Developing of discrimination experiment to find most adequate model of plant’s multi-nutrient functional response

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Abstract. To create reliable Closed Ecological Life Support Systems (CELSS) it is necessary to have models which can predict CELSS dynamic with good accuracy. However it was shown that conventional ecological models cannot describe CELSS correctly if it is closed by more than one element. This problem can be solved by means more complex models than conventional ones - so called flexible metabolism models. However it is possible that CELSS also can be described correctly in "semi-conventional" framework – when only one trophic level is described by flexible metabolism model. Another problem in CELSS modeling is existence of different and incompatible hypotheses about relationships between plants growth rate and amounts of nutrients (functional responses). Difficulty of testing these hypotheses is associated with multi-nutrient dependency of growth rate and comprehensive experimental studies are expensive and time-consuming. This work is devoted to testing the hypothesis that "semi-conventional" approach is enough to describe CELSS, and to planning the discrimination experiment on selecting correct type of the plant's functional response. To do that three different models of plants (one flexible and two conventional) were investigated both in the scope of CELSS model, and in hemostat model. Numerical simulations show that each of the models has typical patterns which can be determined in experiment with real plants.

1. Introduction

The usage of Closed Ecological Life Support Systems (CELSS) in space is possible only if the range of working regimes is estimated correctly. It is obvious that estimation of CELSS reliability has to be based on CELSS testing at different working conditions and external influences to simulate dangerous situations (similar to car crash-tests). However “crash-tests” of full-scale CELSS with humans are impossible due to obvious reasons.

This problem can be solved by mathematical models. In that case parameters of system can be measured in non-catastrophic experiments, after that estimation of working range of CELSS is conducted by an adequate model. Since there is no uniform method of CELSS models creation, some semiformal procedure of their creation has to be developed if we want to have a technology (not art) of CELSS creation.

To simulate CELSS it’s necessary to find mathematical description which can describe processes in the system correctly. It’s obvious that biological components of CELSS are hardest for description.

A number of experimental papers which are devoted to biological part of CELSS exist now [1,2,3,4]. This articles show some dynamical properties of ecosystems, at least near steady state. Articles on CELSS modeling [5,6,7,8,9,10] are devoted to the description of steady state of CELSS. In
the same time experimental and model analysis of transit and critical regimes of CELSS had not conducted.

Creation of adequate dynamic models of CELSS is connected with steady-state stability analysis and analysis of system sensibility to big external disturbances. Since CELSS as usual includes several species the objective of ensuring their consistent and stable operation raises. This objective is not trivial since biochemical compound and required proportions of chemical elements consumption (similar to stoichiometry in chemistry) are different for each species. It has previously been shown [9] that if biological part of CELSS built in tradition Lotka-Volterra framework with fixed proportions of substrate consumption then in general case the ecosystem cannot be closed with respect to more than one element. Traditional ecological models can be used if there is no switching between limiting elements during the life-time of ecosystem. But our task is study of transition and, especially, critical processes and there is not any reason to exclude possibility of limiting element changing. It means that it is necessary to use models with different structure then traditional one to describe CELSS properly.

As was shown earlier [9,10] the problem of closing can be resolved by using models with “flexible” metabolism. In these models organisms can change their diet in accordance with their needs and nutrients distribution. Models with similar properties of biological components (humans and plants) were used for calculations of optimal stationary configurations of CELSS [6,8]. However these models were not studied in dynamic case in these works. Dynamic flexible models were studied in [9,10] but these models are rather complex and have number of hardly-estimated parameters. It makes the question on the necessity to use “flexible” approach to describe all species in CELSS or only part of them very actual.

Adequate description of real CELSS is associated with another important objective – selecting proper trophic function of plants included. This task becomes fundamentally important to build realistic scenarios at extreme situations.

In accordance with well-known Liebig’s law of the minimum [11] plant’s growth rate is depended by element which is insufficient. Since “to be insufficient” is Boolean (can be only “yes/true” or “no/false”) property distinctive feature of models based on Liebig’s law is discontinues functional response to changes of substrate concentration. At the same time in practical applications including models of CELSS [5] smooth multiplicative function was used as functional response. Therefore there are at least two widely used versions of mathematical descriptions of nutrient consumption by plants. In this paper for the sake of brevity the model with discontinuous trophic function is called Liebig's models and smooth - Mitscherlich's models [12].

This work has the following goals:
1) to test the hypothesis that for the closure of the ecosystem with respect to several biogenic elements only one "flexible" trophic level is sufficient;
2) to reveal characteristic features of the dynamics of Closed Ecological Systems for different functional responses (functions, which describe dependence between growth rate and nutrients concentrations);
3) to formulate conditions of discrimination experiments to choose the most adequate type of functional response.

Our goal at this stage is not to get new fundamental results, but developing tools for creation and, which is more important, practical usage of CELSS models.

2. Models description
As a real prototype of models of CELSS we consider a system that ensures the crew only by plant food since introducing animals into CELSS is very complex objective, and the data on the functioning of such systems is not sufficient [4]. Then CELSS can be decomposed into fundamentally different mass components:
1) Biogenic elements in the inorganic form
2) Photoautotrophs (plants) that consume inorganic nutrients.
3) Inedible parts of plants and organic waste produced by man. A feature of CELSS as an ecosystem is that the biomass of the consumer (human) has to be almost constant. This greatly simplifies the model by excluding dynamics of human biomass. Furthermore it is possible to consider human metabolism as "black box" - it consumes plants at a constant rate, and disposes of wastes containing biogenic elements in the same amount they were consumed (a necessary condition of organism homeostasis and the steady state of the ecosystem). The details of human diet are not considered here.

Although in the CELSS model the rate of consumption and waste production of crew can be considered as constants, for a more accurate description of extreme situations it makes sense to use a variant of the well-known function Mono with small semi-saturation constants:

$$H_i(X_1,...,X_n) = \frac{H_i \prod_j X_j}{\prod_j (h + X_j)}$$

where \(H_i\) is rate of consumption of plant \(i\) at normal conditions, \(h\) – semi saturation constant and \(X_j\) is biomass of plant species \(i\).

This function provides practically constant specific consumption rate of photoautotrophs \(X_j\) for all \(X_j > h\). If the biomass of one of the plant species decreases to \(h\) then human consumption of all plant species simultaneously decreases. It is the simplest way to describe the influence of non-balanced diet.

In reality human organism can adapt to the deficiency of one of the foodstuff. However in the scope of objective - the construction of a minimal model of CELSS providing closure - this simplification is acceptable since it gives the evaluation of the lower boundary of CELSS stability.

In this work the processing detritus into inorganic substances is supposed to be performed by chemical reactor. Obviously reactor is not sensitive to the absence of any detritus nutrient element unlike bacteria decomposers. However the reactor has a limited capacity and therefore can be saturated by substrates. On this basis the reactor can be described quite adequately by following system of equations:

$$\begin{align*}
\frac{dC}{dt} &= \frac{RD_C}{(r + D_C + D_N + D_P)} \\
\frac{dN}{dt} &= \frac{RD_N}{(r + D_C + D_N + D_P)} \\
\frac{dP}{dt} &= \frac{RD_P}{(r + D_C + D_N + D_P)}
\end{align*}$$

where \(R\) is maximum detritus processing rate; \(D_C, D_N\) and \(D_P\) are element content of the detritus; \(C, N\) and \(P\) are biogenic elements (carbon, nitrogen and phosphorus) in inorganic form.

General view of the model of CELSS closed by three biogenic elements including three types of producers taking into account the above adjustments is as follows:
\[
\begin{align*}
\frac{dC}{dt} &= \frac{RD_C}{(r + D_C + D_N + D_P)} - \sum_{i=1}^{i\leq 3} f_{Ci}(C, N, P, X_i) \\
\frac{dN}{dt} &= \frac{RD_N}{(r + D_C + D_N + D_P)} - \sum_{i=1}^{i\leq 3} f_{Ni}(C, N, P, X_i) \\
\frac{dP}{dt} &= \frac{RD_P}{(r + D_C + D_N + D_P)} - \sum_{i=1}^{i\leq 3} f_{Pi}(C, N, P, X_i) \\
\frac{dX_1}{dt} &= g_1(C, N, P, X_i) - \frac{H_i X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} \\
\frac{dX_2}{dt} &= g_2(C, N, P, X_1) - \frac{H_i X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} \\
\frac{dX_3}{dt} &= g_3(C, N, P, X_1) - \frac{H_i X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} \\
\frac{dD_C}{dt} &= \frac{W_1 X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} - \frac{RD_C}{(r + D_C + D_N + D_P)} \\
\frac{dD_N}{dt} &= \frac{W_2 X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} - \frac{RD_N}{(r + D_C + D_N + D_P)} \\
\frac{dD_P}{dt} &= \frac{W_3 X_1 X_2 X_3}{(h + X_1)(h + X_2)(h + X_3)} - \frac{RD_P}{(r + D_C + D_N + D_P)} \\
\end{align*}
\]

where \( f_{Ci}(C, N, P, X_1) \), \( f_{Ni}(C, N, P, X_1) \), \( f_{Pi}(C, N, P, X_1) \) and \( g_i(C, N, P, X_1) \) are functional responses; \( W_i \) is human dissimilation rate of element \( i \).

In this work three different types of functional response of plant species were investigated: “Inner regulatory pool” (IRP) which was considered in [9,10] early:

\[
\begin{align*}
 f_{Ci}(C,X_i) &= k_{Ci} C^c_i (X_i^{\text{max}} - c_i) \\
 f_{Ni}(N,X_i) &= k_{Ni} N^c_i (X_i^{\text{max}} - n_i) \\
 f_{Pi}(N,X_i) &= k_{Pi} P^c_i (X_i^{\text{max}} - p_i) \\
 g_i(C,N,P,X_i) &= \mu_{c,n,p} \\
\end{align*}
\]

where \( k_{Ci} \), \( k_{Ni} \) and \( k_{Pi} \) are specific rates of element absorption by plant, \( X_i^{\text{max}} \) are maximum plant species biomass, \( c_i^{\text{max}}, n_i^{\text{max}} \) and \( p_i^{\text{max}} \) are maximum concentrations of biogenic elements in the inner regulatory pools. Variables \( c_i, n_i \) and \( p_i \) are amounts of elements in inner regulatory pools of plant and can be calculated by following ordinary differential equations system:
where $\mu_i$ is specific rate of element consumption by the metabolism of $i$-th plant and $A_{ci}+A_{ni}+A_{pi}=1$ are normalized stoichiometric coefficients of plants or proportions of carbon, nitrogen and phosphorus therein. Summands $\frac{c_i}{X_i} \prod_{j}^H \left( \frac{X_j}{h+X_j} \right)$, $\frac{n_i}{X_i} \prod_{j}^H \left( \frac{X_j}{h+X_j} \right)$ and $\frac{p_i}{X_i} \prod_{j}^H \left( \frac{X_j}{h+X_j} \right)$ describe decreasing of biogenic elements in their pools due to the consumption of plants by the crew.

Another variant of plant metabolism description was Mitscherlich’s model:

$$\begin{align*}
f_{ci}(C,X_i) &= A_{ci}g_i(C,N,P,X_i) \\
f_{ni}(C,X_i) &= A_{ni}g_i(C,N,P,X_i) \\
f_{pi}(C,X_i) &= A_{pi}g_i(C,N,P,X_i) \\
g_i(C,N,P,X_i) &= \frac{v_i C NP}{(V_{Ci} + C)(V_{Ni} + N)(V_{Pi} + P)}(X_i^{max} - X_i)X_i
\end{align*}$$

where $v_i$ is maximum growth rate and $V_{Ci}$, $V_{Ni}$, $V_{Pi}$ are semi saturation constants.

The third variant was classic Liebig’s model:

$$\begin{align*}
f_{ci}(C,X_i) &= A_{ci}g_i(C,N,P,X_i) \\
f_{ni}(C,X_i) &= A_{ni}g_i(C,N,P,X_i) \\
f_{pi}(C,X_i) &= A_{pi}g_i(C,N,P,X_i) \\
g_i(C,N,P,X_i) &= \min \left( \frac{v_i C}{(V_{Ci} + C)}, \frac{v_i N}{(V_{Ni} + N)}, \frac{v_i P}{(V_{Pi} + P)} \right)(X_i^{max} - X_i)X_i
\end{align*}$$

As can be seen from the equations of Mitscherlich’s and Liebig’s models the consumption of biogenic elements by plant is proportional to the rate of growth of the organism, and the coefficients of proportionality are constant. As shown in [9] ecosystem models built only on the base of these principles cannot be closed with respect to more than one element. However in all three models plants are consumed by human, and it is considered that human consume plants in proportion which allow him to live. In this way, human is “flexible” part of models but mechanism of its flexibility is not described now. Consequently there is reason to expect closure achieving when using Liebig’s and Mitscherlich’s models.

Constants and variables from all three models are represented in Table 1.
Table 1. Model’s constants and variables.

| constant or variable | units       | short description                                                                 |
|----------------------|-------------|------------------------------------------------------------------------------------|
| \( H_i \)            | g*day\(^{-1}\) | rate of consumption of plant by human at normal conditions                          |
| \( h \)              | g          | semisaturation constant                                                             |
| \( R \)              | g*day\(^{-1}\) | maximum detritus processing rate                                                   |
| \( r \)              | g          | semisaturation constant                                                             |
| \( k_{Gi}, k_{Ni}, k_{Pi} \) | g\(^{-2}\)*day\(^{-1}\) | specific rates of element absorption by plant                                       |
| \( c_i^{\text{max}}, n_i^{\text{max}}, p_i^{\text{max}} \) | g          | maximum concentrations of biogenic elements in the inner regulatory pools          |
| \( A_{ci}, A_{ni}, A_{pi} \) | g          | normalized stoichiometric coefficients of plants or proportions of carbon, nitrogen and phosphorus therein |
| \( \mu_i \)          | g\(^{-2}\)*day\(^{-1}\) | specific rate of element consumption by plant’s the metabolism                     |
| \( V_i \)            | g*day\(^{-1}\) | maximum growth rates (Mitscherlich)                                                 |
| \( V_{Gi}, V_{Ni}, V_{Pi} \) | g          | semi-saturation constants                                                           |
| \( W_i \)            | g*day\(^{-1}\) | rate of waste of plant by human at normal conditions                               |
| \( X_i^{\text{max}} \) | g          | maximum plant’s biomass                                                            |
| \( v_{Gi}, v_{Ni}, v_{Pi} \) | g*day\(^{-1}\) | maximum growth rates (Liebig)                                                      |
| \( C, N, P \)         | g          | biogenic elements (carbon, nitrogen and phosphorus) in inorganic form               |
| \( X_i \)            | g          | plant’s biomass                                                                    |
| \( c_i, n_i, p_i \)   | g          | amounts of elements in inner regulatory pools                                       |
| \( D_{Ci}, D_{Ni}, D_{Pi} \) | g          | element content of the detritus                                                    |

For a correct comparison of these three models production characteristics of the simulated plants were made practically equal. Equations were solved with Scilab 5.4.1 with default method of ode() function (automatically selects between nonstiff predictor-corrector Adams method and stiff Backward Differentiation Formula (BDF) method) with default relative and absolute estimated errors (10\(^{-5}\) for relative one and 10\(^{-7}\) for absolute).

3. Results
The study was conducted by numerical simulations of CELSS models with random set of plant parameters. Some parameters of plants and the ranges of variation for others are shown in Table 1. Uniform distribution was used in the random number generator.

Table 2. Basic parameters of the plants in the test model of CELSS.

| Parameters | Abstract plants | Plant1 | Plant2 | Plant3 |
|------------|-----------------|--------|--------|--------|
| \( A_{ci} \) | \( n_i^{\text{max}} \) | \( p_i^{\text{max}} \) |        |        |
| 0.9883     | 0.0105          | 0.0012 | 0.0012 | 0.0062 |
| 0.0105     | 0.00077         | 0.0011 | 0.0012 | 0.0062 |
| 0.0012     | 0.0011          | 0.0012 | 0.0062 |        |
| 0.1        | 0.1             | 0.1    | 0.1    | 0.1    |
| 0.01       | 0.01            | 0.01   | 0.01   | 0.01   |
| 0.001      | 0.001           | 0.001  | 0.001  | 0.001  |
The stoichiometric ratios are chosen close to the real [13].

Typical results for all 100 numerical simulations of the plants dynamics in CELSS model with parameters ranges from table 2 are presented in Figure 1.

| $k_{Ci}$, $k_{Ni}$, $k_{Pi}$ (g•2*day$^{-1}$) | 1 – 2 |
| $\mu$ (g•2*day$^{-1}$) | $2\times10^6 – 3\times10^6$ |
| $x_{i}^{\text{max}}$ (g) | 10 – 20 |

![Graphs showing the dynamics of plant components of CELSS models. Line with colons - IRP, dot-dash - Mitscherlich model, solid - model Liebig.](image)

**Figure 1.** Dynamics of plant components of CELSS models. Line with colons - IRP, dot-dash - Mitscherlich model, solid - model Liebig.

From the results of numerical experiments it can be concluded that the closure of system (steady state of plant biomass is assumed) is possible for all three models in a wide range of parameters.
Hence the hypothesis on the possibility of closure of the ecosystem within the models accounting the flexibility of the metabolism of only one trophic level is confirmed.

3.1. Description of the experiment to determine the type of functional response of several variables

The experiment in the CELSS is rather complicated and expensive. However, as shown in Figure 1, the differences in plant biomass dynamics for different models mean differences in the type of functional response of plant to changes of the amount of nutrients. So we have an ability to differentiate the type of plant functional response in experiments. The measurements of the growth rate of plants can be conducted in a phytotron with intensive carbon dioxide flow. At sufficiently high rate of CO₂ input, its concentration in the phytotron can be considered as constant. If influx of carbon dioxide is turned out its concentration (measured in real-time by gas analyzer) begins to decline as a result of plants life. It is possible to determine the specific rate of consumption of carbon dioxide by plant from the curve of reduction of carbon dioxide concentration.

In this work, numerical simulation of experiment on determination of the trophic functions of plants was conducted. To do that, values of functions which describe consumption by the mechanisms of IRP, Liebig’s, and Mitscherlich’s models with varying amounts of nutrients were calculated. Four "computer experiments" were conducted as illustration. In each experiment the values of N and P were fixed while C concentration was varied. The dependence of growth rate on C concentration is presented by graph. The values of N and P in each of the experiments were taken as follows:

Table 3. Changing of amounts of nitrogen and phosphorous in experiments.

| Experiment | N   | P   |
|------------|-----|-----|
| 1          | N_{min} | P_{min} |
| 2          | N_{min} | P_{max} |
| 3          | N_{max} | P_{min} |
| 4          | N_{max} | P_{max} |

where N_{min}, P_{min}, N_{max}, and P_{max} are the smallest and largest amount of nitrogen and phosphorus used in these experiments. Values N_{min}=1, P_{min}=0.1, N_{max}=7, P_{max}=0.7 were used in computer experiments. Selection of these values of nitrogen and phosphorous content is similar with nitrogen-phosphorous ratio in real plants.

Results of computer experiments are shown on Figure 2. Computer experiments consisted of identifying "concentration of CO₂ - specific growth rate" relation for 10 randomly generated sets of model parameters of IRP and the corresponding parameters of Liebig’s and Mitscherlich’s models. For better clarity the figure shows the typical curves for each of the three models. The next parameters of plants were varied: \( \mu_i \) in the range \( (2 \times 10^6-3 \times 10^6 \text{ day}^{-1} \text{g}^{-2}) \) and \( k_C, k_N, k_P \) in the range \( (1-2 \text{ day}^{-1} \text{g}^{-2}) \).

As can be seen from Figure 2, IRP, Liebig’s, and Mitscherlich’s models have following characteristic patterns which can be detected experimentally:

1) in the framework of the IRP and Liebig's models maximum growth rate are almost independent of the concentrations of nitrogen, but it depends on the concentration of phosphorus if nitrogen-phosphorous ratio;
2) for Liebig’s model the break of the curve when entering the almost horizontal plateau are typical;
3) in Mitscherlich’s model strong dependence of the upper level of "growth rate – CO₂" curve on nitrogen and phosphorus is observed.

3.2. Description of the experiment to determine the type of functional response

The concentration of carbon dioxide in the phytotron with the flow of carbon dioxide is described by the following equation:
\[ \frac{dS}{dt} = D(S_0 - S) - \nu(S), \]  

(11)

where \( S \) is CO₂ concentration in the phytotron, \( S_0 \) is CO₂ input concentration, \( D \) is specific flow rate, \( \nu(S) \) is rate of CO₂ consumption by plants. When the flow rate is much greater than the plant's rate of consumption then concentration of CO₂ in the phytotron is almost equal to input concentration. When flow is off then carbon dioxide concentration begins to fall in accordance with the equation

\[ \frac{dS}{dt} = -\nu(S). \]  

(12)

Obviously plant’s carbon consumption rate can be reconstructed by the shape of the curve of CO₂ concentration falling. In the case of short flow turn off decreasing of CO₂ concentration can be considered as a linear.

There exists the possibility that with increasing CO₂ concentration plants need some time to adaptation to a new concentration. To check existence of this period an additional experiment should be made:

0) Increasing of carbon dioxide input concentration.
1) Switching off CO₂ flow and measuring of CO₂ curve of fall.
2) Switching on CO₂ flow. CO₂ concentration return to initial level.
3) Repeating switching off after several tens of minutes.

If plants need no time for adaptation to the new CO₂ concentration (both curves are identical) then experiments on growth curve determination can be produced through a series of pulsed CO₂ injections with a gradual increase of its concentration. If plant adaptation exist then it is necessary to increase interval between switching off. Series of four curves corresponding to one of the three patterns shown in Figure 2 have to be the result of the experiment.
Figure 2. The results of computational experiments on the measurement of the food curve depending on the amounts of available nutrients. Line with colons is IRP, a line with a point is Mitscherlich’s model, solid is Liebig’s model.

4. Conclusion
In this work the possibility of CELSS closing by several biogenic elements was shown in the model with only one “flexible” species – human being. This result allows in some cases significantly simplify CELSS models.

To select the most adequate model for describing real plants searching for typical patterns for each of the models was conducted. It was shown that the investigated models demonstrate specific patterns allowing experimental discrimination of hypotheses:
1) In the framework of the IRP and Liebig’s models maximum growth rate are almost independent on the concentrations of nitrogen.
2) In the Liebig’s model growth curve has a break point and strictly horizontal "plateau".
3) In the Mitscherlich’s model maximum growth rate in case of saturation by CO2 depends on the concentration of other nutrients.

Preliminary description of the experiment on the determination of the adequate way of describing the functional responses of plants according to one of the above patterns was made.

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