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A Dynamical Model of Environmental Effects on Allocation to Carbon-based Secondary Compounds in Juvenile Trees

S. GAYLER[1],*, T. E. E. GRAMS[2], W. HELLER[3], D. TREUTTER[4] and E. PRIESACK[1]

[1]*Institute of Soil Ecology, GSF–National Research Center for Environment and Health, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany,* [2]*Ecophysiology of Plants, Department of Ecology, Technische Universität München, Am Hochanger 13, D-85354 Freising-Weihenstephan, Germany,* [3]*Institute of Biochemical Plant Pathology, GSF–National Research Center for Environment and Health, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany and* [4]*Unit of Fruit Science, Center of Life Sciences Weihenstephan, Technische Universität München, Alte Akademie 16, D-85350 Freising, Germany*

• *Background and Aims* Patterns and variations in concentration of carbon-based secondary compounds in plant tissues have been explained by means of different complementary and, in some cases, contradictory plant defence hypotheses for more than 20 years. These hypotheses are conceptual models which consider environmental impacts on plant internal demands. In the present study, a mathematical model is presented, which converts and integrates the concepts of the 'Growth–Differentiation Balance' hypothesis and the 'Protein Competition' model into a dynamic plant growth model, that was tested with concentration data of polyphenols in leaves of juvenile apple, beech and spruce trees. The modelling approach is part of the plant growth model PLATHO that considers simultaneously different environmental impacts on the most important physiological processes of plants.

• *Methods* The modelling approach for plant internal resource allocation is based on a priority scheme assuming that growth processes have priority over allocation to secondary compounds and that growth-related metabolism is more strongly affected by nitrogen deficiency than defence-related secondary metabolism.

• *Key Results* It is shown that the model can reproduce the effect of nitrogen fertilization on allocation patterns in apple trees and the effects of elevated CO_2 and competition in juvenile beech and spruce trees. The analysis of model behaviour reveals that large fluctuations in plant internal availability of carbon and nitrogen are possible within a single vegetation period. Furthermore, the model displays a non-linear allocation behaviour to carbon-based secondary compounds.

• *Conclusions* The simulation results corroborate the underlying assumptions of the presented modelling approach for resource partitioning between growth-related primary metabolism and defence-related secondary metabolism. Thus, the dynamical modelling approach, which considers variable source and sink strengths of plant internal resources within different phenological growth stages, presents a successful translation of existing concepts into a dynamic mathematical model.

Key words: Plant growth, carbon-based secondary compounds, plant defence hypotheses, simulation model, phenolic allocation, nitrogen, carbon dioxide, *Malus domestica*, *Fagus sylvatica*, *Picea abies*.

INTRODUCTION

Environmental factors such as nutrient supply, temperature, light conditions or atmospheric carbon dioxide concentrations can influence the level of defence-related carbon-based secondary compounds in plant tissues, and, consequently, plant internal partitioning of carbohydrates and energy between growth-related primary metabolism and defence-related secondary metabolism has been the subject of ecological research for more than 20 years (Coley *et al.*, 1985; Herms and Mattson, 1992; Bazzaz, 1997; Wainhouse *et al.*, 1998; Koricheva, 2002*b*; Glynn *et al.*, 2003; Matyssek *et al.*, 2005; Fine *et al.*, 2006). Numerous studies have been performed within the last years to investigate environmental effects on allocation to polyphenolic compounds, which are known to be used for defence-related functions in numerous plant–pathogen interactions across taxa (Dixon, 2001), both as constitutive defence metabolites and as induced

phytoalexins (Mittelstraß *et al.*, 2006). For example, in all parts of Sitka spruce (*Picea sitchensis*), the concentrations of polyphenols were higher in low nitrogen treatments and increased in high light treatments (Wainhouse *et al.*, 1998). Glynn *et al.* (2003) reported a decrease of phenolic metabolite concentrations in leaves of Black poplar (*Populus nigra*) in a high nutrient treatment. A study with paper birch (*Betula papyrifera*) showed that CO_2 enrichment stimulated a pathway-wide increase in carbon partitioning to phenylpropanoids (Mattson *et al.*, 2005). Low-irradiance leaves were correlated with lower concentrations of soluble phenolic metabolites in a study with different plant species carried out by Poorter *et al.* (2006). Additional nitrogen supply decreased phenolic concentrations in potato leaves (Mittelstraß *et al.*, 2006).

During this time, different complementary, and in some cases contradictory (Mattson *et al.*, 2005), plant defence hypotheses were developed to explain patterns and variations in the concentration of carbon-based secondary compounds in plant tissues. The 'Carbon–Nutrient Balance'

* For correspondence. E-mail gayler@gsf.de

(CNB) hypothesis (Bryant *et al.*, 1983; Tuomi *et al.*, 1991) explains that variation in plant defence is based on the environmentally available carbon and nitrogen. This hypothesis predicts that plants growing in nitrogen-poor soils will favour allocation to carbon-based defence-related metabolites, while those plants growing under low carbon availability (e.g. low light conditions) will be more likely to produce nitrogen-based metabolites of similar function. In addition, CNB predicts that in plants growing in low-nutrient conditions, the level of constitutive carbon-based defences will decrease when the availability of nitrogen is increased (e.g. upon fertilization). The 'Growth–Differentiation Balance' (GDB) hypothesis suggests that allocation of plant internal resources is determined by plant internal competition for common substrates and energy, and that patterns of plant defence result from resource trade-offs between 'growth-related processes' and 'differentiation-related processes' depending on the environment in which the plant was grown (Loomis, 1932; Herms and Mattson, 1992; Herms, 2002). According to this hypothesis, any environmental factor reducing growth rate to a greater degree than photosynthesis may increase the resource pool available for allocation to secondary metabolites. The 'Protein Competition' model (PCM) (Jones and Hartley, 1999) focuses on the biochemical regulation of synthesis of proteins and phenylpropanoids, both of which compete for the limiting resource phenylalanine, which is a branch point at the end of the shikimic acid pathway located between primary and secondary metabolism. Consequently, the growth–defence trade-off depends not only on competition for a limited pool of available carbohydrates, but also on competition for nitrogen as a component of common precursor compounds.

These plant defence hypotheses are conceptual models which consider environmental impacts on plant internal demands. Although the volatile dynamics of carbon partitioning are appreciated by these concepts, they cannot address plant growth dynamics as realistically as a good dynamical model. Consequently, the discussion about adequacy of plant defence hypotheses neglects to a large extent the dynamics of plant growth (Bazzaz, 1997; Hamilton *et al.*, 2001; Koricheva, 2002*a*, *b*; Stamp, 2003*a*, *b*; Nykänen and Koricheva, 2004). However, variable source and sink strengths of the plant internal resources of carbon and nitrogen during different phenological growth stages can also affect the patterns of secondary metabolite concentrations in plant tissues (Leser and Treutter, 2005). Therefore, a more realistic view by applying a dynamical model may help to avoid premature conclusions about the validity of such hypotheses.

To develop a tool that can be used to test the various plant defence hypotheses in the dynamic context of plant growth and phenological development, a modelling approach for allocation of carbohydrates to a pool of carbon-based secondary compounds was integrated into the functional generic plant growth model PLATHO (Gayler and Priesack, 2003; Gayler *et al.*, 2004, 2006). PLATHO considers simultaneously different environmental impacts on the most important processes of plant physiology. The present concept for modelling formation of defence-related compounds integrates assumptions of the CNB and GDB hypotheses as well as the PCM. This concept was first parameterized with data from an experiment with young apple trees 'Golden Delicious' (Gayler *et al.*, 2004). The intention of the present study was the evaluation of this modelling concept with additional data across species on the basis of data sets from experiments with juvenile apple (*Malus domestica* 'Rewena'), beech (*Fagus sylvatica*) and spruce trees (*Picea abies*). The available experimental data sets were split into two fractions. One data set of each experiment was used to parameterize the model for the respective plant species. The second fraction was then used for independent model testing. Simulated and measured concentrations of carbon-based secondary compounds in leaves as well as growth of above-ground parts of the plants were compared. Finally, the model was used to analyse the proposed partitioning mechanism for allocation to defence-related compounds in beech and spruce under a wide range of nutrient availability.

METHODS

The plant growth simulation model PLATHO

The model name PLATHO is an acronym for *PLA*nts as *T*ree and *H*erb *O*bjects. The model simulates the general processes common to all plants such as phenological development, photosynthesis, water and nitrogen uptake by roots, biomass growth, respiration and senescence. Different species are handled solely as special cases of the class of plants. Functionally equivalent plant species can be simulated by model re-parameterization, using different species-specific parameters. PLATHO combines concepts from the plant growth models CERES (Jones and Kiniry, 1986), SUCROS (van Ittersum *et al.*, 2003), SPASS (Gayler *et al.*, 2002; Wang and Engel, 2002), FAGUS (Hoffmann, 1995) and TREEDYN (Bossel, 1996) with new approaches to simulate C and N allocation to plant organs and plant internal biochemical pools (Gayler *et al.*, 2004) and to estimate competition effects between individual plants (Gayler *et al.*, 2006). The gain and consumption of C and N for growth- and maintenance-related processes are calculated at every time step, considering the biochemical composition of different plant organs. All conversion processes are estimated in units of glucose using biochemical information on the dominant pathways of the biosynthesis of the most important classes of substances. Up to 20 plant individuals can be simulated simultaneously, where each individual can differ in starting biomass, ecophysiological parameters or species. The occupied crown and soil volumes of each individual are defined as a cylinder with flexible height and diameter. The above- and below-ground parts of the plant are divided into single simulation discs. The vertical distribution of leaf area and root length within each cylinder is described by means of species-specific, rotationally symmetric distribution functions. Plant individuals are positioned in a rectangular grid with periodical boundary conditions. The competition intensity between the individuals is considered by competition

coefficients which are calculated from the overlap of the occupied space of neighbouring plants.

PLATHO is implemented in the modular modelling system Expert-N (Priesack, 2006). This development tool consists of several modules for simulating different processes in the soil–plant–atmosphere system that can be coupled in various combinations. The present simulation study was carried out by coupling PLATHO with the soil water transport modules of the model HYDRUS (Simunek *et al.*, 1998) and with modules for nitrogen transport and turnover of the model LEACHN (Hutson and Wagenet, 1992). The simulation time step of the whole model is governed by the solver of the partial differential equation for soil water transport, and varies between 0·001 and 0·1 d.

In the following section, the modelling approach for the simulation of resource partitioning between growth-related primary metabolism and defence-related secondary metabolism is presented in detail. A complete model documentation of PLATHO including all process descriptions and equations is given in Gayler and Priesack (2003).

The model considers four main biochemical pools: assimilates (glucose), A_{av} (g), structural biomass, W (g), carbon-based secondary compounds, S (g) and reserves, R (g) (cf. Gayler *et al.*, 2004). The model simulates material fluxes between these pools at the plant level depending on actual plant internal availabilities of carbohydrates and nitrogen and on actual demands of these resources for growth- and defence-related processes (source/sink-related approach). A priority scheme of resource allocation is implemented in the model that is based on the following key assumptions. (*a*) The demand for maintenance takes priority over all other processes. (*b*) Growth takes priority over defence (Tuomi, 1991). This is in accordance with the CNB and GDB hypotheses which predict that additional assimilates may be converted to secondary metabolites if carbohydrates accumulate in excess of growth demands or if availability of nitrogen is lower than the nitrogen demand required for growth processes. (*c*) Photosynthesis is less affected by nitrogen deficiency than growth; this is one of the assumptions of the CNB hypothesis (Bryant *et al.*, 1983). (*d*) Potential allocation to defensive compounds is inversely correlated to maximal plant growth rate (Coley *et al.*, 1985). (*e*) The formation of carbon-based defensive compounds (e.g. phenylpropanoids) requires sufficient nitrogen levels due to the requirements for biosynthesis of precursory compounds (Jones and Hartley, 1999). (*f*) Assimilates from actual photosynthesis will be first used for energy-consuming processes before remobilization of reserves (Lötscher and Gayler, 2005).

The realized increases of the biochemical pool sizes in each time step can be limited either by the availability of assimilates or by the availability of plant internal nitrogen. The amount of assimilates which are available for supplying growth processes and biosynthesis of defence-related compounds is calculated from the actual plant photosynthesis rate, P_{act} [g (glucose) d^{-1}], and potential reserve remobilization during the actual time step Δt (d), the demand for maintenance processes, D_M [g (glucose)] and the surplus assimilates remaining from the time step

before, A_{old} [g (glucose)]:

$$A_{av} = (P_{act} + R\,\tau_R)\Delta t - D_M + A_{old} \qquad (1)$$

where τ_R (d^{-1}) is the remobilization rate of the reserves. P_{act} is calculated by integrating the leaf gross photosynthesis per unit leaf area over plant height. The light distribution profile in the canopy is simulated by an enhancement of the method of Kropff and van Laar (1993) accounting for shading by next neighbours (cf. Gayler *et al.*, 2006). The effect of water availability and leaf nitrogen concentration on potential assimilation rate is considered by a minimum factor of both effects. The response of leaf gross photosynthesis to irradiance and leaf internal CO_2 concentration is calculated following the approach of Farquhar and von Caemmerer (Farquhar *et al.*, 1980; von Caemmerer and Farquhar, 1981). Glucose consumption for maintenance processes, D_M, is assumed to be proportional to organ biomass and to depend on temperature following a Q_{10} relationship. A_{av} represents the source strength of carbohydrates, whereas the sink strength results from the potential growth rate of total biomass, D_{pot} [g (glucose)], which represents the total demand of carbohydrates for structural growth and defensive compounds. In the present model, D_{pot} is determined by the maximum growth rate of the plant, r_{max} [g (glucose) g^{-1} d^{-1}], the living structural biomass, W_l (g) and two factors between 0 and 1 that consider the influence of temperature and phenology on plant growth:

$$D_{pot} = r_{max} W_l\,\Delta t\, f_T f_{Ph} \qquad (2)$$

f_T is an optimum function of air temperature and f_{Ph} depends on the phenological stage of the plant, reflecting the seasonal cycle of plant growth.

The amount of nitrogen potentially available for growth processes, N_{av} [g (N)], is calculated as the sum of potential nitrogen uptake from soil, $N_{upt,pot}$ [g (N)], and potential nitrogen mobilization from the nitrogen reserve pool in the plant, $N_{trans,pot}$ [g (N)]:

$$N_{av} = N_{upt,pot} + N_{trans,pot} \qquad (3)$$

Uptake of nitrogen by roots is simulated according to the method used in models of the CERES family (Jones and Kiniry, 1986; Hoffmann, 1995). $N_{upt,pot}$ results from the actual root surface, the availability of nitrogen in the soil and soil moisture conditions. The amount of mobile nitrogen, $N_{trans,pot}$, which is potentially available for retranslocation between plant organs during the time step Δt, is calculated using the nitrogen remobilization rate t_N (d^{-1}) and the difference between actual nitrogen content, N_{act} [g (N)] and minimal nitrogen content, N_{min} [g (N)], in plant organs:

$$N_{trans,pot} = \Sigma_i(N_{act,i} - N_{min,i})\tau_N\,\Delta t \qquad (4)$$

where *i* denotes the single plant organs (*i* = fine roots, gross

roots, stem, branches, leaves). The demand for nitrogen, N_{dem} [g (N)], is derived from nitrogen levels required to realize the potential plant organ growth, where a fixed level of nitrogen concentration in newly formed structural biomass for each plant organ is assumed.

The trade-off in partitioning resources between growth and defence on the whole plant level is considered in the model by enhancement of an equation, which was first suggested in its original form by Coley *et al.* (1985). The total demand of glucose equivalents, D_{pot} [g (glucose)], is divided into one part, which is related to structural growth, and another part, which is related to defence:

$$D_W = D_{pot}(1 - \sigma) \qquad (5)$$

$$D_S = D_{pot}\sigma \qquad (6)$$

This partitioning is given by a species-specific factor $\sigma = \sigma_C + \sigma_I$, consisting of a constitutive part, σ_C, and an inducible part, σ_I. σ_I is assumed to be greater than zero only in the case of actual stress, such as a pathogen attack or high atmospheric ozone concentrations. The constitutive part subsumes a minimal baseline of partitioning to defence-related secondary compounds that are always needed for development, survival and health, and a dispensable part that is only allocated to defensive compounds if carbohydrates are accumulated in excess of growth demands. The difference between maximal and minimal allocation to constitutive defence describes the phenotypic plasticity of a plant species in regard to allocation to carbon-based secondary compounds.

In each time step, the ratios between plant internal supply and demand of carbohydrates and of nitrogen are given by the factors φ_C and φ_N:

$$\varphi_C = \text{Min}\{1, A_{av}/D_{pot}\} \qquad (7)$$

$$\varphi_N = \text{Min}\{1, N_{av}/N_{dem}\} \qquad (8)$$

Both variables depend indirectly on the availability of carbon and nitrogen in the environment and numerous processes in the soil–plant system.

The realized conversion rate of assimilates to structural biomass, G_W [g (glucose)], results from the availability of assimilates and nitrogen:

$$G_W = D_W \varphi_C \varphi_N^{\beta} \qquad (9)$$

and the fraction of the available assimilates that is allocated to growth processes is given by

$$G_W/A_{av} = (1 - \sigma) \varphi_N^{\beta} \qquad (10)$$

where the exponent β ($\beta < 1$) reflects the fact that the growth rate is increased in a non-linear way by additional nitrogen supply. For β, a value of 0·5 is assumed.

If the demand for growth is fulfilled, the remaining assimilates can be converted to defence-related compounds. The increase in the pool of defence-related compounds, G_S [g (glucose)], can be sink limited if the amount of available assimilates exceeds the gain of structural biomass, G_W, plus the demand for defence, D_S. Furthermore, G_S can be limited by nitrogen availability, following the PCM. This is expressed in equation (11) by the factor φ_N^{δ}, where δ accounts for non-linearity in the relationship between formation of defensive compounds and plant internal nitrogen availability.

$$G_S = \begin{cases} D_S \, \varphi_N^{\delta} & \text{if } A_{av} \geq G_W + D_S \\ (A_{av} - G_W)\varphi_N^{\delta} & \text{otherwise} \end{cases} \qquad (11)$$

and

$$G_S/A_{av} = \begin{cases} \sigma(I)\varphi_N^{\delta}/\varphi_C & \text{if } A_{av} \geq G_W + D_S \\ [1 - (1 - \sigma(I)) \, \varphi_N^{\beta}] \, \varphi_N^{\delta} & \text{otherwise} \end{cases}$$
$$(12)$$

As the metabolism of carbon-based secondary compounds is less affected by nitrogen deficiency than growth processes, due to the continuous regeneration of phenylalanine from a limited nitrogen pool during phenolic biosynthesis (Mattson *et al.*, 2005), the exponent δ should be smaller than β and is set to 0·33 in the present simulations.

G_W and G_S are partitioned to the different plant organs leaves, branches, stem, gross roots and fine roots. The calculation of the partitioning factors, which depend on phenological stage and plant internal availability of assimilates and nitrogen, is described in the complete model documentation (Gayler and Priesack, 2003). The total change of living biomass of plant organs results from the biochemical costs of conversion of glucose to structural biomass and carbon-based secondary compounds and the actual loss rate of biomass due to stress or senescence.

In a final procedure a check is made of whether the pool of reserves must be depleted to meet all demands, or if assimilates are still remaining and can be used to refill the pool of reserves.

Experimental data and model parameterization

The parameterization of the single modules of the simulation model PLATHO is based on many experiments which were carried out within the frame of a special research program called 'SFB 607: growth and parasite defence–competition for resources in economic plants from forestry and agriculture' (Mattyssek *et al.*, 2002, 2005, www.sfb607.de). Most of these experiments are documented or referred to in two special issues of the journal *Plant Biology* [2002 (vol. 4, issue 2) and 2005 (vol. 7, issue 6)]. The data presented in this paper result from two experiments with juvenile apple cultivars 'Golden Delicious' and 'Rewena' (Rühmann *et al.*, 2002, Leser and Treutter, 2005) as well as beech and spruce trees (Bahnweg *et al.*, 2005; Kozovits *et al.*, 2005*a*, *b*).

In the experiment with apple trees (1-year-old graftings), the effects of low (N1), medium (N2) and high (N3) levels of nitrogen fertilization on shoot growth and on concentration of phenylpropanoids in leaves were investigated during one vegetation period. In the second experiment, 3-year-old beech and 4-year-old spruce trees of uniform height (about 0·2 m) were grown for two vegetation periods in monocultures and 1:1 beech/spruce mixtures in phytotrons. At the beginning of the experiment, each plant individual had approximately the same crown volume, but the initial biomass of spruce was about four times higher than that of beech. Plants were exposed to ambient or elevated (ambient + 300 ppm) CO_2 concentrations. Dry mass of plant organs (leaves, stems, shoot axes and roots) and concentrations of phenolic compounds in plant organs were determined at the end of the experiment.

For the parameterization of the model for apple, beech and spruce trees, as many parameter values as possible were taken from measurements. Missing parameter values were adopted from other plant growth simulation models or were taken from the literature (Penning de Vries *et al.*, 1989; Thornley and Johnson, 1990; Hoffmann, 1995; Bossel, 1996; Walton *et al.*, 1999; Bouma *et al.*, 2001). However, some species-specific parameter values were not available and the model had to be calibrated. In the case of apple trees, calibration was carried out with data of 'Golden Delicious' (Gayler *et al.*, 2004). For beech and spruce this was achieved using data from the ambient CO_2 treatment, whereas for model testing, data of additional treatments were applied (Gayler *et al.*, 2006).

RESULTS AND DISCUSSION

Allocation pattern

The putative model behaviour in resource allocation to structural biomass and carbon-based secondary compounds is presented in Fig. 1. The fractions of available resources, A_{av}, that are allocated to structural biomass, G_W, and to the pool of carbon-based secondary compounds, G_S, are functions of the relative plant internal availabilities of carbon, φ_C, and nitrogen, φ_N (Fig. 1A). The ratio G_W/A_{av} increases with increasing nitrogen availability and is independent of φ_C, whereas variations of φ_C and φ_N may cause various responses of the system in respect of allocation to defence-related compounds, depending on the actual position on the surface area (Fig. 1B). For example, according to the CNB hypothesis, on the slope at the left side of the surface area enhanced nitrogen availability results in a decreased allocation to defence. Conversely, on the front side of the surface area presented in Fig. 1B, an increase of nitrogen availability results in enhanced carbon allocation to defence. If nitrogen availability is not sufficient to fulfil the demand for growth ($\varphi_N < 1$), carbon allocation to structural biomass is limited and an excess of glucose equivalents remains in the pool of available assimilates. Therefore, in the case of high carbon availability and medium nitrogen supply, the allocation rate to defence-related compounds can exceed σ, which is in accordance with the hypothesis that carbon is disposed to secondary metabolism if it cannot be used for growth-related metabolism (GNB hypothesis, cf. Herms and Mattson, 1992). However, allocation to defence-related compounds is also limited since a certain amount of nitrogen is required for structural protein and enzyme synthesis and N-cycling enzyme activities involved in the biosynthesis of various metabolites, including that of precursor compounds in secondary metabolism as described in the PCM. If the total amount of available assimilates exceeds that used for both synthesis of structural biomass and carbon-based secondary compounds $(G_W + G_S < A_{av})$, the model allocates the remaining assimilates to the reserves pool or assimilates stay in the pool of assimilates. These assimilates are then available for maintenance, growth and defence processes in the following time step (Gayler *et al.*, 2004). This can
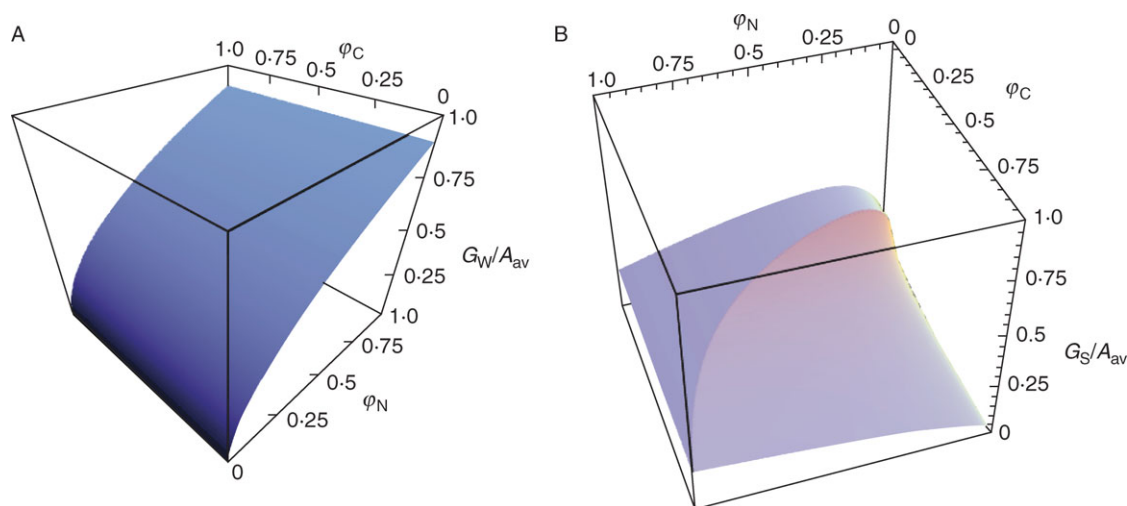

FIG. 1. Fractions of available glucose equivalents (A_{av}) that are allocated (A) to structural biomass (G_W) and (B) to the pool of carbon-based secondary compounds (G_S), depending on plant internal nitrogen and carbon availability factors φ_N and φ_C ($\sigma = 0\cdot15$, $\beta = 0\cdot5$, $\delta = 0\cdot33$).
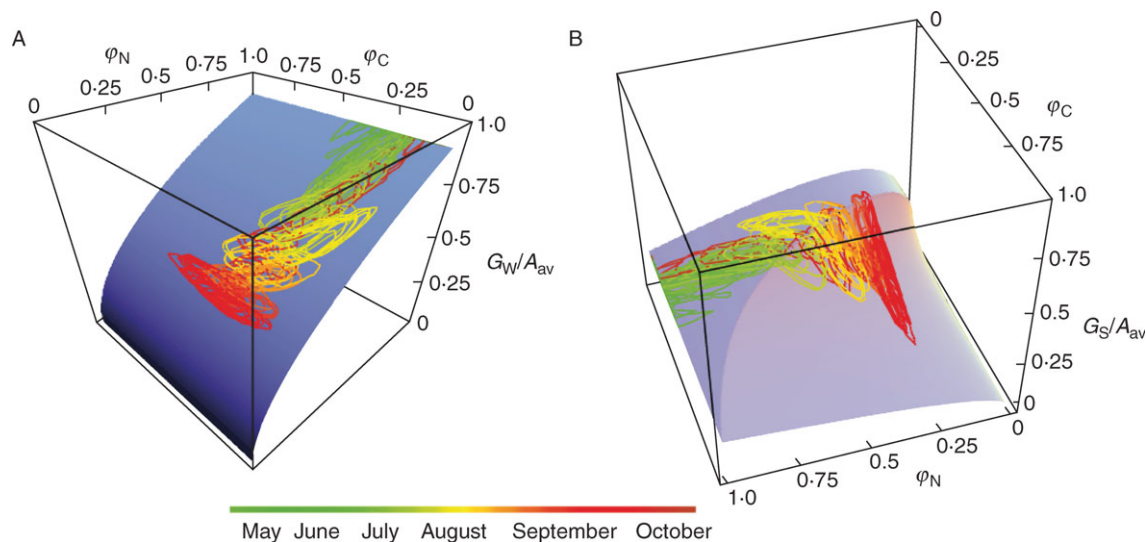
FIG. 2. Simulated dynamic of resource partitioning in medium fertilized (N2) young apple trees 'Golden Delicious' between (A) growth-related metabolism and (B) defence-related metabolism depending on plant internal relative availabilities of carbon φ_C and nitrogen φ_N ($\sigma = 0.15$, $\beta = 0.5$, $\delta = 0.33$). The elapsed time is given by the colour of the trajectory.

be the case if either the carbohydrate supply exceeds the demand for growth and defence or if nitrogen limitation inhibits the allocation to structural biomass or carbon-based secondary compounds.

The relative plant internal availabilities of carbon and nitrogen can strongly fluctuate within the course of a vegetation period as a result of changing demands for carbon and nitrogen at different phenological growth stages and variable carbon and nitrogen supply depending on climate and fertilization scenarios. This model behaviour is visualized in Fig. 2, where a simulation run is shown for a full vegetation period with the parameterization of the model for young apple trees 'Golden Delicious' in a medium fertilization scenario. The time-dependent dynamics of the system are represented by the trajectory, and the elapsed time by its colour shading. Due to the large fluctuations in φ_C and φ_N, the model displays a non-linear allocation behaviour to carbon-based secondary compounds. At the beginning of the vegetation period the model reduces the allocation to defence-related traits with increasing nitrogen availability, whereas this is not the case later on in the vegetation period. In October (end of the vegetation period) the trajectory returns to the initial behaviour of the model.

Effect of N fertilization on apple trees

In order to evaluate the performance of the modelling approach, the simulated allocation to carbon-based secondary compounds as well as the simulated biomass growth were compared with measurements of total concentrations of phenolic compounds in leaves and measured biomass growth in the experiments. Simulated vs. measured biomass of apple shoots at the end of the vegetation period are shown in Fig. 3 for the three different fertilization treatments (N1–N3). The effect of nitrogen fertilization on shoot growth is reproduced by the model for both
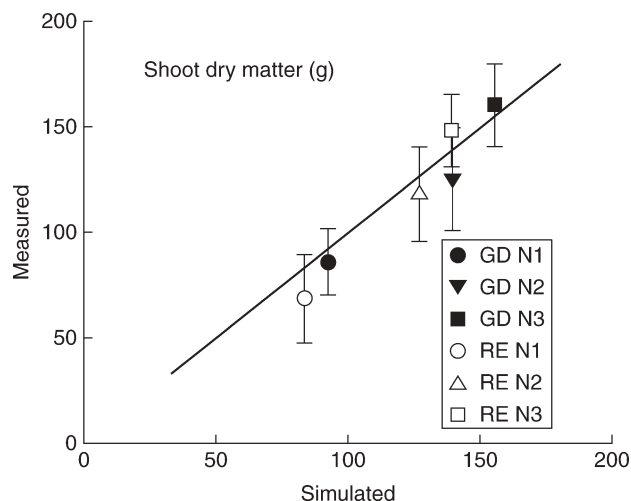

FIG. 3. Simulated vs. measured shoot biomass (dry matter) of apple trees 'Golden Delicious' (GD) and 'Rewena' (RE) at the end of the vegetation period for three different fertilization treatments (N1–N3). The straight line indicates the ideal 1:1 relationship.

apple cultivars, i.e. 'Golden Delicious', for which the model was parameterized, and 'Rewena', for which the independent data set had not been used for model parameterization before. Leaf concentrations of total polyphenols were only measured during the first 4 weeks after leaf unfolding. Simulated leaf concentrations of carbon-based secondary compounds in this period vs. measured concentrations of total leaf polyphenols are shown in Fig. 4. Again, the effect of nitrogen fertilization is satisfactorily reproduced by the simulation results for both apple cultivars. However, the model could not reproduce the almost constant value of approx. 26 % (w/w) polyphenolic metabolites that was measured in the youngest leaves (1 d after
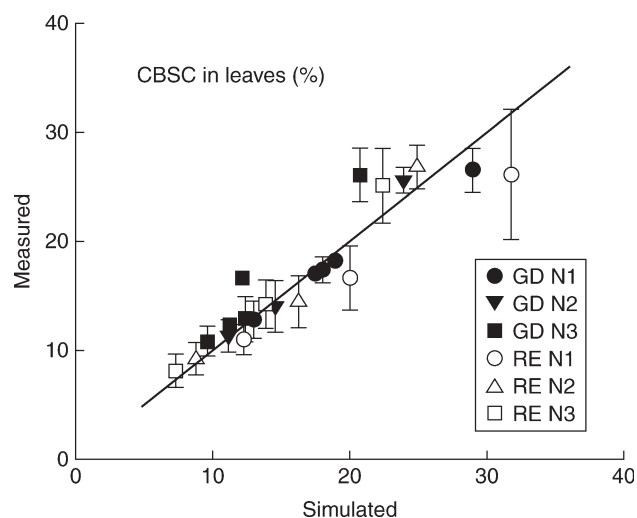
FIG. 4. Simulated vs. measured concentration of carbon-based secondary compounds (CBSC) in leaves of apple trees 'Golden Delicious' (GD) and 'Rewena' (RE) during the first 4 weeks after leaf unfolding for three different fertilization treatments (N1–N3). The straight line indicates the ideal 1 : 1 relationship.
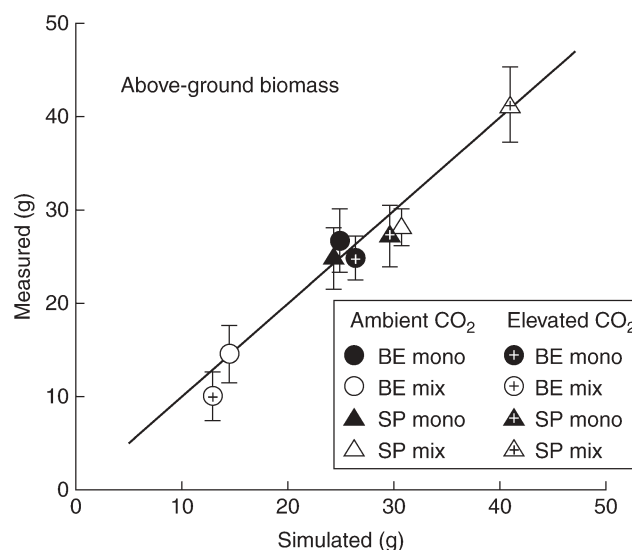

FIG. 5. Simulated vs. measured biomass of total above-ground plant biomass in August 2000 of individual beech (BE) and spruce (SP) in mono- and mixed culture under ambient (370 ppm) and elevated atmospheric CO_2 (according to Gayler *et al.*, 2006). The straight line indicates the ideal 1 : 1 relationship.

leaves were completely unfolded), irrespective of the fertilization scenario. Probably, the high level of chemical protection against pathogens of these leaves, which are living on carbohydrates from the preceding season and which are particularly important for further growth, is genetically determined. The comparison of Figs 3 and 4 demonstrates the trade-off between growth and defence in apple trees in the given scenario. Increased growth rates in the case of high fertilization rates go together with decreased levels of defence-related compounds in leaves, resulting in a putatively higher potential for infection by leaf pathogens.

Effect of elevated CO_2 and competition on juvenile beech and spruce trees

Gayler *et al.* (2006) presented the successful use of PLATHO to simulate growth of juvenile beech and spruce under the given conditions of the present container experiment. The effect of species competition in a mixed stand on the development of above-ground biomass within two vegetation periods was reproduced by the model (Fig. 5). Irrespective of the CO_2 treatment, biomasses of beech and spruce trees were similar after two vegetation periods in monoculture, but spruce dominated beech in the mixture. The reason for this is that at the time of canopy closure in the mixed cultures, spruce was the taller and larger species and therefore was able to dominate the competition for light (Grams *et al.*, 2002; Kozovits *et al.*, 2005*b*; Gayler *et al.*, 2006).

In order to evaluate the modelling approach for resource allocation to defence-related metabolism, simulated and measured effects of growth in mixture and under elevated CO_2 on concentration of carbon-based secondary compounds in leaves at the end of the experiment were compared (Fig. 6). The effect of growth in mixture and under elevated CO_2 was expressed for each species separately relative to growth in monoculture and under ambient CO_2, respectively. In general, simulated effects agree with measurements, except for the examination of the CO_2 effect on the concentration of defence-related compounds in spruce needles, which is overestimated by the model. A putative explanation for this overestimation is a possible acclimation of photosynthesis to elevated atmospheric CO_2 concentrations. This would result in a reduced carbohydrate accumulation. The effect of acclimation, which is not considered in the present model, was observed in long-term studies of growth under elevated CO_2 (Webber *et al.*, 1994). In the mixed stand, the branches of beech were shaded by spruce and consequently the availability of carbohydrates for phenolic metabolites was reduced. Conversely, spruce needles in the mixed canopies were exposed to higher light levels compared with spruce grown in monoculture, resulting in a higher photosynthesis rate and therefore an excess of carbohydrates available for allocation towards polyphenolic material. Elevated CO_2 increased photosynthesis rates for both species. However, the additional carbon gain could not be fully converted into increased biomass growth, presumably due to nitrogen limitation (see Fig. 5). Therefore, an excess of additional carbohydrates was available for allocation to defence-related compounds. Both results are in accordance with the GDB hypothesis (Herms and Mattson, 1992) which predicts that any environmental factor increasing photosynthesis to a greater degree than growth rate may increase the resource pool available for allocation to carbon-based secondary metabolites. A meta-analysis on the basis of >50 single studies (Koricheva *et al.*, 1998) also disclosed a decreasing effect of shading and an increasing effect
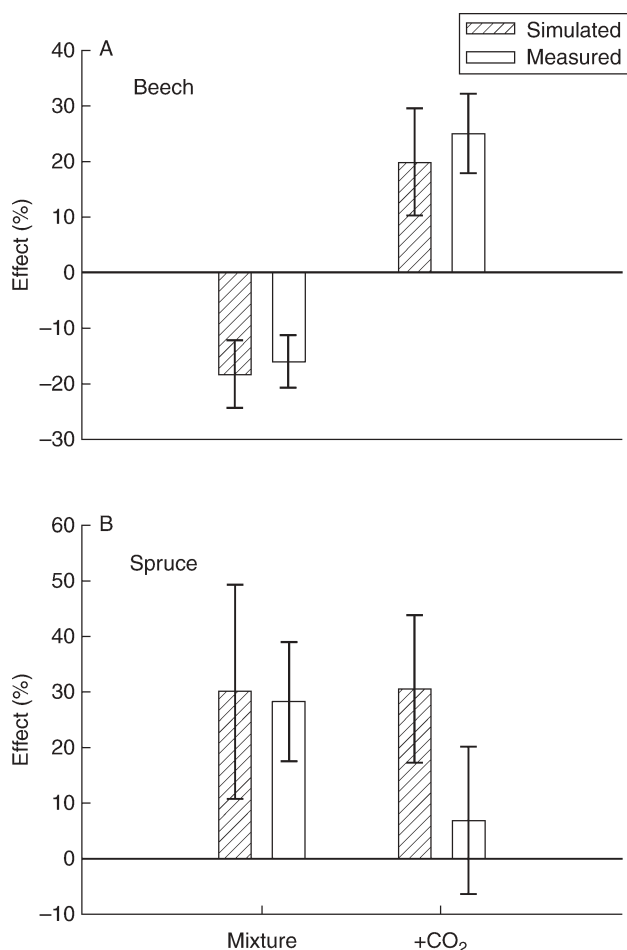
FIG. 6. Simulated and measured effects of growth in mixture (compared with monoculture trees) and elevated atmospheric CO_2 (compared with ambient CO_2) on concentrations of carbon-based secondary compounds (CBSC) in leaves of beech and spruce.
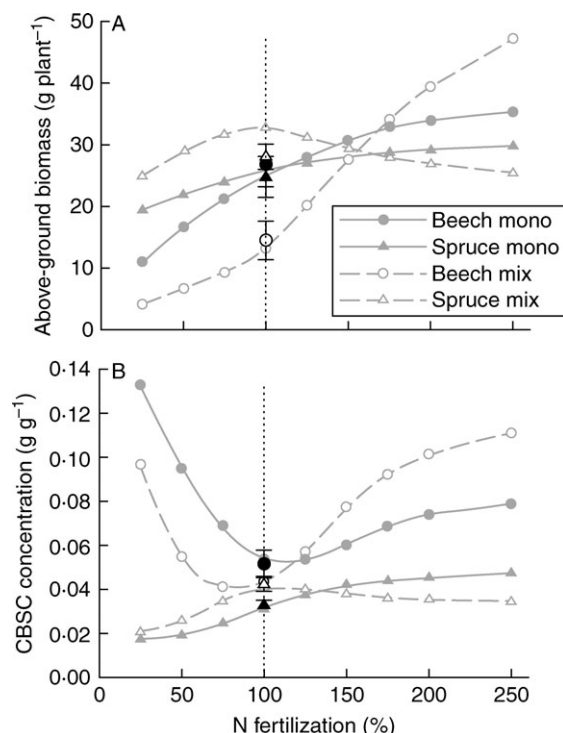

FIG. 7. Effect of nitrogen (N) fertilization on (A) simulated above-ground biomass of beech and spruce grown in mono- and mixed culture, and (B) simulated concentrations of carbon-based secondary compounds in leaves in August 2000. The fertilization rate is given as a percentage of the fertilization rate given in the experiment. The dotted lines indicate the amount of fertilization in the experiment (0·35 g per plant). Black symbols are measurements; grey lines with small symbols are simulations.

of elevated CO_2 on carbon-based secondary compound concentrations in tissue of woody plants.

Simulated effect of N availability in a mixed canopy of juvenile beech and spruce trees

To analyse the putative effect of nitrogen availability on biomass growth and allocation rates towards secondary compounds for beech and spruce in the present experiment, the simulated nitrogen fertilization rate was varied between 25 and 250 % of the amount supplied in the experiment (0·35 g N per plant within the two vegetation periods). The model predicts an increasing competitive advantage of spruce in the mixed culture under reduced nitrogen fertilization (Fig. 7). This can be explained by the lower nitrogen demand of spruce compared with beech (Kozovits *et al.*, 2005*a*, *b*; Luedemann *et al.*, 2005). The simulated advantage of the species with lower nitrogen demand under limited conditions in the simulation calculation is in accordance with results found for herbaceous plants (Aerts, 1999). If nitrogen fertilization exceeds 150 % of the value given in the experiment, the model predicts a

dominating beech under the given experimental conditions (Fig. 7A). This is due to a stronger increase of beech growth rates by additional nitrogen fertilization than that of spruce, which leads to a dominant position when canopy closure occurs. The model differs in the effect of nitrogen availability on allocation to carbon-based secondary compounds between the two species. In the case of low soil nitrogen availability, increased fertilization rates result in decreased allocation to defence in beech grown in monoculture in accordance with the CNB and GDB hypotheses (Fig. 7B). In the case of high nitrogen fertilization, growth of beech is not limited by nitrogen deficiency. Therefore, in the model, the additional nitrogen supply is largely available for the synthesis of defence-related compounds, increasing their concentration in plant tissue. However, carbon-based secondary metabolites are also increased for spruce under low nitrogen levels, because nitrogen limitation of simulated spruce growth is small even under these conditions, as the model simulates sufficient mineral nitrogen delivery by mineralization of soil organic matter to fulfil the moderate nitrogen demand of spruce. For trees grown in mixed stands, these patterns are superimposed on the effect of shading by the respective dominating species. Growth in mixture increases the level of carbon-based secondary compounds for the dominating species and decreases concentrations of defence-related compounds for shaded trees compared with trees grown in monoculture.

TABLE 1. *List of symbols used in the presented subroutines of PLATHO*

Symbol	Description	Unit	Type
A_{av}	Assimilates available for maintenance, growth and defence	kg (glucose)	Output
A_{old}	Assimilate surplus from prior time step	kg (glucose)	Intern
D_M	Demand for maintenance processes	kg (glucose)	Intern
D_{pot}	Potential growth rate of total plant biomass	kg d^{-1}	Output
D_S	Demand for defence-related processes	kg (glucose)	Output
D_W	Demand for growth-related processes	kg (glucose)	Output
G_S	Conversion of assimilates to carbon-based secondary metabolites	kg (glucose)	Output
G_W	Conversion of assimilates to structural biomass	kg (glucose)	Output
f_T	Factor for temperature dependency of growth	–	Intern
f_{Ph}	Factor for seasonal variation of growth	–	Intern
P_{act}	Gross photosynthesis rate	kg (glucose) d^{-1}	Intern
$N_{act,i}$	Actual nitrogen content in organ i	kg (N)	Intern
N_{av}	Available nitrogen	kg (N)	Output
$N_{act,i}$	Minimal nitrogen content in organ i	kg (N)	Intern
N_{dem}	Nitrogen demand for growth-related processes	kg (N)	Intern
$N_{trans,pot}$	Amount of mobile nitrogen in the plant	kg (N)	Output
$N_{upt,pot}$	Potential nitrogen uptake from soil	kg (N)	Intern
R	Reserves	kg	Intern
r_{max}	Maximum plant growth rate	d^{-1}	Input
t	time	d	Intern
W_l	Living structural total plant biomass	kg	Output
β	Factor accounting for non-linearity in the relationship between growth and φ_N	–	Input*
δ	Factor accounting for non-linearity in the relationship between formation of defensive compounds and φ_N	–	Input*
σ	Factor of partitioning to defence-related metabolism	–	Intern
σ_C	Constitutive part of σ	–	Input
σ_I	inducible part of σ	–	Intern
τ_N	Nitrogen remobilization rate	d^{-1}	Input
τ_R	Reserves mobilization rate	d^{-1}	Input
φ_C	Plant internal availability factor for carbohydrates	–	Intern
φ_N	Plant internal availability factor for nitrogen	–	Intern

Variables of the type 'Intern' are calculated in subroutines of the model, which are not described in this paper but are documented in Gayler and Priesack (2003). All output and internal variables are time-dependent functions, whereas input variables are constant values during one simulation run. Input quantities marked with an asterisk are estimations without direct measurements.

CONCLUSIONS

The simulation results show that the presented modelling approach for resource partitioning between growth-related primary metabolism and defence-related secondary metabolism is suitable to reproduce observed patterns in carbon-based secondary compound levels in leaves of juvenile trees. The underlying hypotheses that growth processes have priority over allocation of carbon into secondary compounds and that growth-related metabolism is more strongly affected by nitrogen deficiency than defence-related secondary metabolism are corroborated by this modelling study. Thus, the presented mathematical model, which is able to describe plant growth dynamics, can be seen as a good dynamic realization of existing conceptual models that consider environmental impacts on plant internal demands. It was shown that, with respect to allocation to carbon-based secondary compounds, different responses of the model are feasible, depending on phenological stage and nitrogen fertilization. Consequently, the simulated growth dynamics stress that plant defence strategies can change during the vegetation period and therefore with the developmental stage of the plant.

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