{-5}$. This prevents their effects on growth from being too strong and to behave within a similar magnitude to other feedbacks and the model output without any feedbacks. Feedbacks 3-6 required no additional parameters.

Figure 8 and 9 show the model output when implementing each of the six feedbacks. These show that a negative feedback on nitrogen uptake rate with high root nitrogen concentration (feedback 2) has the largest effect on plant growth by reducing total dry weight from $0.06358g$ (without any feedbacks) to $0.03982g$ after 20 days (Fig. 8a). This is followed in effect by a positive feedback on root growth rate with high carbon (feedback 4) at $0.08535g$ and a positive feedback on carbon uptake rate with high nitrogen (feedback 5) at $0.08476g$. Feedback 6 (high carbon increases nitrogen uptake rate) has the least effect on growth such that total plant dry mass is only reduced by 10^{-9}. Feedback 1 (high carbon reduces carbon uptake rate) has the second weakest effect on growth, reducing total dry plant mass by $4 \times 10^{-5}g$, followed by feedback 3 (high nitrogen increases leaf growth) at $0.07989g$. This shows that feedbacks dependent upon intermediate nitrogen concentration have the largest effect on growth, since nitrogen concentrations are much higher than carbon.

Similarly, feedback 2 (high nitrogen reduces nitrogen uptake rate) has the strongest effect on shoot to root ratio, reducing the proportion of shoot growth by 2.14 when compared to without any feedbacks (Fig. 8d). Feedback 3 (high nitrogen increases leaf growth) has the second strongest effect on allocation, increasing shoot to root ratio to 5.447. Both feedbacks 4 (high carbon increases root growth) and 5 (high nitrogen increases carbon uptake rate) also increase leaf growth in relation to root growth. Although feedback 4 is meant to increase root growth, because root carbon concentration is so low, it is unable to alter shoot:root ratio. Feedbacks 3 and 4 further increase the disparity between leaf and root mass, whereas feedback 2 makes leaf and root mass more similar. Although feedback 5 increases leaf growth, it also increases root growth. This is also the case for feedback 4, although the purpose of this feedback is to increase root growth, this only occurs at 16.5 days as root carbon is not high enough for the feedback to take into effect. Increasing nitrogen uptake rate with high root carbon has little effect on the ratio of shoot to root mass.

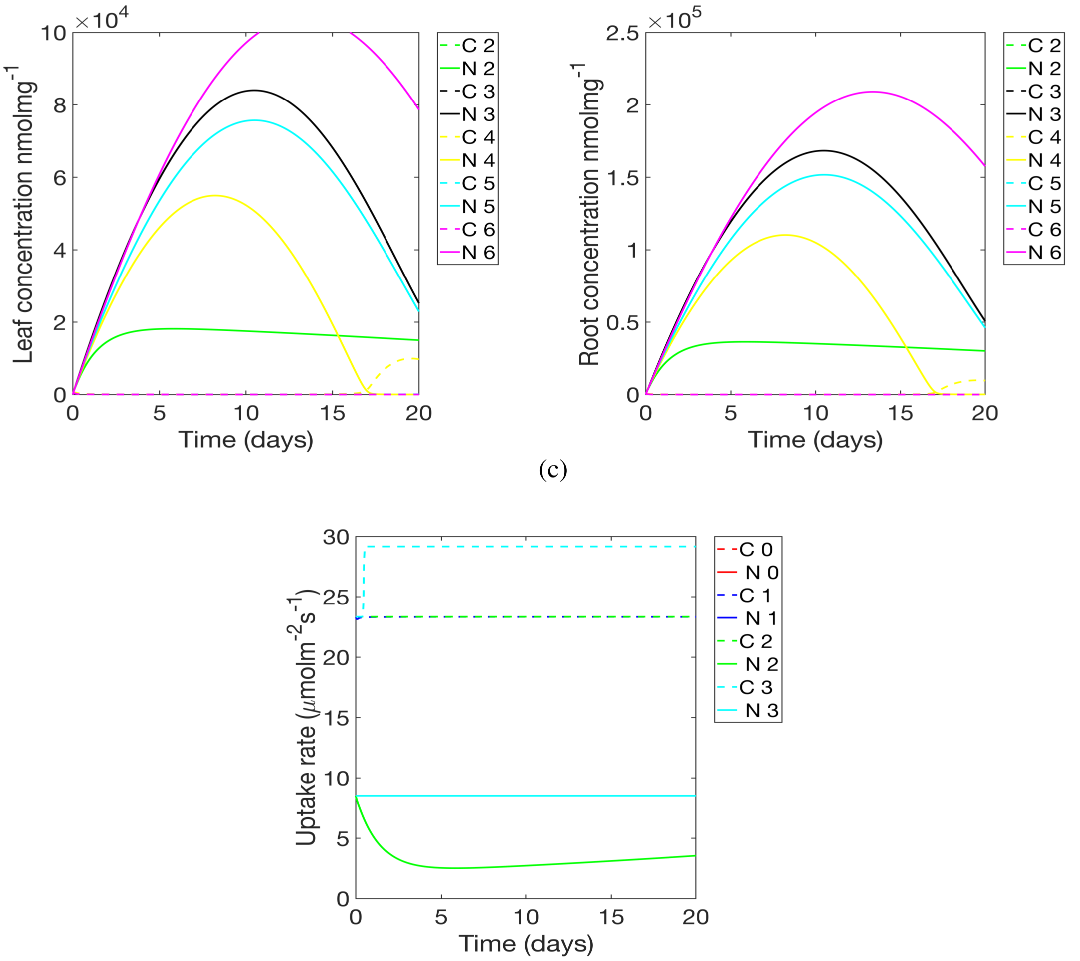
The strength of these feedbacks can be explained by the intermediate concentrations (Fig. 9a & b). In both the leaves and roots, nitrogen reaches a maximum concentration of $10^5 nmolmg^{-1}$, which is much higher than total intermediate carbon concentration, therefore feedbacks which make processes dependent upon nitrogen and in particular, root nitrogen will have the strongest effect (feedback 2). This is a consequence of the parameter set such that, carbon consumption is much higher for growth than nitrogen and carbon uptake rate is higher than nitrogen uptake rate. Feedback 6 (high root carbon increases nitrogen uptake rate) has very little effect on plant growth because root carbon concentrations are so low (Fig. 9a). Along with feedback 2, increasing carbon uptake rate with leaf nitrogen has a strong effect on concentrations. Intermediate concentrations reflect the behaviour of RGR over time (Fig. 8b & c). Feedback 2 has such a strong effect on intermediate concentrations, that leaf RGR is reduced and root RGR is increased. Similarly, feedback 4 (high root carbon increases root growth rate) has a strong effect on relative growth rates, increasing leaf RGR to $0.6 day^{-1}$ by 20 days.

Figure 9c shows the effect of each feedback on carbon and nitrogen uptake rates. All feedbacks except 3 and 4 alter uptake rate, and all but one visibly alters them. The strongest are feedbacks 2 and 5 since they are dependent upon nitrogen content. Carbon uptake rate is slightly reduced with carbon concentration but intermediate carbon concentration is not large enough to implement a positive feedback on nitrogen uptake rate. Therefore, Feedback 2 (high nitrogen reduces nitrogen uptake rate) has the strongest effect on growth by reducing leaf growth rate and increasing root growth, making S:R and carbon and nitrogen concentrations more balanced, whilst feedback 6 (high carbon increases nitrogen uptake rate) has the smallest effect on growth.



(a) (b) (c) (d)

Fig. 8 a) The effect of each feedback on total dry plant mass, b) The effect of each feedback on leaf RGR. c) Root RGR over time with different feedbacks implemented. d) Shoot:root ratio over time when implementing each feedback. For all four subfigures (a - d) feedback 6 (magenta line) overlaps without any feedbacks (red line) and feedback 1 (blue line). When $c_a = 400$, $N = 400$, with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8 nmolmg^{-1}, C_{r0} = 63 nmolmg^{-1}, N_{l0} = 0.1 nmolmg^{-1}$, $N_{r0} = 7.54 nmolmg^{-1}$. Feedback 1: carbon uptake rate decreases with high carbon concentration; feedback 2: nitrogen uptake rate decreases with high nitrogen concentration; feedback 3: leaf growth increases with high nitrogen content; feedback 4: root growth increases with high carbon content; feedback 5: carbon uptake rate increases with high nitrogen; feedback 6: nitrogen uptake rate increases with high carbon. Parameters used from Table 2. 0 - 6 represents feedback type.



(a)

(b)

(c)

Fig. 9 a) Intermediate leaf carbon and nitrogen concentration without any feedbacks and each feedback implemented separately, feedback 6 (magenta line) overlaps without any feedbacks (red line) and feedback 1 (blue line) and carbon with feedback 6 overlaps carbon feedbacks 0-5. b) Intermediate root carbon and nitrogen concentration comparing the effects of each individual feedback, feedback 6 (magenta line) overlaps without any feedbacks (red line) and feedback 1 (blue line) and carbon with feedback 6 overlaps carbon feedbacks 0-5. c) The effect of each feedback on carbon and nitrogen uptake rate, carbon uptake rate feedback 2 (green dashed line) overlaps carbon uptake rate without any feedbacks (red dashed line), nitrogen uptake rate feedback 3 (cyan solid line) overlaps nitrogen uptake rate without any feedbacks (red solid line) and feedback 1 (blue solid line). When $c_a = 400$, $N = 400$, with with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$. Feedback 1: carbon uptake rate decreases with high carbon concentration; feedback 2: nitrogen uptake rate decreases with high nitrogen concentration; feedback 3: leaf growth increases with high nitrogen content; feedback 4: root growth increases with high carbon content; feedback 5: carbon uptake rate increases with high nitrogen; feedback 6: nitrogen uptake rate increases with high carbon. Parameters used from Table 2. 2. 0 - 6 represents feedback type.

5 All feedbacks

Incorporating all feedbacks at once allows the model to respond to different behaviours simultaneously. This increases total dry plant mass at 20 days by $0.0009g$ when compared to the model without any feedbacks, increasing total dry plant mass by 1.42% (Fig. 11a). Figure 10 shows that making processes such as leaf and root growth rates and carbon and nitrogen uptake rates dependent upon internal concentrations enables carbon and nitrogen to become more equal within the leaves and roots. This significantly reduces the concentrations of nitrogen in the leaves and roots, whilst leaf and root carbon is increased. Although nitrogen content is reduced, carbon is the limiting resource for growth, the inclusion of the feedbacks increase carbon uptake rate, leading to higher carbon concentrations and increasing growth rates (Fig. 11d & e). The initial high availability of nitrogen and low carbon, enables feedbacks 2 and 5 to take effect; this increases carbon uptake rate and reduces nitrogen uptake rate (Fig. 11e). As the plant continues to grow with an enhanced carbon uptake and reduced nitrogen uptake, internal carbon concentration begins to increase. At this point in time, carbon uptake rate reduces (feedback 1), nitrogen uptake rate increases and root growth increases. This produces a more balanced shoot:root ratio of 2.423 than without any feedbacks, reducing total leaf mass and increasing root mass (Fig. 11a - c).

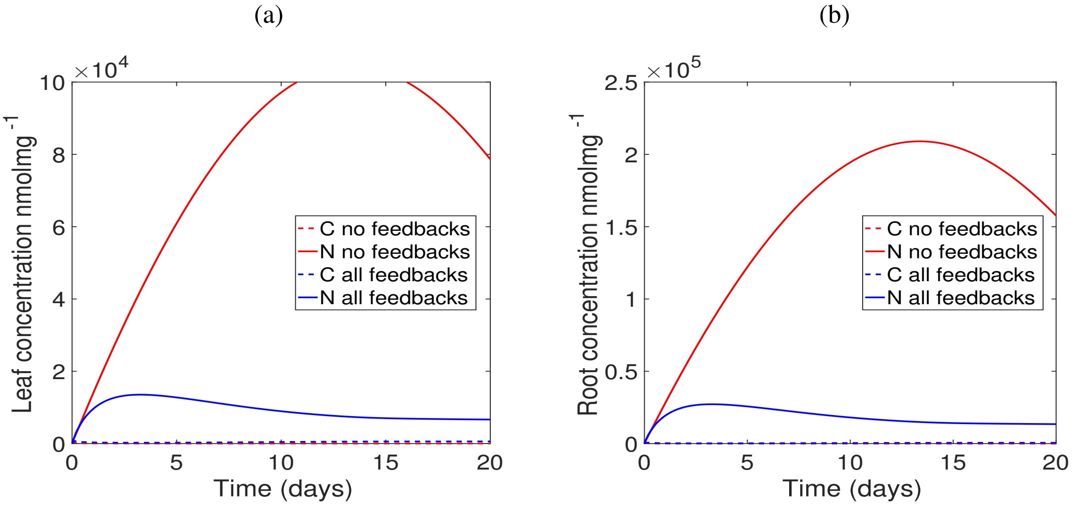


Fig. 10 a) The effect of implementing all six internal feedbacks on intermediate leaf carbon and nitrogen. b) The effect of implementing all six internal feedbacks on intermediate root carbon and nitrogen. The model was ran with parameter values from Table 2 when atmospheric CO_2 is $400ppm$ and soil nitrogen of $400\mu mol$ with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

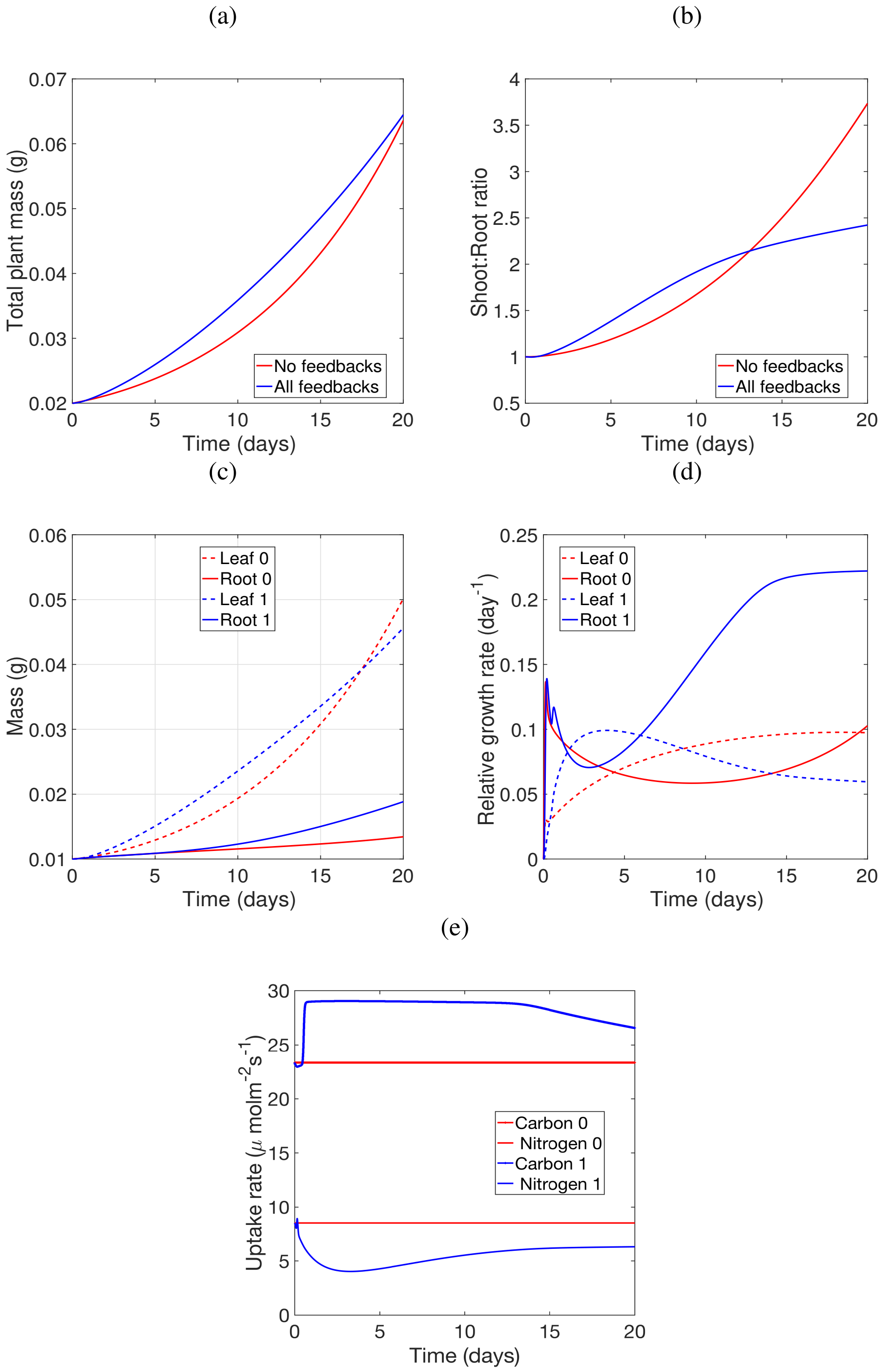


Fig. 11 a) The effect of implementing all feedbacks on total dry plant mass. b) The effect of implementing all feedbacks on shoot:root ratio. c) The effect of implementing all feedbacks on leaf (dashed lines) and root (solid lines) mass. d) The effect of implementing all feedbacks on leaf (dashed lines) and root (solid lines) RGR. e) The effect of all feedbacks on carbon (dashed lines) and nitrogen (solid lines) uptake rates. Ran with parameter values from table 2 when atmospheric CO_2 is $400ppm$ and soil nitrogen of $400\mu mol$ with initial leaf mass of $l_0 = 0.01g$ and root mass $r_0 = 0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$. 0 - represents without any feedbacks. 1 - represents with all feedbacks.

6 Atmospheric CO_2 and nitrogen experiment

By comparing the model output when using two soil nitrogen concentrations and two atmospheric CO_2 concentrations to the patterns observed in experimental data (Coleman et al., 1993; Farage et al., 1998; Rogers et al., 1998; Ainsworth et al., 2003; Butterly et al., 2015), it is possible to determine if the simulated plant with imposed internal feedback mechanisms responds to changes in environment in a similar way to a real plant. Plant growth is simulated for two CO_2 concentrations: $350 ppm$ and $700 ppm$ for a high soil nitrogen treatment ($400 \mu mol$) and a low soil nitrogen treatment ($200 \mu mol$).

Figure 12 shows that increasing atmospheric CO_2 concentration increases total plant mass in both soil nitrogen treatments. Higher soil nitrogen concentrations increases total plant mass and also the effect of CO_2 on total plant mass by 11% such that, increasing atmospheric CO_2 is stronger on total plant mass than with a lower soil nitrogen treatment (high CO_2 creates a 30% change in high nitrogen whilst in low nitrogen the change is 19%). This behaviour replicates a general result from CO_2 and nitrogen experiments (Coleman et al., 1993; Curtis and Wang, 1998; De Graff et al., 2006), such that both nitrogen and CO_2 availability have a positive relationship with plant growth.

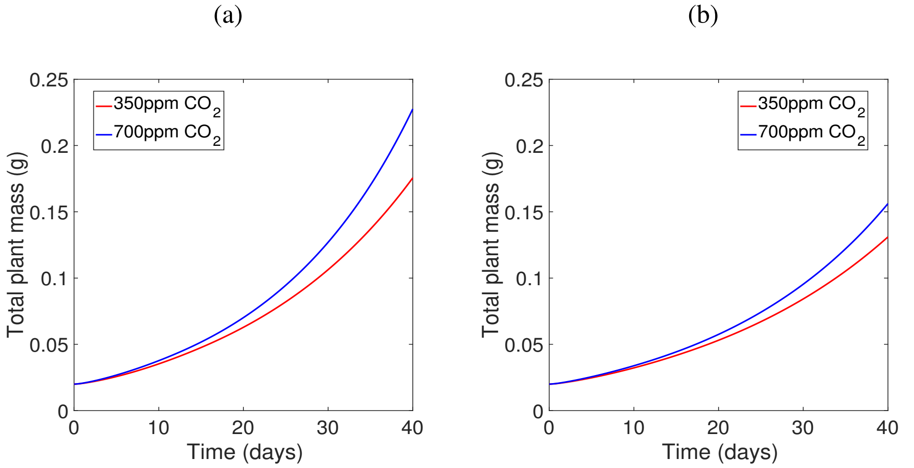


Fig. 12 a) The relationship between plant mass over time and two atmpsheric CO_2 treatments ($350 ppm$ and $700 ppm$) with a high soil nitrogen (N=400 using Eq (19)) treatment. b) The relationship between plant mass over time and two atmpsheric CO_2 treatments ($350 ppm$ and $700 ppm$) with a low soil nitrogen treatment (N=200, using Eq (19)). Both ran with initial leaf mass $l_0 = 0.01$ and root mass $r_0 = 0.01$ and initial concentrations $C_{l0} = 92.8 nmol mg^{-1}, C_{r0} = 63 nmol mg^{-1}, N_{l0} = 0.1 nmol mg^{-1}$, $N_{r0} = 7.54 nmol mg^{-1}$.

Naturally, plants growing in a higher soil nitrogen content have a higher percentage of nitrogen in the whole plant (Fig. 13). Increasing atmospheric CO_2 reduces plant nitrogen concentration initially and as the plant continues to grow, a higher atmospheric CO_2 produces a higher percentage of nitrogen. After 40 days nitrogen concentration is at similar levels for high and low soil nitrogen treatments. Applying a soil nitrogen of $200\mu mol$, at 40 days, nitrogen is 3.4% of total plant mass when CO_2 is high. Reducing CO_2 lowers nitrogen percentage to 3.09%. Increasing nitrogen treatment reduces total nitrogen for high (3.38%) and low (3.01%) CO_2, although this effect is not very strong. When plotting percentage of nitrogen within the whole plant against total plant mass (Fig. 13c-d), the same behaviour occurs except that for a low soil nitrogen treatment, the plant produced is smaller. This implies that a higher soil nitrogen treatment produces a larger total plant mass and therefore nitrogen percentage reduces with plant size.

Carbon uptake rate depends on atmospheric CO_2, leaf carbon and leaf nitrogen. Given final leaf carbon and nitrogen concentrations when running the model for the combination of high and low CO_2 and soil nitrogen for a period of 40 days, curves of carbon uptake rate over intercellular CO_2 (A/c_i) are produced (Fig. 14a-b). This is done by substituting final leaf carbon and nitrogen concentrations at $t = 40 days$ for both CO_2 and nitrogen treatments into Eq (27) to plot carbon uptake rate against intercellular CO_2 between 0 and $1000\mu mol mol^{-1}$. Creating these plots aids the comparison of responses to environmental change since (A/c_i) curves are commonly used. Both types of treatments have a positive effect on carbon uptake rate. High CO_2 levels and soil nitrogen increase carbon and nitrogen uptake. When soil nitrogen is low ($200\mu mol$), atmospheric CO_2 only marginally alters the shape of the (A/c_i) curve and increasing soil nitrogen maximises the effect of CO_2 on the shape of the (A/c_i) curve. The effect of atmospheric CO_2 is stronger with a high soil nitrogen treatment for both carbon and nitrogen uptake rate. In the first fifteen days, CO_2 has a large effect on nitrogen uptake rate, as the plant continues to grow, this effect on uptake rate still occurs but diminishes. For a low nitrogen treatment, the effect of CO_2 after 15 days becomes much smaller than when $n = 400\mu mol$ (Fig. 14c-d).

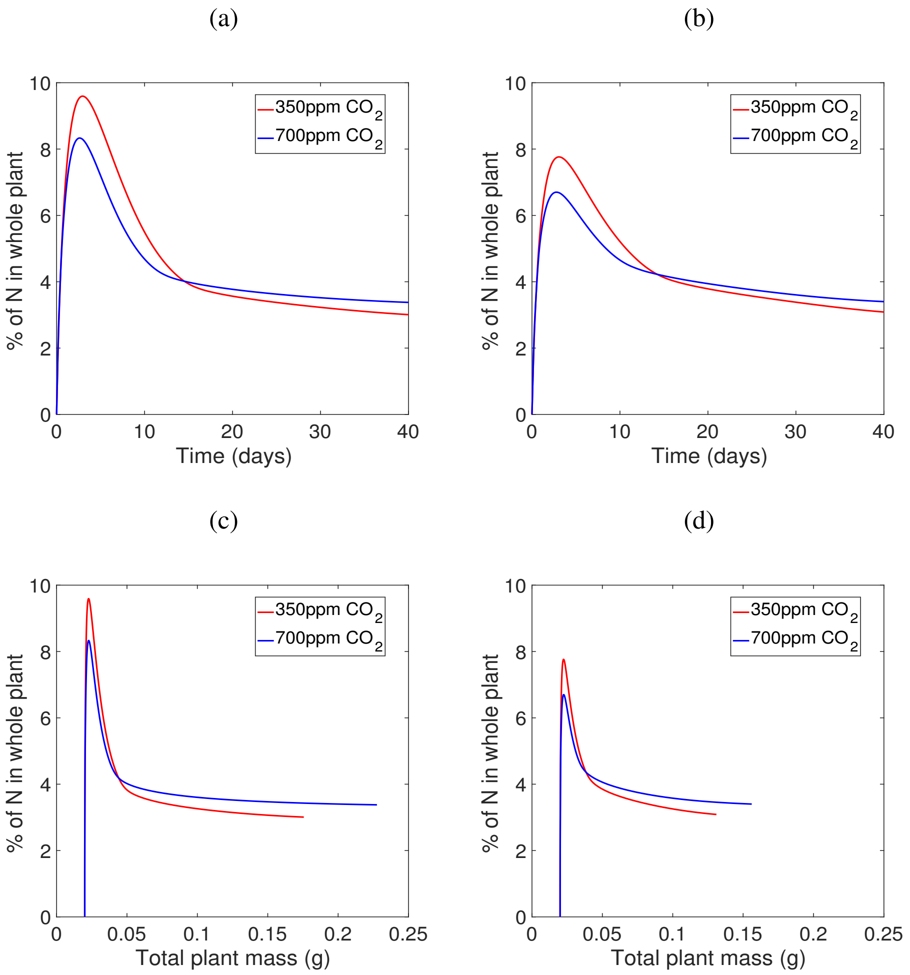
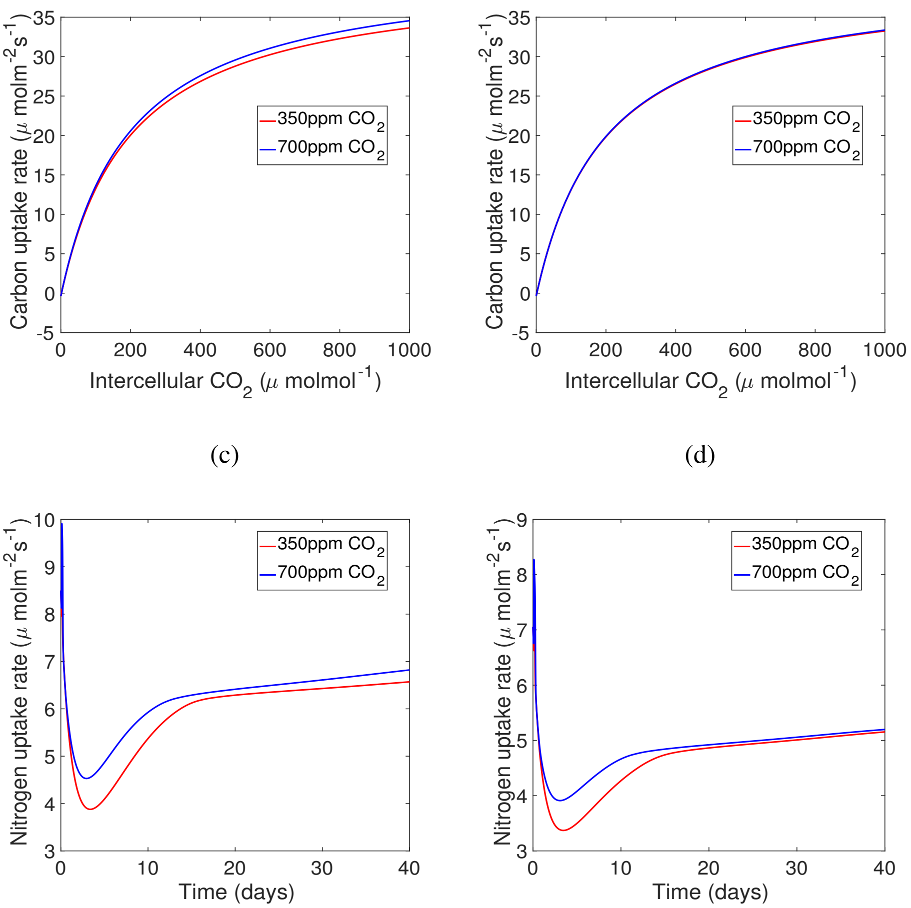

(a) (b) (c) (d)

Fig. 13 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 ($n = 400\mu mol$) b) low soil nitrogen ($n = 200\mu mol$). 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 ($n = 400\mu mol$). d) low soil nitrogen ($n = 200\mu mol$). Ran for 40 days with initial leaf mass of $0.01g$ and root mass of $0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}, C_{r0} = 63nmolmg^{-1}, N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

Figure 15 shows the ratio of root to shoot mass (R:S) as the plant grows for 40 days. The seedlings starts off at equal mass for leaves and roots ($l = 0.01g, r = 0.01g$). As the plant begins to grow, root growth is favoured but this allocation swaps to leaves very quickly and R:S tends towards 0.3 when soil nitrogen is high and CO_2 is low. Initially for high nitrogen conditions, increasing atmospheric CO_2 has little effect on R:S. High atmospheric CO_2 begins to increase the proportion of roots in relation to leaf mass from 5 days. Low nitrogen treatments increase root growth and the effects of CO_2 treatment emerge sooner than when soil nitrogen is high. Low nitrogen treatment overall produces a higher R:S than a higher nitrogen treatment (Fig. 15b). This



(a) (b) (c) (d)

Fig. 14 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 ($400\mu mol$). b) when soil nitrogen is low ($200\mu mol$). These curves are created by substituting final leaf carbon and nitrogen concentrations at $t = 40days$ into Eq (27) 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 ($400\mu mol$) d) when soil nitrogen is low ($200\mu mol$). The model was ran for 40 days with initial leaf mass of $0.01g$ and initial root mass of $0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

reflects the environmental plasticity of the feedback model since when nitrogen availability is high, less roots are produced and when it is lower, more roots are produced. Changes in atmospheric CO_2 have the same effect on R:S under both high and low nitrogen conditions.

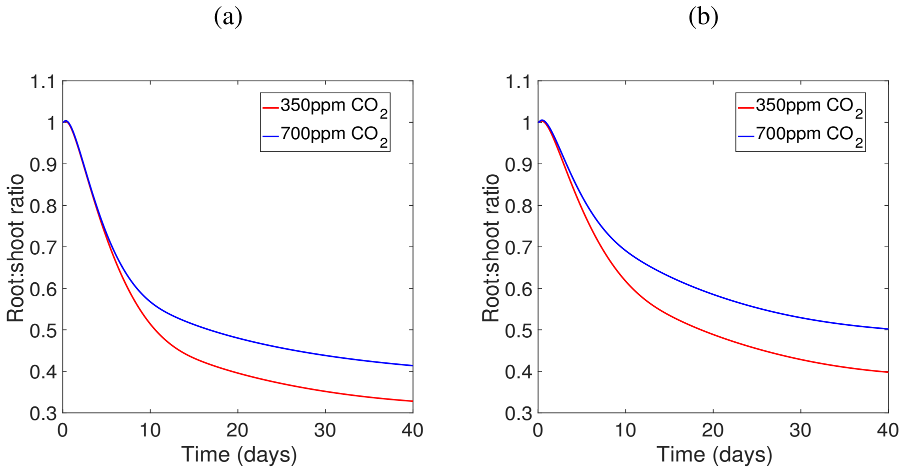


Fig. 15 a) The relationship between root:shoot ratio over 40 days and two CO_2 treatments ($350ppm$ and $700ppm$) when soil nitrogen is high ($400\mu mol$). b)The relationship between root:shoot ratio over time and two CO_2 treatments ($350ppm$ and $700ppm$) when soil nitrogen is low ($200\mu mol$). With initial leaf and root mass of $0.01g$ respectively and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.

The relationships between carbon and nitrogen availability on R:S are a consequence of their relationship with leaf and root relative growth rates. When soil nitrogen is high, increased atmospheric CO_2 simply increases both leaf and root RGR, slightly shifting RGRs. The effect of CO_2 is stronger on root RGR than leaf and therefore leads to an increase in R:S (Fig 12a). When soil nitrogen is low, atmospheric CO_2 also increases individual growth rates but leaf growth is only slightly increased and root growth is increased greatly, rectifying the difference in R:S.

The effect of CO_2 and soil nitrogen treatments on total plant mass still holds when running the experiment on the model without any feedbacks. The removal of internal feedbacks allows intermediate nitrogen concentration to increase and therefore nitrogen initially accounts 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 reduces nitrogen percentage within the plant. Removing the feedbacks produces a similar R:S for all combinations of soil nitrogen and CO_2 treatments such that R:S lies between 0.2 and 0.4. Initially the lower CO_2 treatment has a higher R:S but by 40 days, high CO_2 increases R:S when compared to a lower CO_2 treatment. Due to the lack of internal feedbacks, carbon and nitrogen uptake rate remain constant throughout nutrient availability manipulations (data not shown).

Removing feedback 2 (high nitrogen reduces nitrogen uptake rate) produces a plant with total nitrogen percentages within a range of $10-20\%$ and increasing CO_2 reduces total nitrogen percentage when plotted over 40 days or against total plant mass. The effect of CO_2 and nitrogen treatment on R:S is the same as without any feedbacks. The treatments have a stronger effect on R:S than without any feedbacks but not as strong as with all feedbacks. Additionally removing feedback 4 does not alter the behaviour of total plant mass over time when varying carbon and nitrogen availability. The same occurs for carbon and nitrogen uptake rates over time, removing feedback 2 does not alter the effect of CO_2 and nitrogen treatments. The only differences between the model output is that there is more nitrogen available and nitrogen uptake rate is not reduced so much by feedbacks, and nitrogen percentage is reduced with increased atmospheric CO_2.

7 Defoliation and CO_2 experiment

Defoliation is simulated to further determine how the model responds to different external perturbations. The CO_2 and nitrogen treatments investigated the effect of varying resource availability whereas simulating defoliation investigates how the plant reallocates resources once source size is reduced. To simulate a defoliation experiment, the model was run for 10 days from the same initial conditions used throughout this chapter ($l_0 = r_0 = 0.01g$, $C_{l0} = 92.8nmolmg^{-1}, C_{r0} = 63nmolmg^{-1}, N_{l0} = 0.1nmolmg^{-1}$ and $N_{r0} = 7.54nmolmg^{-1}$) with soil nitrogen of $400\mu mol$. 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 ($350ppm$ and $700ppm$).

Defoliation reduces total plant mass and increases the effect of atmospheric CO_2 on growth (Fig. 16a). At 20 days, CO_2 increases total plant mass by $0.0073g$ without any defoliation (Fig. 12a) whereas plant mass is increased by $0.0065g$ with defoliation (Fig. 16a). Therefore defoliation increases the positive effect of high CO_2 with an increase in 15.% of total dry plant mass. This implies that the model with feedbacks is reacting to a halving of the carbon source size (defoliation). Initially, a lower CO_2 treatment produces a plant which is investing more of its resources into leaf growth than a high atmospheric CO_2, after defoliation, both treatments appear to be investing into leaf growth at similar rates (Fig. 16b).

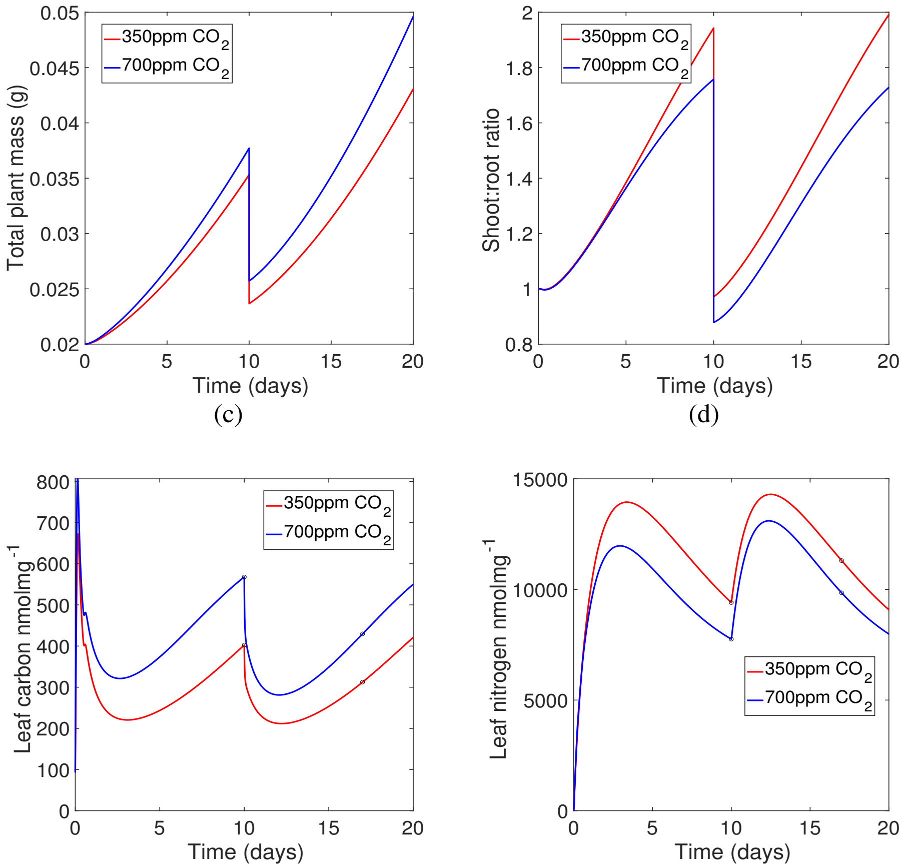


Fig. 16 The effect of defoliation (when total leaf mass is halved at day 10) with all internal feedbacks and a high ($700ppm$, blue lines) and low ($350ppm$, red lines) CO_2 treatment on a) Total plant mass over 20 days of growth. b) Proportion of leaf mass compared to root (shoot:root) over 20 days. 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 $400\mu mol$ 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 $N_{r0} = 7.54nmolmg^{-1}$.

For both CO_2 treatments, carbon uptake rate increases initially due to an initial imbalance between nitrogen and carbon use for growth and respiration and soon reaches a plateau (Fig. 17a). At 7 days after defoliation, carbon uptake rate increases slightly, with a difference of $0.107\mu molm^{-2}s^{-1}$ when CO_2 is high and $0.048\mu molm^{-2}s^{-1}$ when CO_2 is low. Defoliation has a much stronger effect on nitrogen uptake rate than for carbon. At 7 days after defoliation, nitrogen uptake rate is reduced by $0.6926\mu molm^{-2}s^{-1}$ when CO_2 is high and $0.6238\mu molm^{-2}s^{-1}$ when CO_2 is low (Fig .17b). Similarly, 7 days after defoliation, leaf carbon is reduced and leaf nitrogen increased for both CO_2 treatments (Fig. 16c-d).

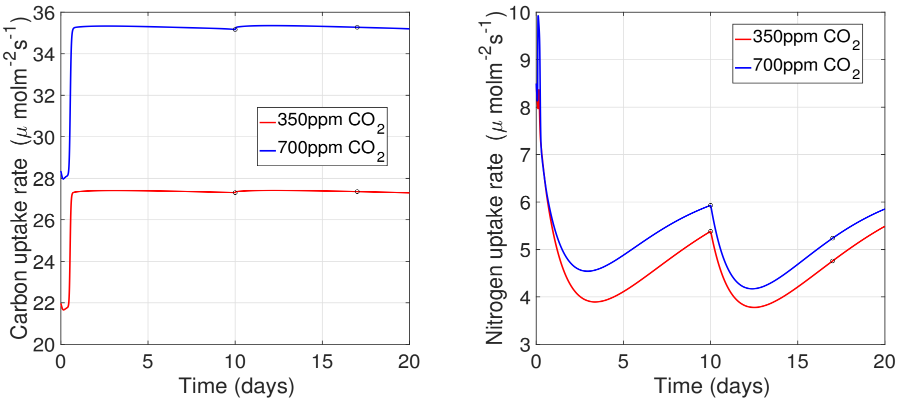

(a) (b)

Fig. 17 The effect of defoliation (when total leaf mass is halved at day 10) with all internal feedbacks and a high ($700ppm$, blue lines) and low ($350ppm$, 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 $400\mu mol$ 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 $N_{r0} = 7.54nmolmg^{-1}$.

Carbon concentration decreases 7 days after defoliation when simulating the experiment without any internal feedbacks on growth and uptake. This also applies for intermediate nitrogen concentration such that after 7 days, nitrogen is higher. Removing the feedbacks on intermediate concentration does not alter the effect of defoliation on concentration. However, defoliation does not alter carbon and nitrogen uptake rates since without any feedbacks they are only dependent upon external CO_2 and nitrogen respectively.

The model was ran without feedback 2 to determine which feedbacks are responsible for the responses to external perturbations. Removing only feedback 2 (high nitrogen reduces nitrogen uptake rate) does not produce the same behaviour as the model with all feedbacks such that, leaf carbon concentration is lower 7 days after defoliation for high CO_2 but higher for low CO_2, leaf nitrogen is lower 7 days after defoliation and carbon uptake rate is also increased 7 days after defoliation for high CO_2 but decreased for low CO_2 (See appendix B).

8 Discussion and conclusions

The aim of this chapter was to implement the feedback framework developed in Chapter 3 to a model which has been validated experimentally (Thornley, 1972) and to evaluate whether it responds to changes in carbon and nitrogen availability 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). This was carried out by simulating two levels of atmospheric CO_2 and two soil nitrogen treatments collectively and a defoliation experiment where the total above ground biomass was halved after 10 days and growth was simulated for an additional 10 days following defoliation. The chapter showed that the model results show the same positive effect of soil nitrogen and CO_2 on total plant mass as experimental results, however the model produces higher nitrogen concentrations and an increase in net carbon uptake rate against intercellular CO_2 with elevated atmospheric CO_2, which does not match experimental results. Additionally the model also reflects experimental results that defoliation increases the positive effect of high CO_2 on growth. This also showed that CO_2 and nitrogen have positive effects on total plant mass, carbon uptake rate, nitrogen uptake rate and total nitrogen content. Additionally, increased atmospheric CO_2 leads to a larger root:shoot ratio and increased soil nitrogen leads to a lower proportion of roots than ambient CO_2 and nitrogen treatments respectively. Imposing defoliation enables higher leaf nitrogen, lower leaf carbon and higher carbon uptake rates. Therefore, a model which simulates internal feedbacks on source and sink strengths with changes in carbon and nitrogen is able to mostly reflect behaviours observed in experiments on source availability. This model takes one step closer at modelling the mechanisms responsible for allocation.

Many models simulate the dependence of growth on two nutrients or more (Ågren et al., 2012; Cheeseman, 1993; Siddiqi and Glass, 1986) and their response to environmental heterogeneity (Yang and Midmore, 2005; Pao et al., 2018) or defoliation (Thornley et al., 1997). Some of which, incorporate dependencies upon nutrient status but do not necessarily address their specific effects on growth by comparing feedback types or including multiple feedback mechanisms. In particular, no previous models simulate the combination of resource dependencies on uptake rates and growth rates, whilst simulating transport. Using a model (Thornley, 1972) which has been widely applied to different plant species and environmental conditions (Wann and Raper Jr, 1984; Rastetter and Shaver, 1992; Minichin et al., 1994; Dewar et al., 1994) enables an easier comparison of how the feedbacks respond to changes in nutrient availability.

Ågren et al. (2012) simulate the partitioning of carbon, nitrogen and phosphorus into pools of labile and structural resources. Nitrogen and phosphorus uptake is dependent upon intermediate carbon concentration and carbon uptake is dependent upon intermediate nitrogen concentration but they have not simulated any negative feedbacks on uptake if intermediate concentrations become too high. Ågren et al. (2012) investigate the effect of varied nitrogen and phosphorus availability but do not look at the shifts in above and below ground activity and therefore is limited in simulating the mechanisms responsible for allocation.

Models which extend the work of Thornley (1972) to make processes such as carbon and nitrogen uptake rates dependent upon the environment (e.g temperature, light flux density, CO_2, water and nitrogen (Wann and Raper Jr, 1984; Rastetter and Shaver, 1992; Minichin et al., 1994; Dewar et al., 1994)) do not incorporate any further dependence of processes which determine growth on internal carbon and nitrogen concentrations. Some assume that the allocation of carbon and nitrogen between leaves and roots is controlled by a functional balance which can be used to optimise growth or control the ratio of carbon to nitrogen (Reynolds and Thornley, 1982; Mäkelä and Sievänen, 1987; Bartelink, 1998; Feller et al., 2015). However, functional balances do not necessarily represent the known mechanisms responsible for resource allocation.

Others have modelled the dependence of the rates of resource acquisition on internal carbon and nitrogen concentrations (Buckley and Roberts, 2006; Drouet and Pagès, 2007). Pao et al. (2018) investigate the effect of varying light on photosynthetic acclimation with nitrogen treatments using a model strictly looking at above ground biomass. Therefore, the extent to which previous models have simulated the various observed feedbacks which describe allocation is by simulating dependencies of nutrient status on growth processes and increasing source activity. No previous models have included feedbacks of nutrient status on sink strength. This chapter extends this sort of work by implementing even more dependencies of carbon and nitrogen which alter the capacities source and sink strengths along with simulating the movement of resource between plant compartments.

The transport mechanism within the Thornley (1972) model allows the plant to actively balance carbon and nitrogen levels within the plant. Although this mechanism allows transport of resource between leaves and roots within hours, this process must occur several times between compartments before carbon and nitrogen become equal. The transport function works to rectify the differences in size between carbon and nitrogen concentration by making internal concentrations dependent upon one another, but this does not make any other processes such as uptake or growth rate dependent upon them.

The results of this chapter show that the feedback responsible for simulating a reduction in nitrogen uptake rate when intermediate root nitrogen is high (feedback 2) had the strongest effect on growth by reducing total plant mass. Feedbacks which depend on internal nitrogen concentration also had a stronger effect on plant growth than feedbacks dependent on carbon. This a consequence of surplus intermediate nitrogen within leaves and roots due to the requirements for carbon being much higher than nitrogen for growth (λ_1 & λ_2). These feedbacks were used without any additional parameters to aid easy comparisons of which alters growth the most. Therefore, the strength of these feedbacks could be manipulated to control which feedback determines the overall plant's response to environmental changes. The feedback model produces a larger total plant mass than the Thornley model without feedbacks, implying that the feedbacks optimise the allocation of resources based on its environmental conditions to improve growth.

Both atmospheric CO_2 and soil nitrogen concentration have a positive relationship with total plant mass. High levels of soil nitrogen increase the effect of CO_2 on total plant mass. Coleman et al. (1993) show the same results in their experiment when treating two annual species of different photosynthetic type (*Abutilon theophrasti* and *Amaranthus retroflexus*) using CO_2 treatments of $350ppm$ and $700ppm$ with a high and low fertiliser treatment. This behaviour also occurs in other experiments with similar magnitudes of CO_2 and nitrogen treatments (Curtis and Wang, 1998; Farage et al., 1998; De Graff et al., 2006; Kirschbaum and Lambie, 2015).

The percentage of total nitrogen within the plant is a similar proportion to that in Coleman et al. (1993) whilst simulating a CO_2 and nitrogen treatment experiment. Although the magnitude of nitrogen concentration is similar to experimental results, the effect of CO_2 on nitrogen concentrations are not. The feedback model shows that high levels of CO_2 overall increase total nitrogen percentage while Coleman et al. (1993) shows that increasing CO_2 overall reduces nitrogen percentage. Both the model results and experimental results show an overlap in the two CO_2 treatments, implying that the effects of high CO_2 on total nitrogen percentage are not so distinct. Many other studies have shown that high CO_2 reduces nitrogen content (Cotrufo et al., 1998; Curtis and Wang, 1998; Norby et al., 1999; Jablonski et al., 2002; Ainsworth and Long, 2005; Taub et al., 2008). The model shows that increasing nitrogen availability increases total nitrogen content which is observed experimentally (Vicente et al., 2015). Hirose (1986) showed that the allocation of biomass and nitrogen to plant compartments is linearly related to total nitrogen concentration within the plant.

Both increasing CO_2 and nitrogen concentration increase carbon uptake rate. Nitrogen increases the effect of atmospheric CO_2 on carbon uptake rate. Farage et al. (1998) show that increasing CO_2 reduces carbon uptake rate against intercellular CO_2 and increasing nitrogen treatment reduces this effect. Ainsworth et al. (2003) replicate the result that increased atmospheric CO_2 reduces carbon uptake rate against intercellular carbon but show that high nitrogen content increased this effect. Both nitrogen and CO_2 increase nitrogen uptake rate in the model. This can be an example of where the feedback mechanisms do not fully represent a plant's response to environmental heterogeneity.

The results of this chapter show that high levels of atmospheric CO_2 promote an increase in root:shoot ratio, whilst increasing soil nitrogen reduces root:shoot ratio. This is to be expected within the model as high levels of carbon increase root growth and conversely high levels of nitrogen increase leaf growth. This is imposed by the internal feedback mechanisms. Lacointe (2000) shows that increasing CO_2 increases root:shoot ratio, whereas Butterly et al. (2015) found that high atmospheric CO_2 reduces the number of roots per leaves and high soil nitrogen increases root:shoot ratio. Dybzinski et al. (2011) also found that increasing nitrogen availability reduces root:shoot ratio using tree growth model when allocation carbon to the leaves for growth is dependent upon nitrogen uptake. Vicente et al. (2015) found that increasing nitrogen reduces root:shoot ratio. Therefore there are conflicting ideas on whether root:shoot ratios should increase or decrease from changes in CO_2 and soil nitrogen availability.

Leaf nitrogen is increased 7 days after defoliation whilst leaf carbon is reduced 7 days following defoliation. This agrees with the work of Rogers et al. (1998) which show the same qualitative changes in leaf carbon and nitrogen following a defoliation event. Carbon uptake rate increases 7 days after halving total above ground biomass in the model. This result also matches that of several experimental papers (Von Caemmerer and Farquhar, 1984; Rogers et al., 1998; Eyles et al., 2013). Most defoliation experiments are tested over multiple years with several cutting instances (Von Caemmerer and Farquhar, 1984; Bryant et al., 1998; Rogers et al., 1998; Ainsworth et al., 2003; Eyles et al., 2013). They also only measure metabolites at a distinct time point, either immediately or at a fixed interval (7 days) after and metabolite levels are unknown at any other point in time (Von Caemmerer and Farquhar, 1984; Rogers et al., 1998; Ainsworth et al., 2003).

Incorporating feedback mechanisms into the model allows the carbon and nitrogen uptake rates to become responsive to internal concentrations and consequently changes in carbon and nitrogen availability. Testing the Thornley (1972) model with changes in external CO_2 and nitrogen shows that it is able to respond in certain aspects such as changes in percentage of total nitrogen within the plant but carbon and nitrogen uptake rates remain constant. Similarly, when simulating a defoliation experiment, the plant is able to respond correctly to a reduction in source strength by correcting its internal carbon and nitrogen concentrations. This is due to the transport mechanism within the model but carbon uptake rate remains constant without the imposed feedbacks. Running the feedback model without feedback 2 (high nitrogen reduces nitrogen uptake rate) allows the plant to respond more like experimental results when comparing plant nitrogen content, such that increasing atmospheric CO_2 reduces nitrogen percentage whilst the response to defoliation is different to the model with all feedbacks and experimental results. This implies that removing feedbacks enables certain responses to behave more like experimental results but in doing so, this prevents other responses to resource availability.

This model can be extended to include other known feedback mechanisms for instance on the rate of transport of substrate between leaves and roots (Chiou and Bush, 1998). The feedbacks chosen 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) and low sugars can also stop the transcription of nitrate reductase (Stitt and Krapp, 1999; Klein et al., 2000; Kaiser et al., 2002; Reda, 2015). This presents other feedbacks which could be incorporated in the the model. Further detailed analysis is needed on which feedbacks are more essential than others, for instance, what is the fewest number of feedbacks required to simulate reasonable responses to changes in environment? Are additional feedbacks required to make the model behave more reasonably?

Overall, the results of this chapter confirm that the model with internal feedback mechanisms based on internal carbon and nitrogen concentrations is able to reproduce most of the behaviours seen in experiments varying carbon and nitrogen availability in plants addressed here. This work provides insight into how a combination of feedbacks work together to allocate resources to above and below ground biomass and identifies which feedbacks play a larger role in environmental responses. This chapter provides a framework model to investigate the dynamics between internal feedback mechanisms which control the partitioning of biomass and uptake of external resources. Furthermore, this chapter strengthens the need to know more about the physiological mechanisms underpinning resource allocation, as understanding the mechanisms behind allocation can provide new areas of focus to manipulate the net primary productivity of plants.

Chapter 5

General discussion

1 Summary of findings

The aim of this thesis was to investigate how plant growth can be improved through the simulation of resource allocation between above and below ground biomass. Specifically, the work looked at how the processes defining growth (i.e resource uptake rates, respiration, growth rates) are coordinated together through a combination of internal responses to carbon and nitrogen. Here, the main findings of each chapter are identified and their potential application to crop productivity is discussed.

Chapter 2 used a simple model of carbon allocation to show that the costs of leaf and root maintenance respiration have a large impact on how allocation between leaves and roots alters total plant mass. When leaf maintenance respiration costs twice as much carbon than that of the roots, increasing allocation towards the roots can increase total plant size. Additionally, the costs of maintaining leaves and roots has an impact on the range of allocation strategies which produce similar final plant masses.

Chapter 3 developed a framework model of internal feedback mechanisms on carbon and nitrogen uptake rates, and leaf and root growth rates, when carbon and nitrogen are high. This showed that stepwise functions best simulated all three contrasting environmental cases when increasing uptake rates, whilst a linear function best simulated negative feedbacks on uptake and a scalar function best simulated feedbacks on individual relative growth rates. Decreasing carbon or nitrogen uptake rate when internal carbon or nitrogen concentrations are high (Feedbacks 1 & 2) respectively have the strongest effects on total plant mass. When implementing all six feedbacks into the model, plant growth increases and allocation towards the leaves increases when carbon availability is hindered and similarly root growth increases with a deficiency in nitrogen. Therefore the framework model is able to respond appropriately to changes in carbon and nitrogen availability.

Chapter 4 showed that the framework of feedback mechanisms can overall reflect observed plant responses to changes in CO_2, soil nitrogen concentrations and leaf defoliation when applying it to a widely supported, parameterised transport resistance model (Thornley, 1972). Specifically, the feedback model produced an increase in total plant mass, total plant nitrogen, carbon uptake rate and nitrogen uptake rate with high CO_2 and soil nitrogen. This shows that nitrogen increases the effect of high CO_2 on total plant mass, which agrees with experimental results. The model results also show that high atmospheric CO_2 reduces nitrogen content however high CO_2 has a positive effect on the model. Experimental results show that high nitrogen reduces the negative effect of CO_2 on carbon uptake rate whereas the model results show that high soil nitrogen increases the positive effect of high CO_2 on carbon uptake rate. Additionally, the model results matched experimentally observed behaviours when implementing defoliation, such that 7 days after halving total leaf mass, leaf nitrogen and carbon uptake rate increase and leaf carbon decreases. Therefore, a model which simulates feedbacks on source and sink activity with changes in internal carbon and nitrogen mostly behaves the same as experimentally observed responses to changes in source strength.

2 A general theoretical approach

Initially a general theoretical approach was taken to determine the overall effects of allocation between leaves and roots on total plant mass. By taking a theoretical approach toward carbon balance within the plant, the limits to which plants can allocate growth to leaves and roots was determined (given by a range of potential leaf and root masses). Chapter 2 uses a simple allocation assumption that the ratio between leaf and root growth is constant throughout the plant's life to investigate the effects of allocation strategies on growth. Comparing the effect of the ratio between leaves and roots on total plant size is not explicitly visited in most plant growth models. By keeping the model simple, it made it easy to determine the combined impacts of allocation strategies with photosynthesis or respiration on total plant mass, which might otherwise be constrained heavily by data.

This work additionally investigated the effect of specific allocation strategy during vegetative growth on the effect of reproductive output (i.e seed mass) and flowering time. Many plant growth models investigate the causes of flowering time but do not look at the impacts of allocation strategy on this. The combined outlook on vegetative and reproductive growth gives a wider perspective of allocation on growth.

3 Maintenance respiration

The simple approach of Chapter 2 enabled the effects that maintenance respiration have on allocation and growth to emerge, recommending that maintenance respiration should be a research focus in improving the efficiency of biomass production. This work hypothesises that for plants with higher leaf maintenance respiration, reducing the leaf canopy size can increase total plant mass.

Growth (the costs of tissue synthesis) and maintenance (the costs of tissue turnover) respiration equally consume roughly 20%-30% of carbon acquired through photosynthesis (Gifford et al., 1984; Amthor, 1989; Cannell and Thornley, 2000; Amthor et al., 2019), therefore if respiration can be optimised, this is represents a large potential increase for yield improvements. The cost of respiration in the leaves and roots varies a lot. Across 39 grassland and savannah species, leaf respiration ranges between 10.4 and 22.9 $nmolg^{-1}s^{-1}$ roots is 4 -19.3 $nmolg^{-1}s^{-1}$ (Tjoelker et al., 2005), showing higher mean respiration rates for leaves than roots. Conversely, Johnson (1983) argues that root respiration should be higher due to the costly nature of nitrogen and anion uptake and assimilation.

Respiration rates are determined by the consumption of ATP (Plaxton and Podestá, 2006) and are sensitive to environmental conditions (Black et al., 1987; Theodorou and Plaxton, 1993; Møller, 2001; Podestá and Plaxton, 2003; Plaxton and Podestá, 2006; McDonald and Vanlerberghe, 2008). Ryan (1991) states that maintenance respiration is more sensitive than growth respiration to environmental and internal changes. Growth respiration is more dominant during the fast-growing earlier stages of a plants life and maintenance respiration is more dominant when plants are older (Amthor et al., 2019).

The complexity and numerous processes involved in respiration cause attention to be directed more towards photosynthesis research (Bonner, 1962; Loomis and Williams, 1963; Ainsworth and Ort, 2010). However, advances in respiration research now make it possible to identify specific processes involved in respiration to be optimised. Amthor et al. (2019) identify several hypothesised actions to take such as: moving nitrate reduction from the roots to the leaves to make use of surplus energy available in the leaves (Andrews et al., 2004); moving nitrate assimilation from roots to leaves (Andrews et al., 2004; Shaw and Cheung, 2018); reducing protein damage by replacing vulnerable amino acids using proteome datasets (Bilova et al., 2017; Soboleva et al., 2017); replacing inefficient pathways with alternative ones (Amthor et al., 2019) and moving processes which occur overnight to the daytime, when surplus photosynthetic energy is available (Ishihara et al., 2015; Verbančič et al., 2018; Brauner et al., 2018).

Some of the strategies that Amthor et al. (2019) propose to reduce carbon loss via respiration involve moving processes which primarily occur in the root to the leaves. The processes of nitrate reduction and assimilation are described as maintenance costs which scale with plant size (Amthor, 1989; Cannell and Thornley, 2000; Litton et al., 2007; Amthor et al., 2019). Since, for some plant species, leaf respiration is higher than the roots, moving respiratory processes into the leaves would increase the likelihood of leaf maintenance respiration costing twice that of the roots.

The analysis in Chapter 2 could be extended to further investigate the effects of different ratios of maintenance costs between leaves and roots on allocation and growth. This work only looks at when maintenance respiration is equal and when leaf maintenance respiration is twice that of the roots. To be able to refine these boundaries on allocation, the model needs to be parameterised and compared against experimental data. Such parameterisation will enable these predictions to become testable hypotheses for plant growth experiments.

4 Modelling feedbacks

Many plant growth models simulate the use of carbon and nitrogen whilst investigating the effects of environmental heterogeneity (Reynolds and Thornley, 1982; Cheeseman, 1993; Buckley and Roberts, 2006; Drouet and Pagès, 2007; Feller et al., 2015). However, the allocation of resources for growth is not often an emergent property brought about through the simulation of feedback responses of carbon and nitrogen concentrations. Some models simulate one or two dependencies of carbon on nitrogen on each other. For instance, Thornley (1972) simulates the translocation of carbon and nitrogen between sources and sinks to balance their respective pools of concentration. Drouet and Pagès (2007) simulate a nitrogen uptake rate which increases with carbon availability and decreases with nitrogen availability along with a carbon uptake rate which increases with nitrogen availability and declines with carbon availability. Although these are similar feedbacks used in this work, no previous models have simulated the combination of positive and negative feedback effects on uptake rates along with a transport mechanism. Additionally, no models have simulated positive feedbacks on leaf and root growth capacity with high levels of carbon and nitrogen. The framework of feedbacks developed in this thesis extends the work of other models by including more types of feedbacks than any other plant growth model. By incorporating more of the observed feedbacks, this work provides a closer representation to how allocation should be modelled.

Models which simulate feedbacks based on carbon or nitrogen concentrations make assumptions on how they should be simulated and do not look at the type of function that should be used. In order to develop a framework of multiple feedbacks, each feedback was simulated using five functions to determine which behaved the most reasonably when varying carbon and nitrogen availability. After the most reasonable function was determined for all three environmental cases, the feedbacks were implemented into a simple carbon and nitrogen model. This work extends that of others by investigating which out of the six feedbacks alter growth the most by looking at total plant mass, shoot:root ratio, internal concentrations and uptake rates.

The framework was developed to build a balanced set of responses to carbon and nitrogen such that carbon and nitrogen uptake rate and leaf and root growth rate are sensitive to internal concentration.

These responses are paired so that if carbon uptake rate can be increased, there is a feedback available for nitrogen uptake rate to increase to match it. This framework model can be used to directly test the effects of specific feedbacks on growth by controlling the relative “strengths" of each feedback in the model. In future this could be used to make predictions which can be used to investigate these feedbacks experimentally.

The framework model developed within this thesis provides a tool to analyse known feedbacks on growth processes. When implementing the framework into a parameterised model, results showed that the model responded to changes in source availability in the same way to a number of key experimental observations from the literature. Specifically, carbon uptake rate and leaf nitrogen increases, whilst leaf carbon decreases 7 days after defoliation. This is the same as experimentally observed responses to defoliation. The combined positive effects of CO_2 and soil nitrogen on total plant mass matches experimental results. However, the model was unable to reproduce the negative effect of high CO_2 on total plant nitrogen and the negative effect of high CO_2 on carbon uptake rate against intercellular CO_2 as the model shows positive responses to CO_2. Experimental results show that high nitrogen reduces the negative effect of CO_2 on carbon uptake rate whereas the model results show that high soil nitrogen increases the positive effect of high CO_2 on carbon uptake rate. This suggests that this framework adequately, but not entirely, describes the allocation mechanisms but not entirely.

There are other observed feedback responses to carbon and nitrogen concentration which have not been investigated in this thesis. In particular, the models in this thesis do not include any feedbacks which respond to low levels of carbon and nitrogen. Low sugar levels repress the transcription of nitrate reductase (Stitt and Krapp, 1999; Klein et al., 2000; Kaiser et al., 2002; Reda, 2015), consequently lowering the rate of nitrate assimilation. This acts as a mechanism to lower carbon consumption when carbon source strength is low. Klein et al. (2000) show that when leaf sugar is lower than $5\mu mol$ hexose equivalents g^{-1}, the transcription of nitrate reductase stops entirely in Tobacco plants. Carbon levels are typically higher than this lower bound, but can reach these values when in extended dark periods, in short day conditions, low light or in plants with reduced photosynthetic rates (Matt et al., 1998; Stitt and Schulze, 1994; Klein et al., 2000).

Sugars can also stop meristem growth (Lastdrager et al., 2014), such that carbon source strength can inhibit carbon sinks, when carbon is low. When there are enough sugars available they promote T6P production, this inhibits SnRK1 activity which represses processes involved in growth. When sugars are low, SnRK1 is not inhibited. Additionally, Nunes et al. (2013) and Lastdrager et al. (2014) show that when nitrogen concentrations are low, less glucose is needed to stop seedling development, suggesting that nitrate can inhibit the effect of sugar signalling. This implies that, not only are there feedbacks which are sensitive to carbon or nitrogen, but these processes are much more complicated and are co-dependent upon carbon and nitrogen concentrations.

A simplification used in the models in this thesis is that the accumulation or depletion of carbohydrate intermediates triggers these known feedback behaviours, whereas it is much more complicated than that. Some feedbacks are induced specifically by the presence of starch, glucose or sucrose. An important part of the regulation of sources and sinks is the allocation of carbon into starch and sucrose (White et al., 2016). Carbon can be stored in reserves in the form of starch and broken down to provide additional energy when needed. The levels of sugars and starch alter the enzymes responsible for starch synthesis and degradation (Smith and Stitt, 2007). Sugar accumulation can also have a negative effect on phloem transport. Vaughn et al. (2002) show that high levels of sucrose in the phloem reduces phloem loading capacity through the down-regulation of the sucrose symporter gene.

Knowing there are other observed feedbacks shows that there is still much work to be done in order to understand the mechanisms behind the allocation between sources and sinks. This framework can be extended by incorporating additional feedbacks and / or by using an entirely different set of feedbacks. The stepwise functions could be adapted to have upper and lower bounds on concentrations to simulate feedbacks which respond to a low and a high nutrient status. It is not obvious whether these additional types of feedbacks would enable the model to respond more like experimental results.

Some experiments state a threshold value in which the feedback occurs but not all results are so quantitative (Klein et al., 2000; Nunes et al., 2013). Most address the timescale of the measurements but do not focus on the time it takes from the sensing of resource to the overall effect on plant growth. Additionally, these experiments are not performed all on one species and these threshold values could therefore be species-specific. For feedbacks to be modelled effectively, more information is needed about their behaviour. Specifically, the following questions need to be answered: 1. Are these feedbacks continuous or do they act like a switch? 2. How long does it take from sensing the signalling molecule to directly altering growth processes? 3. Do different feedbacks behave the same or are some faster than others? 4. How strong are the feedback responses and how sensitive are they to changes in carbon and nitrogen concentrations? To understand how these feedbacks work, experiments need to be carried out which measure leaf and root metabolites along with measurements of carbon and nitrogen uptake rates throughout. Information is needed on how plant metabolites change when environmental conditions change and how this consequently alters other processes within the plant. This would provide a fuller picture of when concentrations become too high or low, and show the internal changes which arise over time. Measuring uptake rates with this could provide a better understanding on how the feedbacks directly alter them.

5 Future directions

The work addressed in this thesis can be extended by spending more time investigating the individual effects of each feedback on the responses to atmospheric CO_2 and nitrogen availability. Specifically, future work should look at the minimum number of feedbacks required to simulate an observed response to changes in the environment. A possible route to look at this is by using optimal control theory and it could be used to investigate which combination of feedbacks produces a larger plant. Further work can be done in understanding which feedbacks are more responsible for allocation than others, by looking into how well transport resistance models are able to respond to changes in resource availability. Does the simulation of the movement of resources between leaves and roots do the same things as some of the feedbacks imposed in the feedback framework? The work addressed here could be extended to investigate how the framework model reacts to environmental changes with additional feedbacks mentioned in this discussion. Would the inclusion of more feedbacks enable a plant to respond more or less like experimental data? Do some feedbacks make others redundant?

With information from experimental data on how these feedbacks work, individual feedbacks can be modelled more specifically. This would require information on whether the feedback incrementally reacts to changes in concentrations or if it is an instant switch-like mechanism. It would need to be determined if there are any threshold values which prompt the feedback responses. Once the behaviour of each feedback is qualitatively known, they can be simulated and combined into a parameterised model. The relationship between feedbacks can be investigated and the sensitivity of each mechanism on growth and allocation can be determined within in a parameterised setting. Feasible predictions on which behaviours could be targeted in order to optimise growth.

To make direct links between this work and making predictions on how crop yield can be improved, the framework needs to be implemented into a crop scale model. Such crop growth models can simulate the individual leaf or root based processes and scale up to canopy level (Lizaso et al., 2005, 2011; Evers and Vos, 2013). They are able to simulate plant architecture and competition for resources within stands (Evers and Vos, 2013) and can make predictions for crop management strategies. Crop growth models are useful tools for agricultural management decisions in selecting appropriate species and predicting yield responses to environmental changes (Bryant and Snow, 2008; Deryng et al., 2011; Di Paola et al., 2016). Boote et al. (2013) argue that crop growth models need more mechanistic detail with regards to allocation of carbon and nitrogen among plant organs and environmental responsiveness. Zhu et al. (2016) argue for a unification of plant growth models to apply highly detailed mechanistic models which focus on different aspects of plant growth into one to simulate a virtual plant.

The feedback framework should be relatively easy to apply to other crop-scale models which scale up from individual organs, as each feedback is simply a modification of growth processes (photosynthesis, nitrogen uptake, leaf growth, root growth) at the leaf or root level. These processes should already be expressed within these models and would only require additional terms so long as carbon and nitrogen content can be calculated.

Overall, the work in this thesis provides two different approaches for simulating the allocation of growth to sources and sinks. First, a general theoretical approach is used to show that the cost of maintaining leaf and root tissue can greatly alter the effect of allocation on total plant mass.

Second, a much more mechanistic approach is used to simulate a framework of feedback mechanisms which have been observed through changes in carbon and nitrogen concentrations. This provides a framework tool which includes more feedback mechanisms than any other previous plant growth model to illustrate the processes behind allocation. The results of this work found that the framework model mostly reflects the behaviour in CO_2, soil nitrogen and defoliation experiments. This work argues the need to further understand how these feedbacks work, as without knowing how to quantify the rate or strength of the feedback, the assumptions upon which these feedbacks are modelled can vary widely.

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Appendix A

Chapter 2 appendix

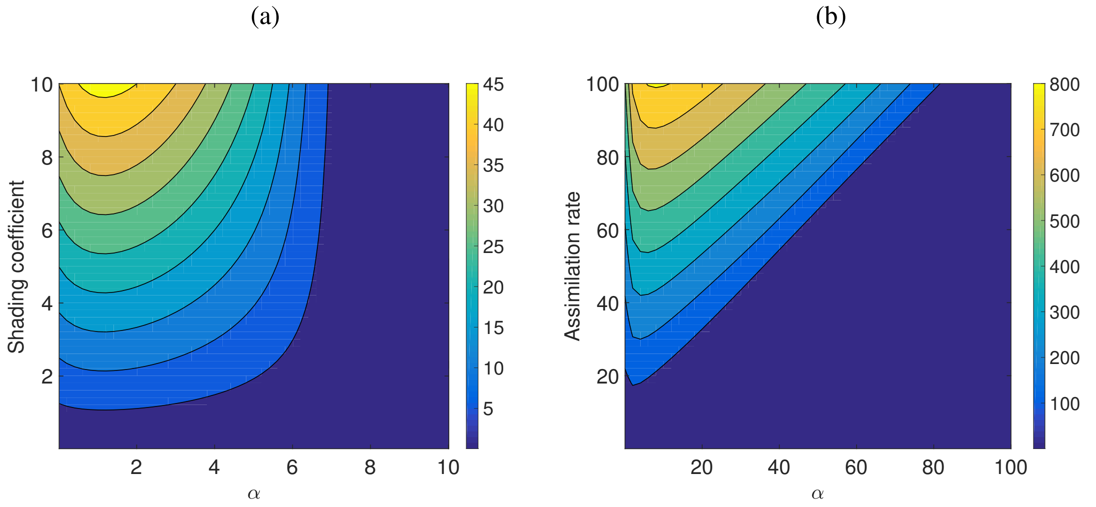


Fig. 1 a) Sensitivity analysis of final plant size when varying shading coefficient and allocation strategy when $A_0 = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 1$. b) Sensitivity analysis of final plant size when varying assimilation rate and allocation strategy when $\theta = 10$, $m = 1$, $R_1 = 2$ and $R_2 = 1$. Using initial leaf and root tissue of $l_0 = 0.01$ and $r_0 = 0$. The black lines represent contours of constant final plant size. Colour bar represents different final plant sizes. All model parameters are dimensionless.

The black lines represent parameter space where final plant size remains the same and the colours represent different final plant sizes (yellow is high plant biomass and blue is virtually no plant material). For low shading coefficient values, the black lines of constant final plant size are horizontal for the majority of allocation strategies (Fig. 6a). As allocation strategy continues to increase after $\alpha = 2.7$, these lines become almost vertical. As the shading coefficient increases, the region where the black line is approximately horizontal for a given shading value decreases in size and is much smaller using $\theta > 7$. This implies that the shading coefficient reduces the level of plasticity within the model by increasing light penetration into the leaf canopy.

Appendix B

Chapter 4 appendix

1 Table of conversions

Parameter	Thornley units	Data value	Data units	Conversion	Definitions
A_c - C uptake rate	$kgmolm^{-3}s^{-1}$	40	$\mu molm^{-2}s^{-1}$	$\frac{A_c}{a} \times 10^{-3}$	$\mu mol = 10^{-9}kgmol$
Leaf thickness (a)	-	120	μm		$\mu m = 10^{-6}m$
A_n - N uptake rate	$kgmolm^{-3}s^{-1}$	61	$\mu molkg^{-1}s^{-1}$	$A_n b \times 10^{-6}$	$1\mu mol = 10^{-9}kgmol$
Root tissue density (Wahl & Ryster, 2000) (b)	-	0.24	$mgmm^{-3}$		$mg = 10^{-6}kg$
					$mm^{-3} = 10^9m^{-3}$
r_c- C transport resistance	s	-	d	-	$day = 86400s$
r_n - N transport resistance	s	-	d	-	$day = 86400s$
θ - mass to volume (Garnier and Laurent, 1994)	$m^3(kgmol)^{-1}$	-	-	$\frac{1}{x} \times 10^3 \times 12$	$g^{-1} = 10^3kg$
Leaf dry density (x)	-	1×10^6	gm^{-3}		$kg^{-1} = 12kgmol^{-1}$
Leaf dry density (carbon)	$m^3(kgmol)^{-1}$	45%	-	$0.45 \times \theta$	-
Root tissue density (y)	-	0.24×10^6	gm^{-3}	-	$mm^{-3} = 10^9m^{-3}$
V_s - Leaf tissue	m^3	0.01*	g	$\times \frac{1}{x}$	$1 \times 10^6 gm^{-3}$
V_r - Root tissue	m^3	0.01*	g	$\times \frac{1}{y}$	$0.24 \times 10^6 gm^{-3}$
RGR	s^{-1}	-	d^{-1}	-	$s^{-1} = \frac{1}{86400}day^{-1}$
C_l - Leaf carbon	$kgmolm^{-3}$	92.8$*$	$nmolmg^{-1}$	$x \times 0.45$	$nmol = 10^{-12}kgmol$ $mg^{-1} = 10^3g^{-1}$
C_r - Root carbon	$kgmolm^{-3}$	63$*$	$nmolmg^{-1}$	$x \times 0.45$	$nmol = 10^{-12}kgmol$ $mg^{-1} = 10^3g^{-1}$
N_l - Leaf nitrogen	$kgmolm^{-3}$	0.1$*$	$nmolmg^{-1}$	$\times 0.45x \times \lambda_1$	$nmol = 10^{-12}kgmol$ $mg^{-1} = 10^3g^{-1}$
N_r Root nitrogen	$kgmolm^{-3}$	7.54$*$	$nmolmg^{-1}$	$\times 0.45x \times \lambda_2$	$nmol = 10^{-12}kgmol$ $mg^{-1} = 10^3g^{-1}$
R_1 - Leaf maintenance respiration	$kgmolm^{-3}s^{-1}$	15	$nmolg^{-1}s^{-1}$	$\times x \times 10^{-12}$	$nmol = 10^{-12}kgmol$
R_2 - Root maintenance respiration	$kgmolm^{-3}s^{-1}$	15	$nmolg^{-1}s^{-1}$	$\times y \times 10^{-12}$	$nmol = 10^{-12}kgmol$

Table 1 Definitions and formulae used to convert parameter values and initial conditions to SI units.$*$ - initial condition.

2 Responses to changes in resource availability without feedback 2

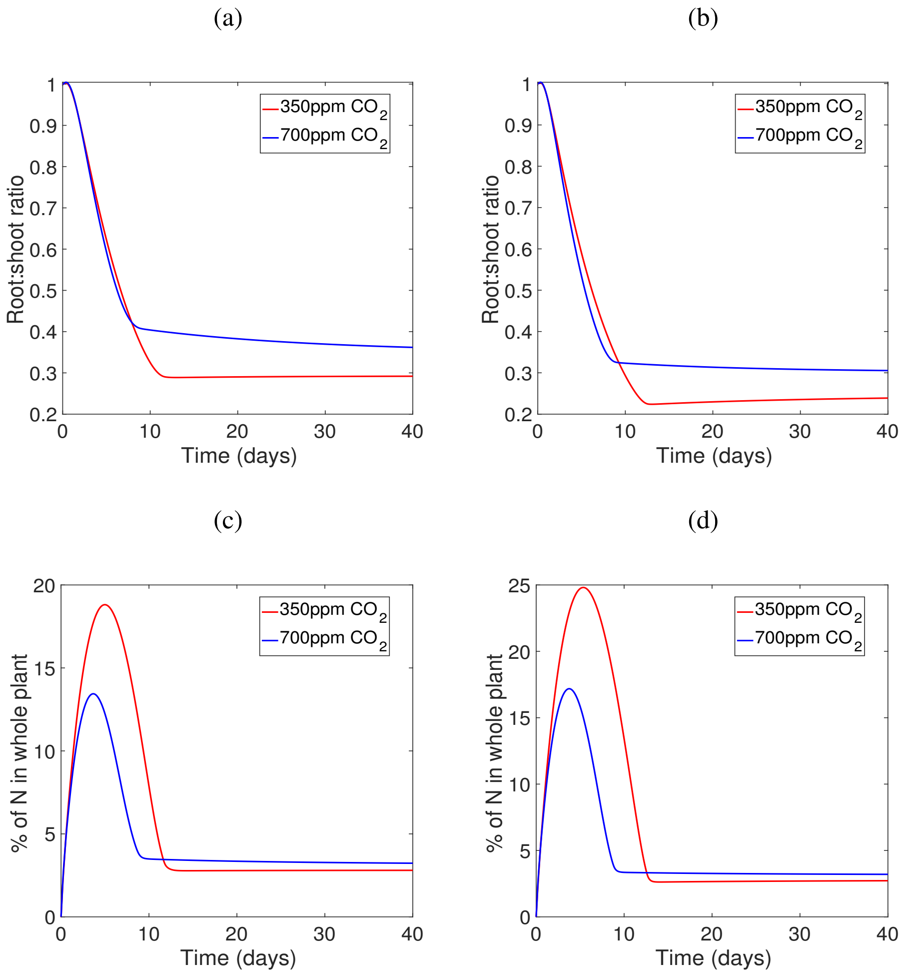
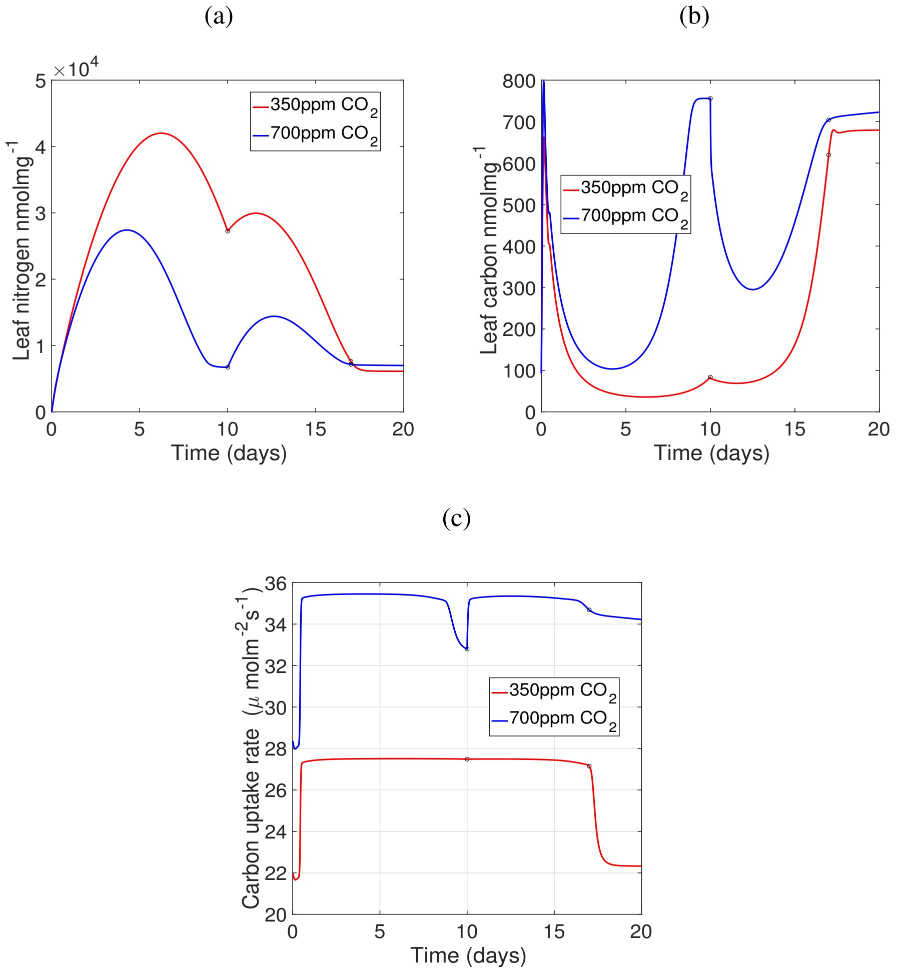

(a) (b) (c) (d)

Fig. 1 The effect of varied CO_2 and soil nitrogen on the model without feedback 2 (high nitrogen reduces nitrogen uptake rate) a) The relationship between root:shoot ratio over 40 days and two CO2 treatments (350ppm and 700ppm) when soil nitrogen is high ($400\mu mol$). b) The relationship between root:shoot ratio over time and two CO2 treatments (350ppm and 700ppm) when soil nitrogen is low ($200\mu mol$). 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 ($n = 400\mu mol$) b) low soil nitrogen ($n = 200\mu mol$). Ran for 40 days with initial leaf mass of $0.01g$ and root mass of $0.01g$ and initial concentrations $C_{l0} = 92.8nmolmg^{-1}$,$C_{r0} = 63nmolmg^{-1}$,$N_{l0} = 0.1nmolmg^{-1}$, $N_{r0} = 7.54nmolmg^{-1}$.



(a)

(b)

(c)

Fig. 2 The effect of defoliation (when total leaf mass is halved at day 10) without feedback 2 (high nitrogen reduces nitrogen uptake) and a high ($700ppm$, blue lines) and low ($350ppm$, red lines) CO_2 treatment on a) Intermediate leaf carbon concentration for 20 days. b) Intermediate leaf nitrogen over 20 days. c) carbon uptake rate over 20 days. Markers signify concentrations of carbon and nitrogen in the leaves and carbon uptake rate at day 10 and day 17 for both carbon and nitrogen plots. All run with soil nitrogen $400\mu mol$ 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 $N_{r0} = 7.54nmolmg^{-1}$.