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"BACWAVE," a Spatial–Temporal Model for Traveling Waves of Bacterial Populations in Response to a Moving Carbon Source in Soil

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ABSTRACT

Previously, we discovered the phenomenon of wavelike spatial distributions of bacterial populations and total organic carbon (TOC) along wheat roots. We hypothesized that the principal mechanism underlying this phenomenon is a cycle of growth, death, autolysis, and regrowth of bacteria in response to a moving substrate source (root tip). The aims of this research were (i) to create a simulation model describing wavelike patterns of microbial populations in the rhizosphere, and (ii) to investigate by simulation the conditions leading to these patterns. After transformation of observed spatial data to presumed temporal data based on root growth rates, a simulation model was constructed with the Runge–Kutta integration method to simulate the dynamics of colony-forming bacterial biomass, with growth and death rates depending on substrate content so that the rate curves crossed over at a substrate concentration within the range of substrate availability in the model. This model was named "BACWAVE," standing for "bacterial waves." Cyclic dynamics of bacteria were generated by the model that were translated into traveling spatial waves along a moving nutrient source. Parameter values were estimated from calculated initial substrate concentrations and observed microbial distributions along wheat roots by an iterative optimization method. The kinetic parameter estimates fell in the range of values reported in the literature. Calculated microbial biomass values produced spatial fluctuations similar to those obtained for experimental biomass data derived from colony forming units. Concentrations of readily utilizable

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substrate calculated from biomass dynamics did not mimic measured concentrations of TOC, which consist not only of substrate but also various polymers and humic acids. In conclusion, a moving pulse of nutrients resulting in cycles of growth and death of microorganisms can indeed explain the observed phenomenon of moving microbial waves along roots. This is the first report of wavelike dynamics of microorganisms in soil along a root resulting from the interaction of a single organism group with its substrate.

Introduction

Distribution patterns of microbial populations within root systems have been investigated extensively [8, 18, 30, 36, 37, 39, 43]. High microbial densities have generally been observed close to the root tip and in middle and upper sections of the roots [36, 39, 43]. Similarly, distribution patterns of photosynthetically derived carbon compounds in the rhizosphere have been widely studied, usually using $^{14}CO_2$ [27, 34, 44]. The distribution of these carbon sources is generally similar to that of microbial populations, concentrated in the vicinity of root tips, at sites of lateral branch formation, and in older root segments where the cortex is decaying [24, 25]. Thus, patterns in microbial density have generally been attributed to corresponding points of exudation, taking root growth into account [21, 34, 36].

Recently, we determined densities of bacterial colony-forming units (CFU) and concentrations of soluble total organic carbon (TOC) at various distances from the tip of seminal roots of wheat plants of different ages [39]. Microbial densities showed three peaks along roots of older plants and fewer peaks along roots of young plants. Concentrations of TOC were high around the root tip and at the root base, but much lower in the middle sections of the root. Thus, the spatial distribution of TOC content along the root was different from the microbial distribution. Because there were no correlations between microbial density and TOC content or lateral root density, we came to the conclusion that there must be another process besides exudation leading to the observed regular microbial distribution along roots.

Harmonics analysis of our data showed that the observed patterns of bacterial CFUs along the root constituted significant waves [39]. Based on spatial shifts in the phases of the waves as the plants grew older, we considered the wavelike patterns as moving waves along the roots. The patterns observed by van Vuurde and Schippers [42, 43] could also be interpreted as waves.

We hypothesized the following mechanism underlying the observed wavelike patterns. Exudation takes place primarily at the root tip. Exudates are absorbed and metabolized primarily by fast-growing bacteria resulting in a rapid increase of these bacteria and a temporary depletion of readily utilizable substrate (RUS). Depletion of RUS may lead to autolysis of bacterial cells and release of readily utilizable nutrients. After accumulation of substrate above the uptake threshold [38] a second cycle of growth and death is initiated. Meanwhile the root tip continues to extend further into the soil, releasing exudates along its path. The growth and death cycles of microbes at any location where the root tip passes results in a wavelike pattern of microbial populations along the root on individual sampling dates, and "running waves" over time. The lack of a correlation between soluble carbon concentrations in the rhizosphere and bacteria population size, expressed as CFUs [22, 39], supports this hypothesis.

One of the means to test the potential of this hypothesis to explain the observed wavelike patterns is the construction of a simulation model that incorporates the hypothetical mechanism sketched earlier. Several models with increasing complexity have been constructed to simulate microbial growth in the rhizosphere. Newman and Watson [26] assumed that root exudation was uniform along the length of a root; they modeled diffusion of exudates into surrounding soil and the associated net microbial growth (dependent on nutrient concentrations) at various distances from the root surface. Simulated microbial populations declined monotonously with increasing distance from the root. Microbial death rates were not specified, but microbial biomass decreased when nutrient supply was less than the maintenance requirements [26]. Darrah [9] made a distinction between a model simulating uniform exudation along the root and one simulating exudation concentrated at the root tip. He found significant differences between these models in soluble carbon concentrations and microbial dynamics. Root growth was considered, but microbial distributions along the length of the root were not explicitly studied. Waves in microbial populations were not obvious from the figures [10]. Growth of microbial biomass was dependent on nutrient concentra-

tions. Live microbial biomass was converted into nonsoluble necromass [9] instead of into soluble carbon instantaneously [26]. However, death rates were again not specified [9]. Scott et al. [37] also included root growth through layers of soil. Root exudation was larger for 1-week old root parts (extended tip) than for older root parts, but was uniform within those two sections. They considered effects of matric potential and diffusion of exudates between soil layers. Bacterial populations were modeled along the root length, starting with genetically marked bacteria applied onto seed. Live biomass and substrate concentrations were modeled without intermediate necromass. Growth rates were again dependent on nutrient concentrations, but death rates were kept constant. Simulated bacterial populations decreased monotonically with depth, while experimental populations showed wavelike patterns with depth [37].

Wavelike patterns have been analyzed and modeled in many scientific disciplines. Running or traveling waves have been documented for growth of fungal colonies [11], distribution of interacting organisms in two-dimensional space [14], and invasion of an organism in an area [12, 20]. Reaction-diffusion models have been employed to simulate these situations [14]. Using sets of interconnected nonlinear equations, Gilligan [14] showed that heterogeneous distributions of a pathogen and its antagonist in soil need not be due to heterogeneous soil conditions. Thus, it is possible that the wavelike distribution patterns of bacteria observed in the rhizosphere are not directly related to similar distributions of carbon sources along the roots, but to growth and death cycles of microorganisms and recycling of carbon compounds in soil. This dynamic concept of wavelike distributions is a revolutionary deviation from the common notion that microbial patterns are directly related to exudation patterns.

In this paper we present a simulation model realizing the concept that wavelike spatial patterns of bacterial populations in the rhizosphere could be due to growth and death of bacteria in response to a moving nutrient source, and investigate by simulation the internal conditions leading to moving waves of microbial populations along a root. In addition, we derive parameter values for variables that are difficult to measure, such as readily utilizable substrate in soil.

Materials and Methods

Experimental Data

For this research we used data on population dynamics (colony forming units) of copiotrophic bacteria and water-soluble total organic carbon (TOC) along wheat roots 2, 3, and 4 weeks after seeding [39]. The data used were geometric means of 3 replicates of 2-cm long root sections at 4-cm intervals along the total root length. Populations (CFUs/g dry soil) were transformed into biomass carbon per 1 cm^3 of soil according to a factor 4.76×10^{-8} [μg C $\times$ g ds/CFU cm^3]. This value was calculated from previously measured cell sizes for copiotrophic bacteria in a cover crop amended soil (0.61 μm^3 per cell; unpublished data) and the assumption that bacterial cells contain 85% water and that the carbon content of dry bacterial biomass is 40%. Soil density was considered to be 1.3 g/cm^3. Thus, the dry mass of a bacterial cell was estimated to be 9.15×10^{-8} μg, which is similar to values used by Atlas and Bartha [1].

Transformation of Spatial to Temporal Dimension

In the experiment mentioned above [39], root growth against a Plexiglas wall was measured weekly. The relationship between time and root length was statistically not significantly different from linear, so that the growth rate could be considered constant at 3.3 cm/day. Thus, measured distances from the root tip were transformed to a time scale using a constant conversion factor. We consider this transformation justified as the microbial community in soil becomes activated and starts to grow after passage of a root tip at any given point in soil. Immediately after appearance of the root tip this point can be considered as belonging to the rhizosphere. Thus, transformed data were expressed as biomass carbon per cm^3 of soil over time in hours passed since the root tip had entered a certain point in soil.

Model Assumptions

The model was based on the following assumptions: The root consists of a one-dimensional line without side branches: The root grows at a constant extension rate (3.3 cm/day). Biomass and substrate carbon (C) change over time at point locations with infinitely small volumes; transport of biomass or substrate is considered negligible between locations. At any point of the rhizosphere there is the same pattern of biomass behavior with time, but because of shifts in the starting time (the root tip appearance) biomass development is shifted in space. There are only three sources of substrate: root tip exudates; a low-level constant background influx, resulting from soil organic matter decomposition, sloughed root cells, and constant background exudation; and bacterial necromass. Exudation of photosynthetically derived organic carbon takes place primarily at the lowest 1 cm above the root tip, resulting in a short-term pulse of substrate at each point due to continuous root elongation. Root exudation rate declines exponentially with time, being practically 0 after 6 hours of root growth since the tip entered a location. Although day/night rhythms in exudation have been demonstrated [7, 44], day/night rhythms are ignored in the model, since the resolution of our experimental data was too coarse to detect day/night rhythms. Substrate originating from exudates and from background flux has the same composition and is readily utilized by bacteria with a constant yield coefficient. Copiotrophic bacteria make up most of the microbial biomass in the rhizosphere of agricultural soils, due to their fast growth rates in response to an

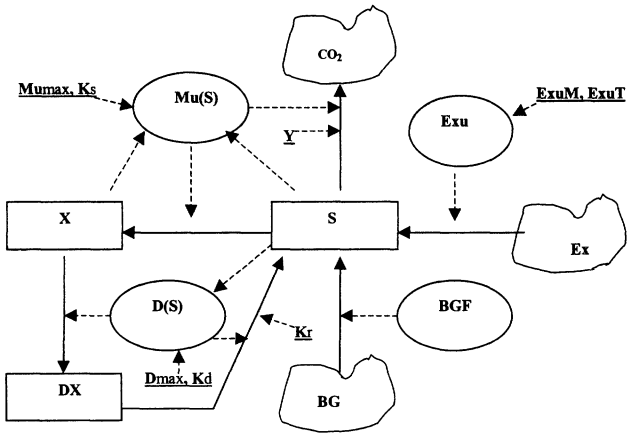

Fig. 1. Diagram of the simulation model BACWAVE with the state variables X (biomass of bacteria, μg C/cm^3 soil), S (substrate concentration, μg C/cm^3 soil), DX (dead biomass of bacteria, μg C/cm^3 soil), Ex (exudate concentration, μg C/cm^3 soil), BG (background carbon, μg C/cm^3 soil), and CO_2 (carbon dioxide, μg C/cm^3 soil); rate variables Exu (exudation rate in μg C/cm^3/hr), BGF (background flux in μg C/cm^3/hr), D(S) (relative death rate of bacteria, hr^{-1}), and μ(S) (relative growth rate of bacteria, hr^{-1}); and auxiliary variables K_r (recyclable biomass fraction, unitless), D_{max} (maximal relative death rate, hr^{-1}), K_d (substrate death constant, μg C/ml solution), μ_{max} (maximum relative growth rate, hr^{-1}), K_s (Substrate constant for growth, μg C/ml solution), Y (yield coefficient, μg C/μg C), ExuM (maximal exudation rate, μg C/cm^3 hr), and ExuT (exudation time constant, hr^{-1}). Solid lines represent carbon flows, and dashed lines information flows.

incoming carbon source, the relatively small size of oligotrophic bacteria (so that their biomass is small even if their numbers are large), and the minor role played by fungi in agricultural soils [5]. Thus, only copiotrophic bacteria are currently considered in our model. Maintenance respiration is considered negligible. Relative growth rate (RGR) of bacteria is related to substrate concentration in the soil solution according to a Monod equation. The maximum relative growth rate and substrate affinity are assumed to be constant during the whole observation period. Relative death rate (RDR) of bacteria depends on substrate concentration according to an inverse Monod equation. Dead cells are instantaneously converted into secondary available carbon compounds by autolysis with a loss of 60% of not easily decomposed material. Bacterial biomass oscillations arise from growth and death cycles dependent on substrate level, not necessarily from competitive or predator–prey interactions.

Model Description

A simplified approach was taken by simulating bacterial populations and readily utilizable substrate (RUS) at one location in time and then translating the results onto space taking root growth rate into account. A diagram of the model is presented in Fig. 1. The model consists of a set of two ordinary differential equations, describing bacterial biomass and substrate dynamics dependent on bacterial biomass growth and death and external sources of substrate (exudation and background flux). The set of equations is presented below:

$$dX/dt = (\mu(S) - D(S)) \times X$$

$$dS/dt = -X \times \mu(S)/Y + Kr \times X \times D(S) + BGF + Exu(t);$$

Where

$$\mu(S) = \mu max \times S/(Ks \times \theta + S),$$

$$D(S) = Dmax \times Kd/(Kd + S/\theta),$$

$$Exu(t) = ExuM \times \exp(-ExuT \times t),$$

and

t = time [hr];
X = biomass of bacteria [μg C/cm^3 soil];
S = substrate content [μg C/cm^3 soil];
μ(S) = relative growth rate of bacteria [hr^{-1}] (dependent on substrate concentration);
μ_{max} = maximal relative growth rate of bacteria [hr^{-1}];
K_s = substrate constant for growth [μg C/ml soil solution];
D(S) = relative death rate of bacteria [hr^{-1}] (dependent on substrate concentration);
D_{max} = maximal relative death rate of bacteria [hr^{-1}];
K_d = substrate constant for death of bacteria [μg C/ml soil solution];
Y = yield coefficient for bacteria [μg C/μg C];
K_r = fraction of dead biomass recycling to substrate [-];
BGF = constant background flux of substrate [μg C/cm^3 soil/hr];
θ = soil water content [ml solution/cm^3 soil];
Exu(t) = exudation rate [μg C/(hr * cm^3 soil)] (dependent on time);
ExuM = maximal exudation rate [μg C/(hr * cm^3 soil)];
ExuT = time constant for exudation, responsible for duration of exudation [1/hr].

The expressions for the specific growth and death rates dependent on substrate content form hyperbolic curves, but oriented in the opposite direction, so that no negative or zero values can occur for μ or D. The curves cross over at a stationary point, where specific growth and death rates of bacteria are equal and no change in biomass occurs. When substrate levels change past this stationary point, net growth will change into net death (or vice versa) resulting in wavelike biomass dynamics.

The model was initially run in the program "Model Maker" (Cherwell Scientific Publ. Ltd., Oxford, UK) to allow easy adjustment and optimization of parameter values. The Runge–Kutta method of integration was selected with a variable time step. The final model was written in PASCAL version 7.0 (Borland International, Inc., Scotts Valley, CA) solving the ordinary differential equations by the Runge–Kutta fourth-order method with a constant time step of 0.01 hr [13]. This model was named "BACWAVE," standing for "bacterial waves." This version is available upon request.

Parameter Optimization

To optimize parameter values, the experimental data for microbial biomass in relation to distance from the root tip, measured two, three, and four weeks after seeding, were combined, assuming that the response of the microbial community to a passing root tip would be similar regardless of the age of the plant. Before optimization, initial biomass was estimated from the biomass observed in bulk soil [39], and substrate originating from the root tip (combination of maximal exudation rate and exudation time constant) was estimated as two times the first rhizosphere biomass peak. Thus, ExuM = 8 and ExuT = 0.8 were chosen to provide 10 μg C/cm^3 soil as total exudation from the root tip for the first 6 hr. The yield coefficient and recyclable fraction of biomass were set at 0.44 and 0.4, respectively [28, 29]. All other parameters were estimated by optimization using the combined data sets (2, 3, and 4 weeks after seeding). Next, the maximal relative growth rate, substrate constant, death rate constant, substrate death constant, and minimal death rate were maintained, while the initial biomass, initial substrate value and background substrate flux were optimized for each individual data set. After optimization of parameter values, simulated patterns of biomass dynamics were graphically compared with observed patterns of bacterial populations in root observation boxes [39]. Parameter values were compared with literature data in as far as they were available. Finally, the model was run for various times and the resulting biomass was plotted versus distance from the static root base rather than the moving root tip, so that the population dynamics at fixed locations could be visualized over time.

Model Validation

The model was validated with the bacterial biomass data from an other root observation box experiment with wheat grown in soil rich in organic matter [39]. For validation, the same parameter values were used while only the background substrate influx was increased.

Sensitivity Analysis

Since the crossover of growth and death curves in relation to substrate concentration was essential to obtain fluctuations in microbial biomass, the effects of varying angles between the curves at the stationary point on microbial biomass dynamics were tested. To evaluate the importance of a moving nutrient impulse versus a constant substrate flux, the ratio of the initial exudation rate (ExuM) and background flux (BGF) was varied.

Results

The simulation model resulted in oscillations in microbial density along the path of a moving substrate source. The simulated waves in bacterial biomass were very close to the wavelike patterns obtained in a root observation experiment with wheat (Fig. 2). Except for slight variations in initial biomass and substrate concentration (which may indeed differ from box to box), the same parameter values (Table 1) resulted in a good fit to two experimental data sets, two and three weeks after seeding (Fig. 2 A and B). Using the parameter values and initial constants that resulted in a good fit between modeled and observed microbial populations along the root of 2- and 3-week old plants did not give a satisfactory fit for the 4-week data set (Fig. 2C). The simulated bacterial biomass curve passed below most experimental points 4 weeks after seeding (Fig. 2C). However, when the background influx was increased from 0.10 to 0.15 μg C/cm^3 soil/hr, the simulated curve was very close to the observed data (Fig. 2D). The same parameter values but a higher background flux (0.35 μg C/cm^3 soil/hr) also resulted in a good fit to the validation data set from an experiment with soil from an organic farm that was much higher in fresh organic matter, with a total carbon content of 1.0% compared to 0.68% in the previous experiment (Fig. 3). Altogether, BACWAVE explained as much of the variance (18–62%) in the experimental data sets as significant harmonics did (25–61%). Additional proof of model adequacy is the fact that for all data sets represented in Fig. 2, there were no significant differences between experimental and simulated data according to a paired *t*-test at 0.05 significance level.

Simulated substrate concentrations had a periodic character similar to that for bacterial biomass but differing in phase compared to the biomass waves (Figs. 2 and 3).

Although it was necessary to adjust initial values of biomass and substrate as well as background flux values to obtain a good agreement of modeled and experimental data, the values of these variables were quite close, ranging from 0.5 to 1.5 μg C/cm^3 for initial biomass, 1.0 to 4.0 μg C/cm^3 for initial substrate, and 0.1 to 0.15 μg C/cm^3/hr for background flux (Table 1). The maximum relative growth rate of copiotrophic bacteria was 0.063 hr^{-1} and the substrate constant for growth was 0.68 ugC/cm^3 of dry soil, equivalent to 3.0 μg/ml soil solution (Table 1). The estimated value for the maximal relative death rate was 0.26 hr^{-1} so that projected death rates were high at substrate concentrations close to zero. However, within the simulated substrate range, the death rate varied from 0.05 to 0.07 hr^{-1} for the basic variant of the model (Fig. 5A).

When biomass was plotted in relation to a fixed point in soil (the root base) for different times, the waves were extended in space, appearing as "running waves" over time (Fig. 4). This simulated behavior was similar to the dynamic

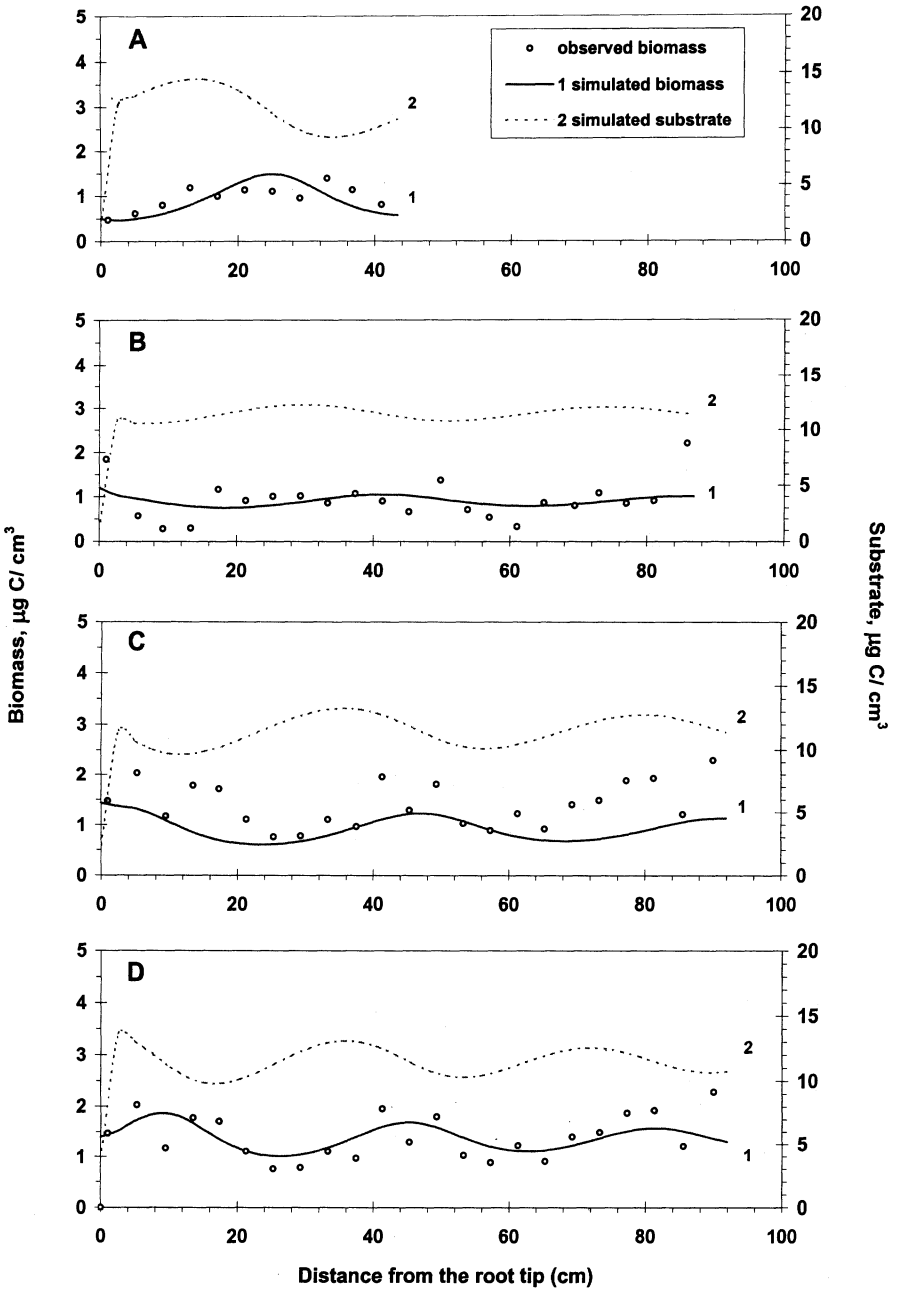

Fig. 2. Simulated (solid line) and observed (open circles) bacterial biomass and simulated (dashed line) substrate concentrations at increasing distances from the tip of the seminal root of wheat (A) 2, (B) 3, and (C) 4 weeks after planting using the same parameter values as in Table 1, including BGF = 0.1 µg C/cm^3 hr, and (D) same as (C) but BGF = 0.15 µg C/cm^3 hr. Experimental data are from the second root observation experiment with soil low in organic matter as described in Semenov et al. [39].

character of the wavelike distribution of bacteria in the experiment with wheat described above. In this experiment, the maxima and minima in bacterial populations shifted in space from 2-week old plants to 3- and 4-week old plants [39].

Variations in growth and death curves in relation to substrate content showed that the substrate concentration at the crossover point must fall within the substrate concentrations attained in the system (simulated data not shown). Oscillation of the substrate level around a switch point (50 µg C/ml soil solution) is associated with switches in microbial behavior from predominant growth to predominant death and vice versa. At this switch point, microbial biomass is temporarily stationary. The frequency and amplitude of the biomass waves depend on the angle between the growth and death curves at this stationary point (Fig. 5, Table 2). The larger the angle, the higher is the frequency. Such a simple rule could not be detected with respect to the amplitude of the biomass waves. The smallest angle between growth and death curves (Fig. 5C) resulted in an intermediate amplitude of biomass oscillations (Fig. 5D), whereas intermediate and large angles in growth and death curves (Figs. 5A and 5E) resulted in small and large biomass amplitudes, respectively (Figs. 5B and 5F).

Table 1. List of parameters, their units, and values used in the standard version of BACWAVE, and optimized to simulate bacterial biomass along wheat roots in experiment 2 of Semenov et al. [39]

Parameter	Symbol	Unit	Value
Internal			
Maximal relative growth rate	μmax	[1/hr]	0.063
Substrate constant	Ks	[μg C/ml]	3.00
Maximal relative death rate	Dmax	[1/hr]	0.26
Substrate death constant	Kd	[μg C/ml]	14.50
Yield coefficient	Y	[μg C/μg C]	0.44
Recyclable fraction of biomass	Kr		0.40
External			
Background substrate influx	BGF	[μg C/cm^3 hr]	0.1–0.15
Maximal exudation rate	ExuM	[μg C/cm^3 hr]	8.00
Exudation time constant	ExuT	[1/hr]	0.80
Moisture of soil	θ	[cm^3/cm^3]	0.23
Initial values			
Biomass	Xi	[μg C/cm^3]	0.5–1.5
Substrate	Si	[μg C/cm^3]	1.0–4.0
Variability explained by the model		[%]	18–62

The amplitude and frequency of biomass fluctuations depend primarily on background substrate flux and to a lesser extent on exudation rate from the root tip: the higher the background flux, the larger the frequency and amplitude (Fig. 6). In all cases, the waves dampen over time, and thus also along the root. The dampening is steeper at higher levels of background flux. Finally, bacterial biomass stabilizes at some stationary level, which is higher at high levels of background substrate flux. The value of this stationary biomass level also depends on the yield coefficient, the value of growth and death functions at the substrate stationary point and the recyclable fraction of dead biomass (data not shown). The location of the first peak relative to the root tip depends primarily on exudation rate (Fig. 6). The higher the exudation rate is, the shorter the distance between the root tip and the first biomass peak. Exudation rate also influences the amplitude of the waves but has only a minor influence on the period of biomass fluctuation.

Discussion

The aim of this paper was to develop a model that can describe and predict patterns of bacterial population dynamics along a root with a moving point of substrate release. We succeeded in attaining this goal and showed that an impulse of nutrients from a growing root tip at any location, together with a low level of background substrate flux, can result in wavelike patterns of bacterial biomass over time. These temporal wavelike patterns can be translated into a spatial wavelike distribution along a root at a particular moment since the start of root growth, similar to the wavelike distribution patterns observed previously for wheat [39]. Relative to a fixed location (the root base), simulated fluctuations in bacterial populations appear as moving waves in the spatiotemporal domain, again similar to the shifting wavelike distributions described for wheat roots [39]. Thus, our discovery of oscillations in microbial populations along a wheat root could be explained on the basis of internal features of a system consisting of a bacterial community and its substrate. These features include root exudation, microbial consumption of substrate, inducing growth and then death of microorganisms, and substrate supplements from recycling of dead biomass and decomposition of soil organic matter.

The main difference between our model and previous rhizosphere models [9, 10, 26, 37] is the assumption that the death rate of microorganisms is dependent on substrate level. This is a plausible assumption for soil conditions with naturally low substrate concentrations, where small fluctuations in substrate level can have a significant impact on biomass dynamics [28]. Another essential difference with previous models is that in our model relative growth and death curves in relation to substrate concentration cross over at a particular substrate level, the so-called "stationary substrate point." If death rates were constant and exceeded growth rates only at almost zero substrate levels, one peak in microbial biomass would be followed by a monotonous decline (simulation data not shown). Indeed, in their simulation model, Scott et al. [37] used low constant death rates compared to high, variable growth rates dependent on nutrient concentrations in the rhizosphere. Their simulated populations globally reflected observed populations, but did not follow the wavelike patterns in their experimental data. We attribute this discrepancy to the slow death rates, independent of nutrient concentration. On the other hand, we were able to obtain wavelike patterns with our model thanks to the crossover of relative growth and death curves in relation to substrate concentration. We also showed that the angle between the curves of growth and death rates plotted against substrate concentrations strongly affects the frequency and to a lesser extent the amplitude of the fluctuations in biomass and substrate contents. The larger the angle,

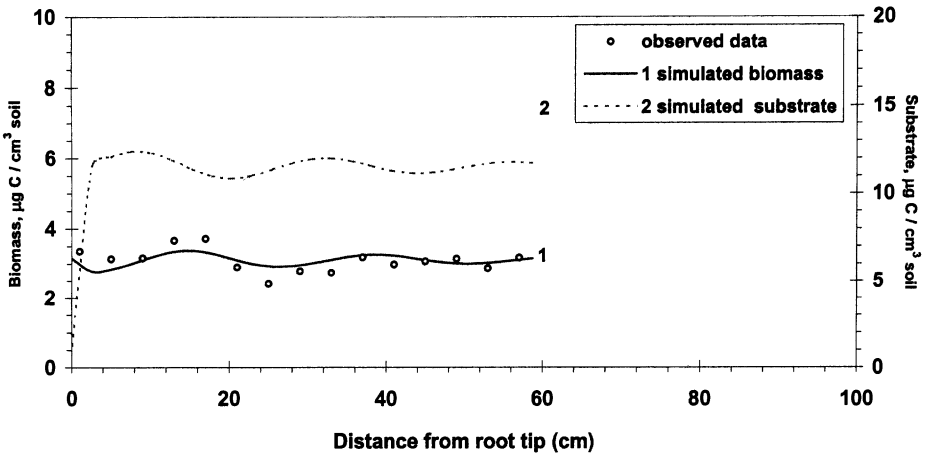

Fig. 3. Simulated (solid line) and observed (open circles) bacterial biomass and simulated (dashed line) substrate concentrations at increasing distances from the tip of the seminal root of wheat three weeks after planting, using the same parameter values as in Fig. 2, but BGF = 0.35 µg C/cm^3 hr. Experimental data are from the first root observation experiment with soil high in fresh organic matter as described in Semenov et al. [39].

the more frequent are the cycles of accumulation and exhaustion of substrate and consequent cycles of growth and death of biomass. Thus, we showed that moving waves can result from the interaction of one organism group with its substrate, and that predator–prey [2, 31, 40] or host–parasite interactions [3] are not a necessary prerequisite for wavelike fluctuations in bacterial numbers or biomass.

The results of our model also confirmed that biomass fluctuations along a root do not necessarily require an additional irregular source of substrate such as lateral root emergence. Fluctuations in substrate level and biomass are initiated by the perturbation created by exudate influx into bulk soil from a moving root tip, but a regular source of background substrate is needed to maintain a relatively large amplitude and frequency of the fluctuations. On the other hand, a very large background flux results in a high frequency but fast dampening of the fluctuations due to substrate loss by respiration and deposition of the nonrecyclable biomass fraction. Only a small background flux (compared to the carbon input from root tip exudates) was needed to fit our model output to the data obtained from a wheat experiment [39]. Thus, the microbial peaks along the middle and upper sections of the roots [39] arose at least partially from recycled carbon sources likely originating from autolysed cells that formed the first peak in bacterial populations immediately after passage of the root tip. This is a likely scenario since autolysis is a common phenomenon in bacteria and fungi [15, 16, 32].

We were able to simulate bacterial biomass fluctuations of different data sets using essentially the same model parameters. Only the initial biomass and substrate values and substrate background flux needed to be adjusted to obtain good fits to the various data sets. In particular, the value of the background flux needed to be increased in the model to mimic observed bacterial biomass as the plants grew older. An increase in background flux with plant age at any location can be explained by rhizodeposition at the root base and downward movement of soluble carbon in the rhizosphere by mass flow resulting from irrigation. The background flux also needed to be increased to mimic fluctuations in bacterial populations in a soil high in fresh organic matter. In that case, the background flux probably originated from decomposing debris in the bulk soil [39]. The fact that a simple increase in background flux resulted in a good fit to the data of this second experiment constitutes a satisfactory validation of our concept of microbial dynamics in the rhizosphere as expressed in the presented model.

The parameter values estimated by optimization were reasonable, and mostly very similar to those reported in the literature [26, 37]. The maximum relative growth rate was relatively low (0.063 hr^{-1}) for copiotrophic bacteria, considering that the estimated doubling time would be 11 hr. In continuous flow culture with an easily utilizable substrate such as glucose, the maximum relative growth rate for copiotrophs would be almost 10 times as high [23, 38]. However, root exudates consist of a mixture of compounds with different decomposition rates [35]. Moreover, our experimental data consisted of relatively large rhizosphere samples compared to the microscale at which nutrient release and consumption take place. Thus, the relative growth rates estimated from these experimental data are averages of microsites with much higher and lower relative growth rates. For similar reasons, the substrate constant was relatively low, but not out of the ordinary [4, 28, 38]. The death rate varied from 0.05 to 0.07 hr^{-1} depending on substrate levels in the range of 35–65 µg C/ml of soil solution. No experimental data are available to verify these death rates, but the rates are similar to those used by Scott et al. [37].

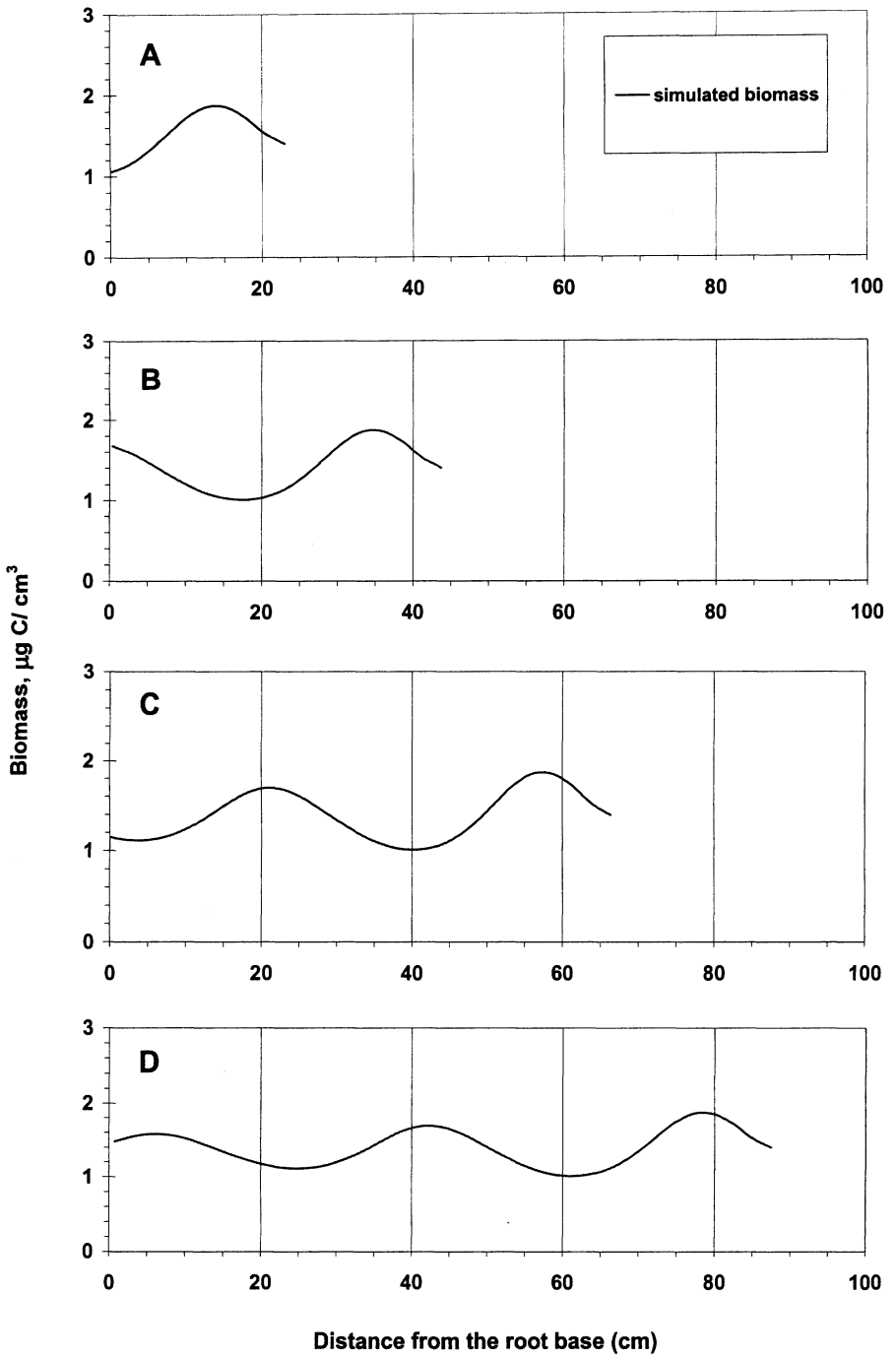

Fig. 4. Simulated running waves of bacterial biomass (µg C/cm^3 soil) from the root base to the tip after runnning BACWAVE for (A) 150 hr, (B) 300 hr, (C) 450 hr, and (D) 600 hr. Standard parameter values were used for these runs (see Table 1).

The estimated initial biomass of copiotrophic bacteria at the root tip corresponded to 1–3 × 10^7 CFUs/g of dry soil. These are reasonable numbers for the rhizosphere, although they are higher than those commonly found in bulk soil [39]. One explanation may be that part of the water-soluble substrate moved downwards in our root observation boxes by mass flow of irrigation water, so that the rhizosphere started ahead of the root tip rather than exactly at the root tip. This would result in a higher biomass at the root tip than expected from bulk soil data. Results from Darrah's model also hinted at a downward flux of exudates beyond the root tip, resulting in an increase in bacterial biomass in the bulk soil just before the root tip entered that location [10]. Another explanation could be that our estimated maximum relative growth rate was lower than the actual maximal growth rate at microsites around the root tip.

In developing our simulation model for microbial dynamics we needed to include a variable called readily utilizable substrate (RUS), or simply substrate. The estimated initial substrate concentration (about 1–4 µg C/cm^3 soil) is

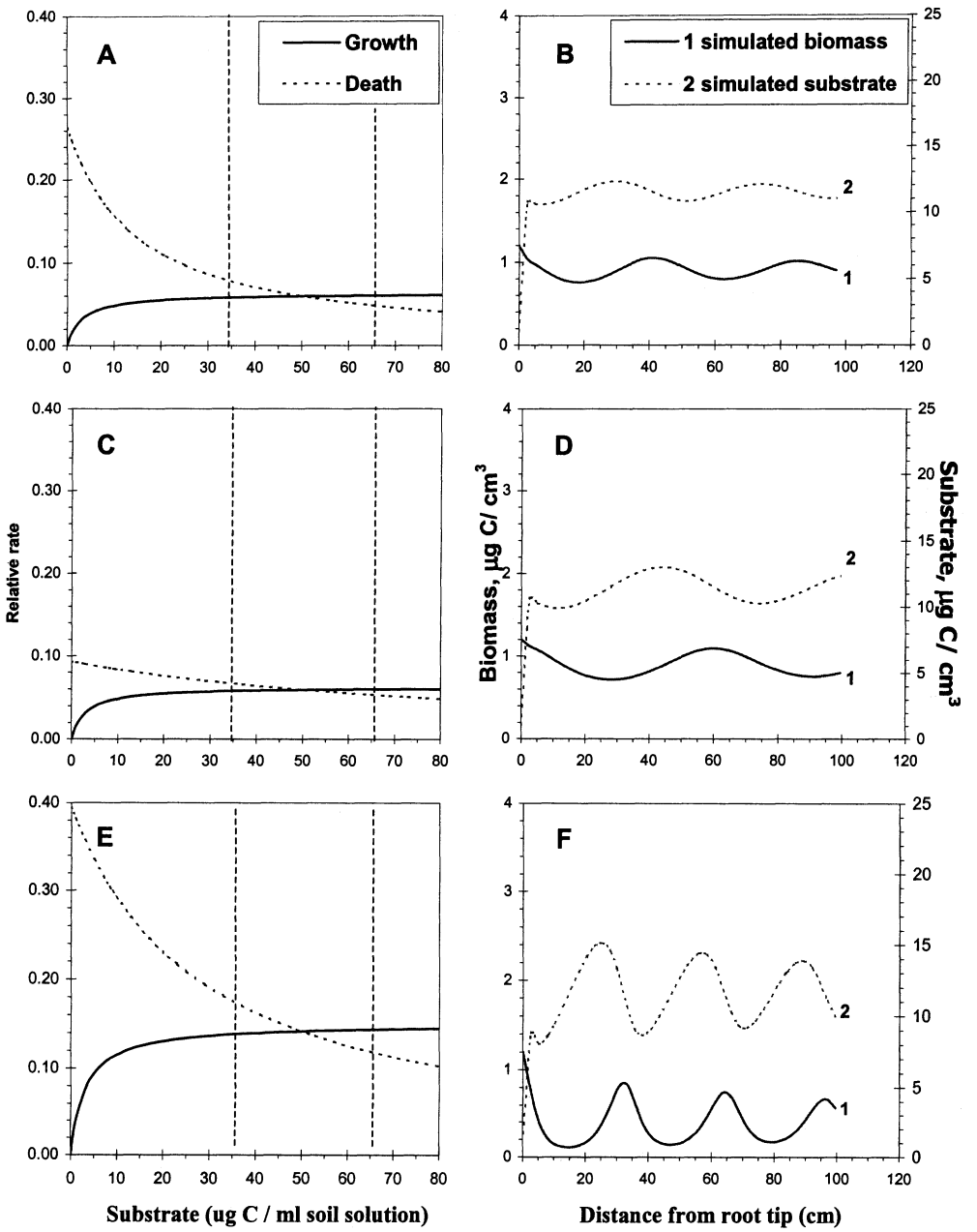

Fig. 5. Effects of varying relationships of relative growth and death rates of bacteria (A, C, and E) with substrate concentrations on fluctuations of simulated bacterial biomass over time as translated into one-dimensional space (B,D, and F). Note that the crossover points of growth and death rates are at the same substrate concentration (50 µg C/ml solution), whereas the angles between the curves at the crossover points vary. Parameter values for growth and death rates relative to substrate concentration are given in Table 2.

reasonable compared to concentrations estimated for bulk soil [9]. However, the maximal simulated substrate concentration (about 15 µg C/cm^3 soil) was much lower than the measured concentrations of water-soluble TOC [39]. Moreover, the fluctuations in simulated substrate concentrations did not correspond to changes in water-soluble TOC along wheat roots [39]. The high concentrations of TOC at the root tip and root base as presented earlier [39] must represent residual compounds that are not easily utilized by microbes. Carbon compounds in the rhizosphere, including TOC, are quite complex [17, 33, 34, 35, 45], and readily utilizable substrates naturally constitute only a small part of these substances. For example, the percentage of simple sug-

Table 2. Values of parameters for relative growth and death rates in relation to substrate concentration used for graphs in Fig. 5 A, C, and E

	Growth		Death	
Graph	µmax [1/hr]	Ks [µg C/ml]	Dmax [1/hr]	Kd [µg C/ml]
A	0.063	3.0	0.26	14.5
C	0.063	3.0	0.09	89.1
E	0.150	3.0	0.40	27.9

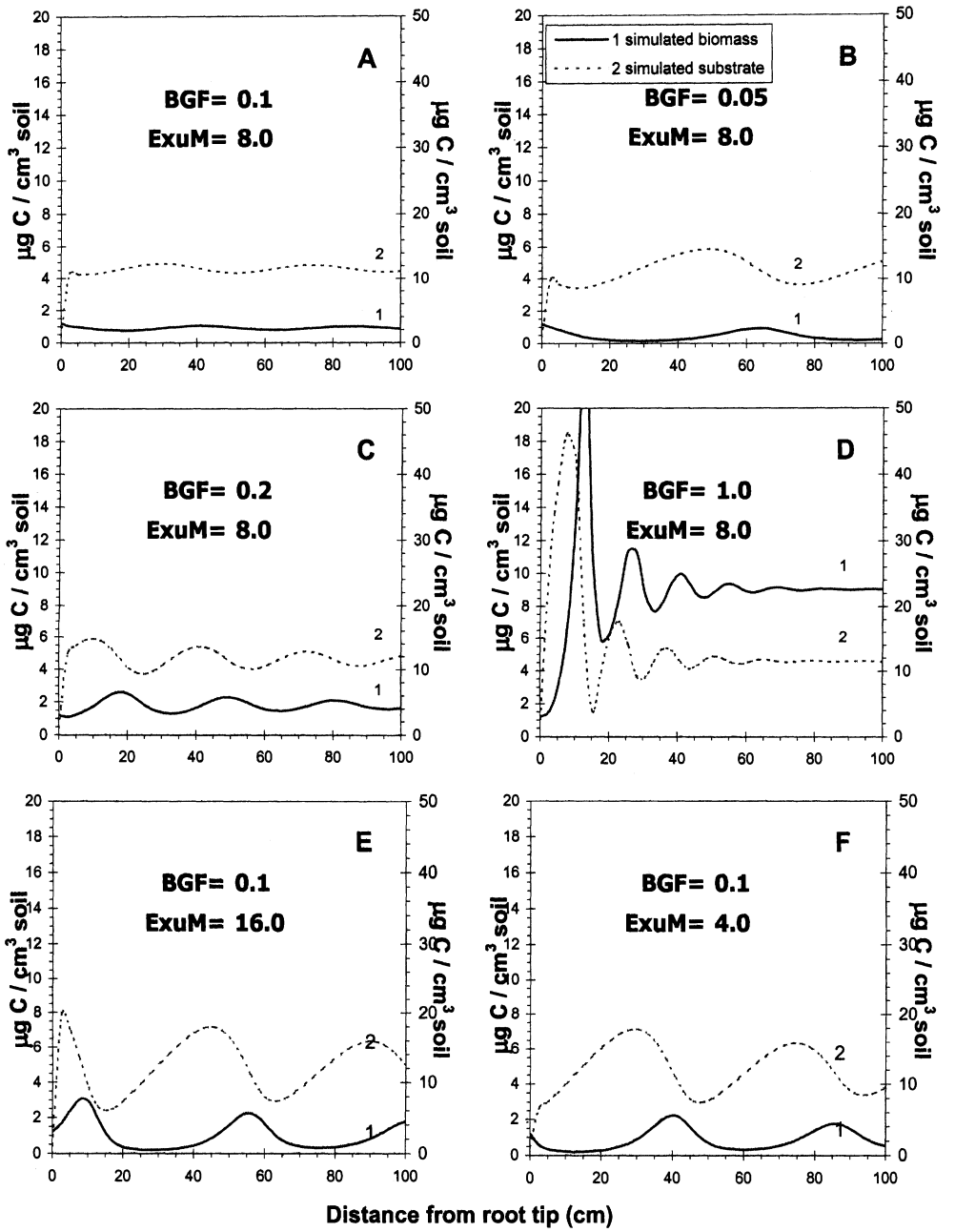

Fig. 6. Effects of varying substrate input from a moving source (ExuM = maximum exudation rate in µg C/cm^3/hr) and background substrate flux (BGF in µg C/cm^3/hr) on fluctuations of simulated bacterial biomass (solid line) and substrate concentration (dashed line) over time as translated into one-dimensional space. (A, B, C, and D) Effects of increasing BGF at constant ExuM; (A, E, and F) effects of varying ExuM at constant BGF. Total amount of carbon released from the root tip in 6 hr is 10 µg C/cm^3 for ExuM = 8.0 µg C/cm^3/hr, and from background flux is 0.6 µg/cm^3 for BGF = 0.1 µg C/cm^3/hr.

ars in released soluble organic matter is often low, varying from 1 to 5% for various crops [19]. A large part of the rhizodeposits that can be extracted by water are polymers, phenolics, and glycosides, which are more difficult to metabolize [19]. Water-soluble TOC also includes fulvic and humic acids coming from the bulk soil or formed in the rhizosphere. These compounds are also not easily metabolized. If a larger part of TOC could be readily utilized, bacterial populations would grow to a much greater biomass, considering that the calculated C content of bacterial biomass was approximately 1–2 orders less than that of TOC. Moreover, in nonsterile soil, RUS is absorbed by microorganisms as soon as the concentration passes the uptake threshold [38]. Thus, the concentrations of residual RUS are expected to be very low, much lower than TOC concentrations in the rhizosphere. In particular, when biomass and substrate content become stationary, the substrate concentration approaches that of the "stationary substrate point," 11.5 µg C/cm^3 soil or 50 µg C/ml soil solution in our model. Although the stationary substrate concentration is always equally low, the stationary biomass concentration can vary considerably dependent on background substrate flux. New-

man and Watson [26] came to a similar conclusion, namely that the substrate concentration is generally low, while the microbial concentration may vary greatly.

In conclusion, we developed a simulation model describing a new concept about microbial population dynamics and resulting wavelike distributions in the rhizosphere. With this model we showed that wavelike patterns can arise from a single moving point of nutrient release plus an evenly distributed substrate background flux, and that peaks in microbial cells along a root are not necessarily the result of corresponding peaks in rhizodeposits, including exudation. We proposed a probable explanation for the development of wavelike patterns in microbial populations along the root, namely periodicity in growth and death of the microbial community in relation to readily utilizable substrate concentrations. More experimental research will be needed to investigate the cause of death in the growth–death cycles. Finally, the relative importance of rhizodeposits from the root tip (including subsequent recycled carbon) and from lateral roots will need to be determined, for example by comparing wild-type roots with mutant roots without laterals [6] and by studying microbial distribution patterns along the path of a moving artificial root without lateral exudation points [41].

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