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Disease suppressive soil has both diverse and uniform ecology: Modeling and characterization from the viewpoint of microbiology and biodiversity

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Soil disease suppression is a worldwide important issue in order to realize stable food supply to people. In spite of that, no established indicators of soil disease suppression have been found out yet. This prevents us from controlling well soil state such that no diseases take place. In this paper, we have proposed a new biological indicator of soil disease suppression; the ability of bacteria to consume carbon resources, which can be automatically observed by Omunilog ID system during a duration of one or two days. This indicator turned out to distinguish disease suppressive soils from others. We have modeled these characteristic time developments of consumption of carbon resources by the simple ecological model where bacteria compete with each other for carbon resources. Measured ecological structure of soil bacteria can fit with the theoretical prediction well. In order to find characteristic features for each of soils, observed time developments are embedded into two dimensional space by non-metric multidimensional method. It results in the almost one dimensional arrangements of embedded points. By analyzing spacial distributions of each carbon resources in the embedded space, healthy soil turns out to have mostly uniform distribution along this one dimensional arrangement. Since sick soil and non-soil example have rather localized distributions, the ecological systems in more disease suppressive soil are both more diverse and more uniform. Since this indicator can be extremely easily and quickly obtained automatically, it is expected to use in order to validate many efforts to try to improve soil before any harvests very much.

1. Introduction

Soil disease suppression is an important issue to maintain sustainable food supply. Soil diseases generally reduce food production by the huge amount all over the world. Thus, if we can suppress the occurrence of soil disease, it will be a great progress to increase the amount of net food production.

In spite of the importance of soil disease suppression, we do not know even how to measure how healthy the soil is. There are many proposals of this criterion[1),2)], but there are no established ones.

Recently, soil biodiversity is regarded to be an important factor to maintain healthy (normal) soils[3)]. Soil microbiology is known to be one of the important parts of this. In this paper, we have found that the amount of consumed carbon resources by bacteria can be a good indicator of soil health. A simple ecological model is also proposed to reproduce time development of consumption processes of carbon resources. The obtained interaction matrix between bacteria and carbon resources is coincident with the previous observations on which bacteria can consume which carbon resources.

The paper is organized as follows. In the next section, we show the time developments of carbon resources' consumption and it is related to disease suppression. Then we propose a mathematical model to reproduce it. Based upon the model computation, we propose interaction map between bacteria and carbon resources, which is compared with and confirmed by real measurements. Finally, feature extraction by non-metric multidimensional scaling is applied to time course data and it is found that the mostly disease suppressive soil keeps both diversity and uniformity in soil ecology.

2. Results

2.1 Measurement of time development of carbon resource consumption by bacteria

Although there are many brand new proposals to quantify soil microbiology diversity based upon genomic technology[4)], here we have employed more classical, phenomenological and macroscopic measure of biodiversity, carbon resource consumption. In order to measure time course development of carbon resource consumption by bacteria, we have used Omunilog ID system. It has 96 wells each of which contains one of 95 kinds of carbon resources plus negative control, water (not shown here).

The amounts of consumption of carbon resources are measured quantitatively by the amount of carbon dioxide exhibited in each well. It is detected as photo

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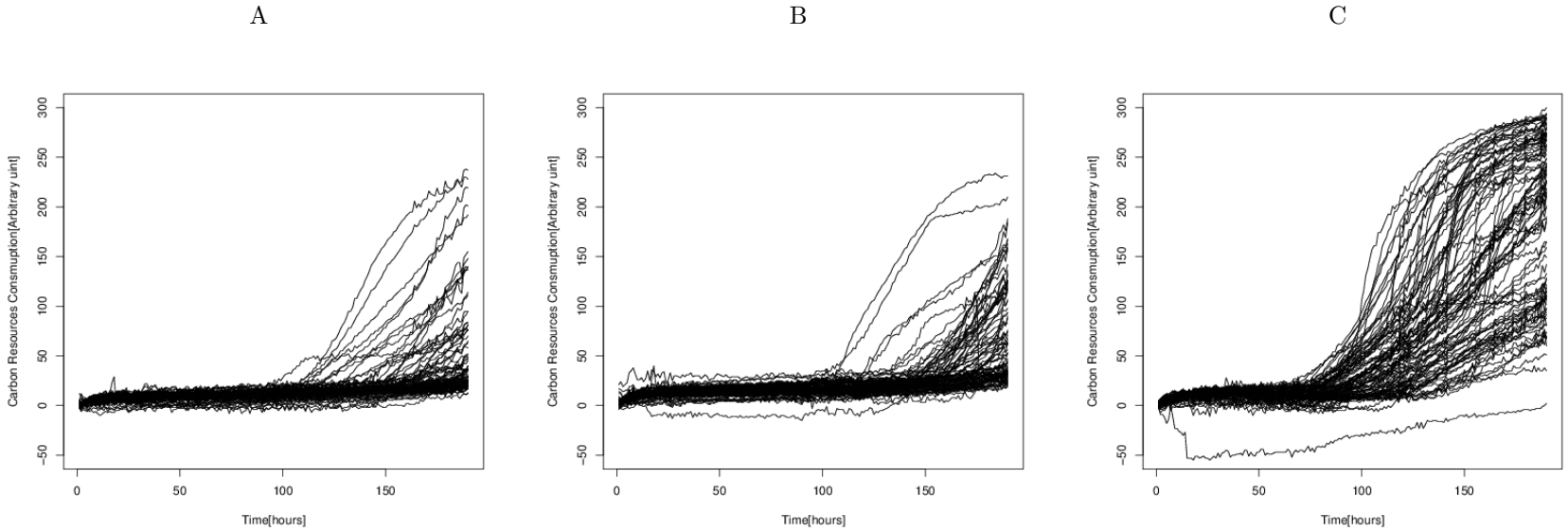

Fig. 1 Time developments of carbon resource consumption. A) Sick soil with the disease probabilities of 100 %, B) that of 69.2 %, C) healthy soil of 19.2 %.

emission using carbon dioxide coloring reagent. Measurements are done every quarter hour after starting time up to half past 47 hours, i.e., almost over two days. Those at time point zero (background) are subtracted from them. Thus, in total, the number of time points is 190 excluding 0 hour, i.e., at the time of starting. These values are integrated from the beginning to each time point. In Figure 1, we have shown typical time developments of the integrated amount of consumed carbon resources in 96 wells up to each time point. Each of plots corresponds to one of soils with the disease probabilities of 100 %, 69.2 % and 19.2 %, respectively. It is clear that their time developments are distinct and dependent upon the disease probabilities. The plant growth rates are strongly affected by disease probabilities (not shown here). How the difference of time developments is related to disease probabilities? Why soil having time development seen in Fig. 1C is more healthy than others? In order to answer these questions, at first we try to construct a model to reproduce these time developments. Then, their conclusion turns out to be coincident with the measurement ten years ago.

2.2 An ecological model of soil microbiology

2.2.1 Model definition

Although there are substantial progresses of modeling in soil microbiology[5], here we have employed classical mean field theories[6]. This is because it is easily treated and understood. Thus, if it works, there are no needs to consider more realistic and complicated models. In order to model the time developments seen in Figs. 1, we have assumed that the difference between the consumption rates of carbon resources is decided by the number of bacteria which can consume them. That is, rapid consumption means more bacterial species to cosume the carbon resources. In order to model this situation as simple as possible, we proposed the following Lotka-Volterra equation like model,

$$\frac{dx_i}{dt} = \left(\sum_{j=1}^{\frac{M_0}{N} i} y_j \right) x_i \tag{1}$$

$$\frac{dy_j}{dt} = \begin{cases} -\left(\sum_{i=\frac{N}{M_0} j}^{N} x_i \right) y_j, & j \leq M_0 \\ 0, & j > M_0 \end{cases}, \tag{2}$$

where $x_i, (i = 1, \ldots, N)$ denotes the populations of ith bacteria and $y_j, (j = 1, \ldots, M)$ denotes the remained (not yet consumed) amount of jth carbon resources. The quantities in parentheses in the right hand side decide the speed of growing of bacteria or that of carbon resource consumption by bacterial species. This means, bacteria which can consume more kinds of carbon resources can grow faster, while the amount of carbon resource consumed by more bacterial species decreases more rapidly. Due to these equations, ith bacteria consumes $\frac{M_0}{N} i$ kinds of carbon resources, while jth carbon resource is consumed by $N - \frac{N}{M_0} j$ kinds of bacterial species. Generally, M_0, which is the maximum number of carbon resources that one bacterial specie can consume, should be less than M, which is the total number of carbon resources. Therefore, there remains $M - M_0$ carbon resources which no bacterial species can consume. In Fig. 2, which hereafter we call as the interaction map, we have illustrated this situation. If the point having the coordinate (i, j) is placed within the shaded region, the term proportional to $x_i y_j$ exists in the right hand side of eqs. (1) and (2). For example, the term proportional to $x_{i_1} y_{j_1}$ does exist in the right hand sides of eqs. (1) and (2), since the point (i_1, j_1) is in the shaded region. On the other hand, that to $x_{i_2} y_{j_2}$ does not exist in the right hand sides of eqs. (1) and (2), since the point (i_2, j_2) is outside the shaded region.

2.2.2 Reproduction of real examples

We have numerically integrated eqs. (1) and (2) from $t = 0$ to $t = T$ with the initial conditions of

$$x_i = 0.01, (i = 1, \ldots, N) \tag{3}$$

$$y_j = 1 - \varepsilon_j, (j = 1, \ldots, M) \tag{4}$$

where ε_j is uniform random number between 0 and 0.1, which was introduced to reproduce randomness seen in Figs. 1, by setting $M = N = 90, T = 150$. Figures 3 reproduce the outcomes seen in Figs. 1 very well if the simpleness of our models is considered. The reason why we used $M = 90$ is simply because ominilog ID system employs 95 carbon resources. Even if we change M a little bit, the results do not change drastically. On the other hand, we have taken $N = M = 90$. It is almost sure that more bacterial species exist in soil and consume carbon resources competitively. However, at least for numerical modeling, it turns out that the number of bacterial species is large enough if it is as many as the number of kinds of considered resources. It is also almost sure that there will be more carbon resources in the soil to be consumed by bacteria. Thus, anyway what we observe and try to reproduce is subsystem of soil ecology. Then, we have decided to omit the contributions from more bacterial species than the number of kinds of considerer carbon resources. We can consider these $N \simeq M$ bacterial species as the representatives that mainly consume the considered carbon resources. Although T can be taken to be any values, we have chosen so as to reproduce the real outcome best. We do not think that this is badly arbitrary, because anyway the period for which measurements were performed is arbitrary.

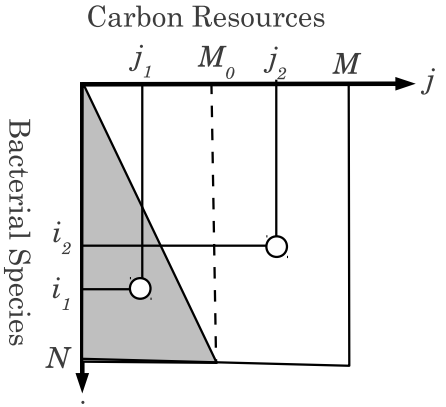

Fig. 2 The interaction map that illustrates the interaction between bacterial species i and carbon resources j. If the point having the coordinate (i, j) is placed within the shaded region, the term proportional to $x_i y_j$ exists in the right hand side of eqs. (1) and (2). See main text for more details.

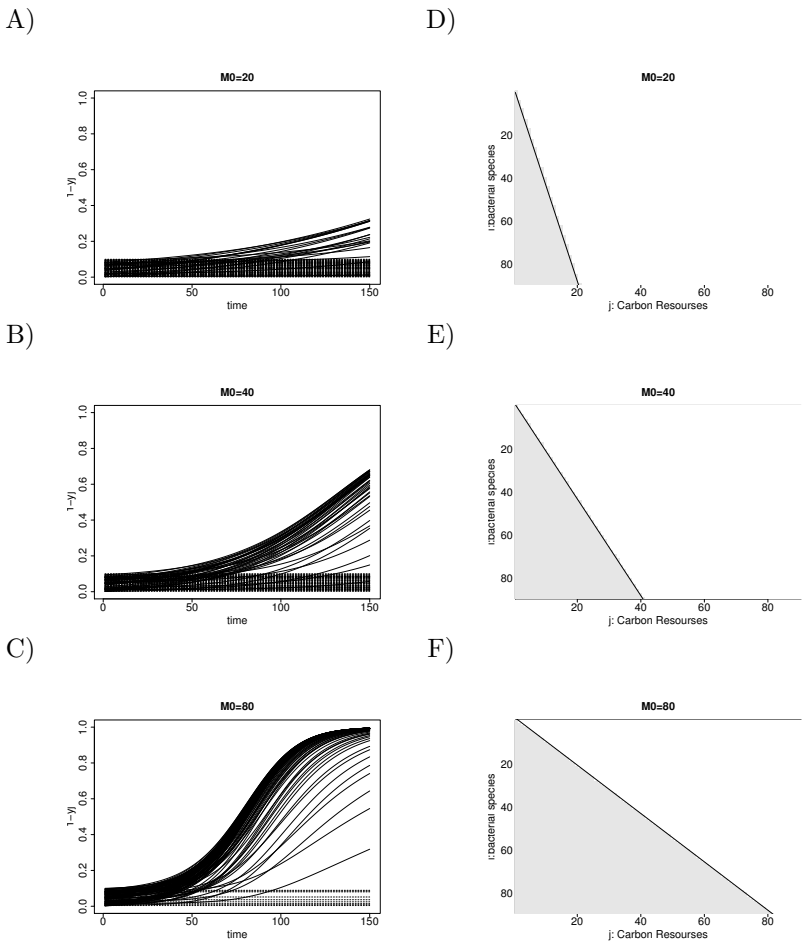

Fig. 3 Numerical simulation of eqs. (1) and (2) for $T = 150, N = M = 90$**.** A)-C) $1 - y_j(t)$ as a function of t for $M_0 = 20, 40$,and 80, respectively. D)-F) Interaction maps accompanied with A),B) and C), respectively. Solid lines for $j < M_0$ and broken lines for $j > M_0$.

2.2.3 Extreme case: $M = M_0$

Although no soils have similar outcomes to those obtained when $M = M_0$; i.e., no carbon resources are consumed by none of bacteria. There are two characteristic features when $M_0 = M$ (not shown here),

(1) The amount of consumed carbon resources starts to increase and saturates in earlier time region if compared with Figs. 3A-C.

(2) In contrast to Figs. 3A-C, there are no carbon resources which remains unconsumed.

Are there no real situations having these above two features? The answer is no. The time developments of carbon resource consumption for a compost, "Kanto-kun" provided by Fujimi Engineering Co., Japan has similar tendencies described in the features (1) and (2) (not shown here). In addition to this, it is well known that the content of compost can be fully consumed by bacteria in contrast to soils. This suggest that interaction map of compost is possibly like when $M_0 = M$, i.e., there are no carbon resources consumed by no bacteria. In conclusion, the situation when $M_0 = M$ is well reproduced in the compost.

2.3 Assumed real interaction map

There are no ways to observe directly interaction maps in the real soil. However, if we can isolate bacterial species one by one, there are some possibilities to observe interaction map indirectly. It can be done if we can have each bacteria to colonize. Of course, since not all bacteria can be incubated, this procedure is not perfect. However, at least, if we can know which carbon resource each of isolated bacteria can consume, it can be a substitute to the interaction map. Almost ten years ago, we have isolated soil bacteria and observe this by biolog system. Although relatively scattered, this kind of observation for healthy soil (not shown here) have similar configuration seen in Figs. 3D-F. As mentioned above, although this cannot be a true interaction map but an assumed one, it does not at least disagree with our model eqs. (1) and (2).

3. Discussion

3.1 Feature extraction

Although our model equations more or less reproduce real outcomes, time development themselves have too much information to compare with each other. It will be better to have more understandable features to distinguish sick soil from healthy one. We have previously proposed[7] integration of area below graph, i.

e.

$$ADS \equiv \sum_{j=1}^{96} \sum_{t=1}^{191} \Delta c_j(t),$$

where $\Delta c_j(t) \equiv c_j(t) - c_j(0)$, as activity-diversity score (ADS) to judge how healthy soil is. $c_j(t)$ is the amount of consumed jth carbon resource up to tth time points. In our model equations (1) and (2), $c_j(t)$ is assumed to be propotional to $1 - y_j(t)$. It turns out that ADS can discriminate sick soil from healthy soil. For example, soils in Figs. 1A-C have ADSs of 306,097, 412,000, 1,114,100, respectively. Thus, ADS can be a measure of how healthy soil is. However, compost "Kanto-kun" has greater ADS of 2,820,201 than soils. Since compost cannot be a soil, simply increasing ADS cannot result in healthy soil but may result in some other state. We need more suitable indicator to judge how healthy soil is.

One possibility to find such features is the usage of multivariate analysis[8]. Multivariate analysis allows us to reduce redundant information into more compact one. For example, principal component analysis[8] can reduce redundant feature vector into the vector with less and important components. However, since this is a linear transformation, the ability of reduction is limited. Recently, Taguchi and Oono proposed a new and efficient algorithm of non-metric multidimensional scaling (nMDS)[9], which can reduce the size of information space in the non-parametric thus non-linear manner.

In order to make use of nMDS, we first have to define the dissimilarity (distance) $\delta_{jj'}$ between objects j and j'. Here we would like to have $\delta_{jj'}$ for the pair of the jth and j'th carbon resources. This is defined as

$$\delta_{jj'} \equiv \sum_{t=0}^{191} \left[\Delta c_j(t) - \Delta c_{j'}(t)\right]^2. \tag{5}$$

Employing $\delta_{jj'}$ defined in eq. (5), we have apply nMDS to the collection of the four time developments in Figs. 1A-C and "Kanto-kun". We have obtained two dimensional embeddings (not shown here) by nMDS employing the dissimilarity eq. (5). For these embeddings, we have embedded all of time developments in Figs. 1A-C and that of "Kanto-kun" together. Thus, in total, we have had $96 \times 4 = 384$ time sequences. However, to understand easier, we have plotted sequences in each figure separately (not shown here). Each point corresponds to one of time sequences.

First of all, they have clear one dimensional structure, although its direction varies one by one. Since nMDS is non-metric method, scales of horizontal/vertical axes are arbitrary. However, the ratio between them is reproducible. Thus, the obtained one dimensional structure is meaningful; i.e., we have successfully reduced the rich information contained in time sequences. That is, these time sequences are ranked in one criterion very well. Remarkably, nMDS embedding can give us clear distinguishable features among them. For example, for sick soils, points concentrated to the limited region. While for healthy soil and compost "Kanto-kun", points distribute over wider region. Wider distribution means more diversity of time sequences. Thus, more healthy soils have tendency to have more diversity of time sequences.

At a glance, compost seems to have more diversity than healthy soil, since points distribute over wider region. In order to see this points, we have computed spatial distribution (not shown here) of points along horizontal axis. It turned out that the distribution corresponding to Fig. 1C, has more uniform distribution than that corresponding to compost "Kanto-kun". Thus, we can conclude that healthy soil has both diversity and uniformity. In other words, healthy soil has the mostly valanced distribution of time development.

3.2 Diversity and uniformity

In this paper, we have shown that healthy soil has time development of carbon resources consumption with very unique features; both diversity and uniformity. Although at the moment, it is impossible to measure how diverse and uniform soil ecological systems are, if we can trust our simple modeling, such a kind of distribution is related to uniform interaction matrix, i.e., having carbon resources ranging from those consumed by all to those by none (Fig. 3F). If this is true, why healthy soil has diverse and uniform time development can be understood as follows. Suppose that disease is caused by overpopulation of some specific bacteria. If interaction map is like Fig. 3F, ecological system is occupied by both specialist which consumes small kinds of carbon resources effectively and generalist which can consume many of carbon resources but less effectively than specialist. If there are more specialists, there will be easier for one species to

grow drastically if it can beat some of specialists, since then it can use full of carbon resources which are consumed by the specialists that it beats. However, if there are generalists, too, it cannot monopolize this resources since generalist will consume it, too. On the other hand, there are only generalists, it is also easy for some bacteria to invade it if it is specialists, because there are no specialists which can consume target resources as well as it can. Thus, if ecological system is occupied by both generalists and specialists, ecological system is more difficult to invade. Although at the moment, there are no way to confirm this conjecture, someday, we believe that some can confirm this conjecture and will succeed in generating soil which are strongly disease suppressive.

4. Materials and Methods

4.1 Preparation of soil suspension

Microorganism is dispersed using 15mM phosphate buffer solution from soil. 1000-fold diluted suspension is dispersed to GN-2 BIOLOG plate. Color reaction (590nm wavelength region) is measured in both wells each of which includes one of 95 carbon resources and the reference well with no carbon resource. Robotic system invented by BIOLOG Co. is used for automatic measurement every 15 minutes (25 °C, 48 hours). Used software for control is OL-PM ver.1.3 and that for analysis is OL-FM1.3. Output from this system is user for further analysis.

4.2 Colonization

50 $\mu\ell$ soil suspension is applied to culture medium (1/10 PTYGA[10]) by Autoplate. It is incubated for four days (25 °C, under dark condition). Bactria is sampled from each of colonies. Since sampling must be random, we have sampled all of colonies in the medium with at least ten or more colonies. Sampling has been done using a disinfected toothpick. Each of samples are applied to a new culture medium again. Medium is incubated over 24 hours. These whole processes are repeated twice and cell suspension (10^7 cells/ℓ physiological salt solution) is deposited to the BIOLOG plate. Permeability of detection light (440 nm) is controlled to be 70 to 80 %. Assimilation by bacteria is detected by the light (590 nm) by comparing control well with no carbon resources and is digitized to 1 or 0, by the software Microlog3N (BIOLOG Co.).

Acknowledgments

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