Spatial and temporal fluctuations in bacteria, microfauna and mineral nitrogen in response to a nutrient impulse in soil

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To

Ariena,

Alexander,

my grandmother Irina,

my mother Roza,

my father Viktor,

my sister Nadiusha

for inspiration, concern, patience, and love

Abstract

Fluctuations of bacterial populations can be observed when frequent and sufficiently long series of samples are obtained for direct microscopic or plate counts of bacteria. Fluctuations in bacterial numbers are especially noticeable after some disturbance of soil such as substrate addition. However, very seldom were bacterial fluctuations subjected to proper statistical analysis to detect significant periodical components in the data. In this thesis, bacterial dynamics and distributions were investigated in short-term (1 month) controlled experiments for rhizosphere and bulk soil after substrate input from plant roots and fresh plant debris, respectively. By using harmonics analysis, significant wave-like oscillations in bacterial numbers were detected along wheat roots and over time after clover-grass incorporation into soil. Oscillations were not related to numbers of lateral roots, soil moisture, mineral nitrogen concentrations, pH or redox potential. Soil fauna was not monitored in the rhizosphere experiments. In clover-grass experiments, nematode populations and composition were monitored daily, while protozoa were enumerated occasionally. Bacterial feeding nematode populations did not oscillate, but daily changes in their numbers did oscillate with a frequency similar to that of bacterial oscillations. Short-term oscillating dynamics of bacterial populations were simulated in two mechanistic models: "BACWAVE" to simulate the wave-like distribution of copiotrophic and oligotrophic bacterial populations along roots and "BACWAVE-WEB" to simulate wave-like oscillations of 3 trophic groups of bacteria (copiotrophic, hydrolytic, and oligotrophic) and their predators (protozoa and nematodes) over time after incorporation of plant residues into soil. In both models relative growth and death rates were non-linearly related to readily utilizable substrate concentrations, so that the cross-over point of the growth and death rate curves occurred at realistic substrate concentrations. A good fit was obtained to the observed data. Mineral nitrogen release was also modeled satisfactorily with "BACWAVE-WEB". Virtual experiments suggested that substrate-bacteria interactions are more important for regulation of bacterial oscillations than predator-prey interactions, and that regulation by protozoa was more influential than that by nematodes. Release of mineral nitrogen was primarily determined by bacteria, and to a lesser extent by predators. Oligotrophic bacteria played an important role in stimulating hydrolytic enzyme production and moderating bacterial fluctuations. The "BACWAVE-WEB" model has good potential to predict responses if microbial communities to a disturbance, which could be used to characterize soil health.

Contents

Chapter 1

Introduction and overview

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Cycles and fluctuations

Living nature operates in cycles, in particular daily, seasonal and annual cycles, as influenced by corresponding oscillations in temperature, humidity and light. There are also strong influences of available sources of carbon and other biogenic elements on living nature. The main cycle of living organisms is birth and death. Birth and death rates are not only determined by availability of resources but also by competition for these sources, predation and parasitism. Under non-limiting environmental conditions, including nutrients or waste products, there would be unlimited growth and random death or death due to ageing. Under limiting conditions, however, more or less synchronic birth and death cycles can be expected at small spatial scales.

Besides these regular cycles, irregular fluctuations in populations have frequently been observed. Unusual or irregular data may have been ignored in the past, but in the last three decades, ecologists have tried to bring order in these data. Temporal and spatial fluctuations have been analyzed and modeled extensively for mammals, insects, and plants (Virtanen et al., 2002; Turchin et al., 1999), but less frequently for microorganisms (Zvyagintsev and Golimbet.,1983; Zvyagintsev, 1987; Vayenas and Pavlou, 2001).

The population dynamics of macroscopic (and to a lesser extent microscopic) organisms has been described as periodic, wave-like or chaotic using deterministic analytical models (Poole, 1977; Vayenas and Pavlou, 2001). Such patterns might have been ascribed to underlying physical, chemical or nutritional factors in the past, but could be totally explained by inter-species interactions (Hassell et al., 1994; Gilligan 1995). Spatially chaotic patterns can revert to regular, wave-like patterns as a result of a perturbation in the form of ‘pulse’ or ‘wave’ control (Doebeli and Ruxton, 1997). Destabilization of spatially homogeneous equilibria can lead to a new steady state behaving as a standing spatial wave of population abundances. Thus, stable pattern formation can arise after a disturbance in both multiple-species and single-species models (Doebeli and Ruxton, 1998).

Fluctuations in microbial populations

There are many publications in which fluctuations in microbial populations are apparent from the figures, but in most cases, no special attention was paid to these fluctuations. When the authors did investigate the fluctuations and tried to explain them, attempts were sometimes made to relate the observed fluctuations directly to similar fluctuations in environmental conditions or such observations were left without any explanation. The data sets were often limited and, with some exceptions (Aristovskaya et al., 1977), statistical analyses were usually not carried out. When oscillations in microbial populations were intentionally studied, these were mostly studied in the context of predator – prey or host – parasite interactions (Gilligan, 1995; Doebeli and Ruxton, 1998).

Microbial dynamics can be classified into three basic types: (1) seemingly random, (2) apparently fluctuating with changes in environmental factors, (3) more or less regular oscillations. Moreover, fluctuations can dampen and reach a plateau or can continue as long as observations are carried out.

The ability to detect microbial fluctuations depends very much on the frequency and duration of the observations. Regular oscillations become especially noticeable when population densities are measured frequently, for example every day. This thesis will be focused on revealing more or less regular oscillations over time and in space, and on the processes that are involved in regulation of these oscillations. Thus, microbial cell cycles based on circadian rhythms or influenced by seasons will not be considered. Temporal and spatial distributions that are obviously directly the result of similar distributions of physical or chemical factors will also be excluded. Instead, this thesis will be primarily concerned with the temporal and spatial response of microbial communities to a disturbance in the form of an impulse of a carbon source to soil. The main functional groups responding to such a disturbance are fast-growing copiotrophic[1] bacteria, including hydrolytic bacteria, and their predators, protozoa and nematodes. Other members of the microbial community, in particular oligotrophic[1] bacteria, will also be considered because they contribute to the regulation of hydrolytic enzyme production (Semenov, 1991).

Temporal microbial fluctuations in soil

Temporal fluctuations in microbial populations in soil have been observed since the beginning of the 20th century (Cutler et al., 1922; Thornton and Taylor, 1935). Both colony-forming units and direct microscopic counts were determined on field samples. Daily fluctuations in these two kinds of measurements did not exactly coincide in time, and were not clearly related to temperature and soil moisture (Thornton and Taylor, 1935), although seasonal plate counts were sometimes related to soil moisture contents (James and Sutherland, 1940). In the 1960's and 70's, a major effort was made to relate microbial fluctuations to environmental conditions in various ecosystems and climatological zones of the former Soviet Union. Several Russian microbiologists investigated the dynamics of bacterial populations in natural soil, as reviewed by Zvyagintsev and Golimbet (1983). Both direct microscopic counts and colony-forming units were determined daily for one month (Parinkina, 1972; Zykina, 1972; Mikhailova and Nikitina, 1972; Schapova, 1972; Bagdanavichene, 1975; Domracheva, 1975). Irregular fluctuations were observed in all these studies, with periods ranging from 2-7 days, and widely varying amplitudes. Large, composite soil samples were used to minimize effects of spatial heterogeneity (Aristovskaya et al., 1977). The coefficients of variation from day to day were indeed significantly greater than those among composite soil samples per day (tested by the author of this chapter), thus excluding the possibility that daily variations could be due to sampling error. According to Aristovskaya (1980), the amplitudes of the day-to-day fluctuations seemed to be larger and periods shorter at higher latitudes than in Southern regions, but detailed statistical analyses were not carried out. Daily variations in fungal biomass were measured by direct counts in one study (Zvyagintsev and Zaytseva, 1979). The frequency and amplitudes of the fluctuations seemed to depend on the season (Zvyagintsev and Zaytseva, 1979). The reasons for seasonal relationships are quite obvious and don't require additional explanations.

[1] Copiotrophic bacteria grow best at high carbon concentrations; they have a relatively high half-saturation constant (low carbon affinity) and high maximal relative growth rate. Oligotrophic bacteria grow best at low carbon concentrations; they have a low half-saturation constant (high carbon affinity) and low maximal relative growth rate (see chapter 4) (Semenov, 1991). This physiological distinction into trophic groups is to some extent analogous to the ecological subdivision into r- and K-strategists.

Fluctuations were also observed for colony-forming units in natural field soil in microcosms in the lab (Khudyakov, 1958; 1972). Regular oscillations were observed under controlled conditions in the presence of predators (Cutler, 1923; Habte and Alexander, 1975), but similar oscillations were also observed in the absence of predators (Thornton and Taylor, 1935).

More recently, daily fluctuations in microbial biomass (calculated from CO_2 emissions after drying and rewetting), photosynthesis and respiration rates of phytomass (barley), total soil respiration, direct counts of protozoa, soluble organic compounds, and soil moisture and temperature were measured during 35 days (Gorbenko and Panikov, 1989). The authors explained the observed oscillations as the effect of spatial or external factors and did not suggest any hypothesis considering internal interactions of the bacterial community with its substrate.

Besides natural microbial communities, specific microorganisms have also been monitored after introduction into sterilized or natural soil. For example, *Pseudomonas fluorescens* marked with *gfp* and *lux* genes was monitored for 30 days after introduction to soil. Although not enough observations were made for time series analysis, peaks in *gfp* cells and luminescence could be distinguished after 3, 6 and 13 days (Unge et al., 1999).

Spatial microbial fluctuations in soil

Very few papers were found in which spatial distributions of microbial populations were studied in soil. Federle (1986) quantified microbial activity and biomass in relation to soil depth using fluorescein diacetate hydrolytic activity and total phospholipid fatty acids directly extracted from soil. There was a linear correlation between the results of both methods used, and similar fluctuations were observed for both measurements with depth in silty and clayey loams, but there were no clear fluctuations in loam or sandy loam. Similarly, Natsch et al. (1996) showed an oscillatory distribution of a rifampicin resistant *P. fluorescens* strain with soil depth. They attributed the spatial distribution to preferential transport through soil macropores in a grassland and wheat plot.

At a micro-scale, spatial distributions of bacteria were studied in seawater. Clusters of bacteria formed around nutrient patches created by cell lysis and excretion by protozoa. Wave-like concentric rings were formed as a result of chemotaxis in relation to nutrient diffusion. At a very small scale, similar concentric rings might be formed in soil (see also Kozhevin, 1989, under Spatial fluctuations in the rhizosphere).

Temporal fluctuations in the rhizosphere and rhizoplane

Similar to the numerous studies on temporal oscillations of microbial populations in soil, some Russian microbiologists also studied temporal fluctuations in the rhizosphere. Samples were taken every day, during one month, and both colony-forming units and bacterial cells were determined per g of rhizosphere and bulk soil. Daily dynamics of total numbers of microorganisms (direct counts) in rhizosphere soil of the whole root system of two wild plant species showed oscillations (Aristovskaya, 1980; Evdokimova, 1973). Oscillations seemed to have periods of 2 -3 days. There were five or six

major peaks both in the rhizosphere and bulk soil. Temperature and soil moisture were monitored, but correlations between temperature or moisture and the abundance of microorganisms were not calculated (Evdokimova, 1973). There were also hints of oscillations in total numbers of bacteria (direct microscopic counts), actinomycete hyphal length, length of fungal mycelium and fungal spore numbers in the rhizosphere (per g of soil) and rhizoplane (per g of root) of barley and pea over a period of 30 days (Polyanskaya et al., 1994a and b). Similar waves were observed in pots with and without ammonium nitrate fertilizer, except that the amplitudes of the waves were sometimes greater in the presence of ammonium nitrate (Polyanskaya, 1994b). Colony-forming units of various physiological groups of bacteria in rhizosphere soil of the total root system also fluctuated over time, but the peaks of different functional groups did not coincide (Jiang and Sato, 1992).

Several investigations were carried out to determine survival of biological control agents in the rhizosphere after introduction onto the seed. Colony-forming units of *Bacillus* sp. and *Pseudomonas fluorescens* introduced in untreated or pasteurized field soil fluctuated over time, when they were isolated from the spermosphere of wheat seed at 3-5-day intervals for 40 days (Kim et al., 1997). The fluctuations were only slightly less pronounced in the pasteurized than in the natural field soil. Total cell numbers, micro-colony numbers, and colony-forming units of *gfp*-tagged *Pseudomonas fluorescens* also seemed to fluctuate over time in the barley rhizosphere (Normander et al., 1999). Peaks mostly occurred after 7 and 14 days, sometimes after 3 and 10 days.

Spatial fluctuations in the rhizosphere and rhizoplane

Spatial distributions of bacterial populations in the rhizosphere have been studied both in perpendicular direction to the root and in longitudinal direction along the root. Microbial populations in the rhizosphere are generally thought to decrease monotonically with distance from the root surface as a result of the rhizosphere effect (Bazin et al., 1990; Darrah, 1991a). However, using immuno-fluorescence techniques at a microscopic scale, Kozhevin (1989) was the first scientist to observe fluctuating populations of *Rhizobium japonicum* in the soybean rhizosphere with perpendicular increasing distance from the root surface. The pattern of the oscillations shifted over time, and these spatial-temporal distributions were described as a "running wave" moving from the soil to the root surface with a speed of 1-2 microns/hour (Kozhevin, 1989). Colony-forming units of *Bacillus* sp. also seemed to fluctuate with distance from wheat seeds (Kim et al., 1997), but the number of distance classes was too small for any statistical analysis. When bacterial and protozoan populations were determined at 2 mm-interval distances from a root mat on a gauze (Badalucco et al., 1996), there appeared to be waves with two peaks at 0 and 2-4 mm distance from the gauze for both bacteria and protozoa, while there were no waves in unplanted soil. Peaks in total aerobic CFU counts coincided with peaks in total protozoa.

Distribution patterns of microbial populations have also been described in longitudinal direction along the root. Generally, colony-forming units and microbial biomass are more abundant near the root tip, at zones of lateral root formation, and in upper parts of the root system (Schippers and van Vuurde, 1978; van Vuurde and Schippers, 1980). However, the distribution of bacterial populations appeared as wave-like distributions along the roots. Similarly, the distribution of *P. fluorescens* (introduced on

seeds) along wheat roots appeared to be wave-like, although this wave-like distribution was not reproduced in a simulation model (Scott et al., 1995).

Potential explanations for microbial fluctuations

From the literature presented above, it is clear that there are a lot of publications where fluctuations or oscillations in microbial populations can be detected in the data presented for various ecological niches. Among the many papers with fluctuations, there are only relatively few with regular, wave-like oscillations. In most cases, the authors did not pay special attention to the fluctuations and perhaps considered them as random noise. Some authors mentioned the fluctuations without giving explanations (Zvyagintsev, 1987). Others tried to relate the fluctuations in microbial populations to fluctuations in external factors such as weather or soil moisture variables (which will not be discussed further). Four main types of biological explanations can be distinguished for the observed oscillations: 1) substrate – consumer interactions, 2) predator – prey interactions, 3) host – parasite interactions, and 4) internal regulation of microbial density by chemical substances produced by microorganisms.

Substrate – consumer interactions

Substrate availability can be temporarily increased by disturbances such as heavy rainfall, tillage or incorporation of organic materials in soil. Such an impulse of readily consumable substrate into the soil ecosystem may temporarily synchronize biological activities throughout the soil and lead to fluctuations in populations of soil biota (Clarholm, 1981). For example, when cover crop biomass was incorporated into soil, fluctuations in bacterial populations and total soluble organic carbon concentrations were observed over time (van Bruggen and Semenov, 2000). However, the number of observations was too small to prove statistically that the observed fluctuations formed significant waves. Nevertheless, bacterial populations increased very fast after cover crop incorporation in soil, declined precipitously within a few days, then rose and declined again (van Bruggen et al., 1997). The reasons behind the decline in microbial populations were not clear, but could be related to exhaustion of nutrients or other limiting factors. A shortage of nutrients could be followed by an increase in nutrients if microbial necromass would become available again for microbial growth.

Spatial fluctuations in microbial populations have traditionally been ascribed to spatial aggregation of nutrient sources. Patches of organic matter at regular intervals in bulk soil, may give the appearance of oscillating microbial populations in space. Similarly, microbial populations may be more numerous at points of exudation along the roots, as mentioned above (Schippers and van Vuurde, 1978). The spatial distribution of microorganisms along the roots has traditionally been explained in terms of a direct reflection of nutrient availability along the root. However, this is a static point of view. An integrated dynamic interpretation of longitudinal spatial patterns of microbial populations in the rhizosphere (taking root growth into account) has been lacking so far.

Micro-scale oscillations in *Bradyrhizobium* populations perpendicular to the root surface were interpreted as resulting from migration of bacteria in response to exudates (Kozhevin, 1989). Similarly, chemotactic bacterial cells were seen in concentric spheres with alternating higher and lower bacterial densities around the point sources, forming wave-like patterns both in space and over

time at scales of a few um and seconds (Blackburn et al., 1998). Small patches with nutrient resources were thought to be formed as a result of cell lysis or excretion by protozoa. The wave-like patterns were attributed to the combined effects of molecular diffusion of the attractant, congregation and subsequent dispersal of the motile bacteria (Blackburn et al., 1998). At very small scales, similar phenomena could probably occur in soil.

Predator – prey interactions.

Temporal oscillations typically occur where two or more populations interact with a negative feedback mechanism (Gilligan, 1995; Ermentrout and Lewis, 1997). Bacterial growth and death cycles could be due to grazing by predators such as protozoa and nematodes (Clarholm, 1981; Cutler, 1923; Venette et al., 1997). Typically, declines in bacterial biomass are associated with increases in populations of amoebae 1-2 days later (Clarholm, 1981), although increases in amoeba biomass often account for much less than 100% of the decreases in bacterial biomass in soil, in particular in unplanted arable soil (Clarholm, 1981). Protozoa rarely exhaust their prey in nature. In liquid culture, several protozoan species responded to large concentrations of *Rhizobium meliloti* with active feeding and multiplication, reducing the bacterial counts by a factor 100 or less (Danso and Alexander, 1975). Predator and prey populations fluctuated at decreasing amplitudes, indicating that prey population rebounded after being grazed. Such growth and death cycles may result in more or less stationary populations at certain predator-prey ratios, so that prey populations rarely go extinct (Danso and Alexander, 1975). On the other hand, when heat-killed *R. meliloti* cells were presented as prey, these cells were not exhausted either (Danso and Alexander, 1975), suggesting that density-dependent signal molecules among predators may initiate encystment.

Although there are examples of regulation of bacterial oscillations by predators, especially in microcosms, careful inspection of published data on population dynamics of bacteria and their predators often reveal incongruities. Increases in protozoa do not always closely follow increases in bacterial populations. For example, Cutler (1922) sometimes observed simultaneous increases in soil bacteria and protozoa but did not pay attention to these observations. Bacterial and protozoan populations also rose, fell, and rose and fell again at the same distances from a root mat on a gauze within 4 mm distance from the gauze (Badalucco et al., 1996).

Cyclic growth of bacteria and their predators may occur in phase or out of phase in different microniches. At a larger scale, total populations may seem stable (Clarholm, 1981) or show small irregular fluctuations, until a strong disturbance that acts on all microniches such as a large momentary input of nutrients or energy may temporarily synchronize biological activities throughout the soil (Clarholm, 1981), resulting in a sequence of distinguishable peaks in various organisms. At a small scale, predators can release nutrients by lysis of prey so that nutrient patches are formed that act as point sources to which chemotactic bacteria can be attracted in a wave-like fashion (Blackburn et al., 1998).

Host – parasite interactions

While fluctuations in bacterial populations are common in the presence of predators, fluctuations in

microbial populations have also been observed in the absence of predators (Thornton and Taylor, 1935; Porro et al., 1988; van Bruggen et al., 2000). In those cases, parasites could have regulated bacterial populations, although these were generally not measured. When parasite and bacterial populations were measured, oscillations were often observed in both species. For example, populations of *Escherichia coli* exposed to a specific bacteriophage in microcosms fluctuated over time (Bohannan and Lenski, 1999). However, an *E. coli* strain insensitive to this phage also fluctuated over time, albeit to a lesser extent than the sensitive strain, especially at high nutrient concentrations (Bohannan and Lenski, 1999). Thus, host-parasite interactions were not the only reason for the observed wave-like patterns. In a recent field experiment with *Serratia liquefaciens* and two different phages (one introduced), there seemed to be fluctuations in phage titers, but not in the introduced or native *S. liquefaciens* strains (Ashelford et al., 2000). There was a decline in introduced *S. liquefaciens* strains over time, but without clear relationship to the phages monitored. The apparent fluctuations in phage titers indicate that other bacterial strains might have been infected by these phages.

Internal biological factors

Microbial oscillations have also been observed in axenic continuous cultures, for example of yeasts. The period of these oscillations can differ from a few hours up to 50 hours, depending on the conditions (Chen et al., 1990). Oscillations in biomass of budding yeasts are the consequence of internal regulation of substrate metabolism and ethanol production, oxygen availability and consumption, pH, or the natural cycle of individual cells (Porro et al., 1988). Oscillatory behavior of budding yeasts does not occur at high nutrient dilution rates, which result in fermentative conditions (Chen et al., 1990), suggesting that fluctuations in oxygen, at low dilution rates, may be debet to biomass oscillations (Davey et al., 1996). Although fluctuations in oxygen concentrations, substrate and ethanol concentrations and pH are factors external to the cells, changes in these factors are regulated by the cells themselves, resulting in spontaneous oscillations (Porro et al., 1988).

Recently, density dependent reactions of microbial communities have also been ascribed to the production and sensing of signal molecules by members of the community. When the concentration of a signal molecule becomes higher than a threshold value, a synchronous response of a microbial population may ensue, resulting for example in massive death analogous to eukaryotic programmed cell death, apoptosis (Hochman, 1997).

Justification for research on oscillations

It may be clear from the above literature review that not enough attention has been paid to fluctuations in microbial populations from a theoretical point of view except for the oscillations observed in continuous budding yeast cultures (Davey et al., 1996) and bacterial distributions in liquid environments (Blackburn et al., 1998). Even papers with models for microbial dynamics in soil generally did not consider fluctuating populations, except for predator-prey and host-parasite models (Bohannan and Lenski, 1999; Gilligan, 1995). This holds both for models simulating microbial dynamics in the rhizosphere or rhizoplane (Darrah, 1991a, b; Scott et al., 1995) and for models simulating residue decomposition in soil (van Veen et al., 1984; Griffiths and Robinson, 1992; Probert et al., 1996). Yet, the observed data sometimes clearly exhibited fluctuations, especially along roots

(Scott et al, 1995).

Both the extension of a root and incorporation of fresh organic matter into soil constitute a major disturbance to the soil microbial community. In both cases, large amounts of easily consumable substrate become available for fast microbial growth, and wave-like oscillations in microbial populations could develop. In this thesis bacterial dynamics will be investigated in detail, both spatially along wheat roots and temporally after clover and grass incorporation into soil. Two trophic groups of bacteria will be distinguished, copiotrophs and oligotrophs (Semenov, 1991), because they likely occur in succession (van Bruggen and Semenov, 2000). Copiotrophic bacteria are expected to respond first to an infusion of substrate, while oligotrophic bacteria likely follow copiotrophs. Moreover, oligotrophs my facilitate organic matter decomposition by catabolite derepression (Semenov, 1991). Yet, detailed population dynamics of oligotrophic bacteria has rarely been studied in rhizosphere or soil (Hu et al., 1997).

A study of microbial dynamics and distribution along roots is important in connection with a better understanding of potential successes or failures of biological control agents for soil-borne plant pathogens (Scott et al., 1995; van Bruggen et al., 2001 and 2002). It may also be important for understanding substrate utilization, nutrient release and availability to the roots. For the same reasons it would be desirable to construct a simulation model that can accurately mimic microbial dynamics and distribution along roots, as well as the actual substrate utilized by the microbial communities in the rhizosphere.

Short-term fluctuations in microbial populations over time, after incorporation of fresh plant material into soil, could have important consequences for nutrient release from this material. Microbial responses can be very rapid and can lead to considerable mineralization of nitrogen during the first week following tillage or incorporation of plant material into soil (Jackson, 2000; Lundquist et al., 1999; Semenov et al., 2002; van Schöll et al., 1997). Hence, detailed knowledge of the dynamics of primary decomposers of plant material in agricultural soil is important to predict nutrient release and availability for a subsequent crop (McKenney et al., 1995, Wyland et al., 1995). Decomposition of fresh organic matter in soil and the role of the soil microorganisms and fauna in release of NH_4^+ and NO_3^- have been studied extensively (Hassink, 1994; Kuikman and van Veen, 1989; Probert et al., 1996; van Veen et al., 1984; Verhoeff and Brussaard, 1990), but detailed and frequent observations immediately after a disturbance like tillage or incorporation of plant material in soil have rarely been made (Calderón et al., 2000; Calderón et al., 2001; Lundquist et al, 1999). Potential loss of nitrogen from soil constitutes an important reason to study short-term effects of disturbances such as incorporation of fresh organic materials into soil (Semenov et al., 2002).

Objectives

The objectives of this thesis research were:

1. to study spatial distributions of copiotrophic and oligotrophic bacterial populations in the rhizosphere along roots in relation to patterns of soluble carbon concentrations;
2. to develop and validate a simulation model of fluctuating bacterial populations in the rhizosphere based on bacteria-substrate interactions;

3. to study short-term temporal dynamics of bacterial populations and mineral nitrogen release in soil after introduction of fresh organic matter;
4. to characterize nematode communities and dynamics of nematodes and protozoa after introduction of fresh organic matter into soil;
5. to develop a model simulating the dynamics of 3 trophic groups of bacteria (copiotrophic, oligotrophic and hydrolytic) and their predators in response to addition of fresh organic matter to soil and to estimate the impact of each bacterial and microfaunal group to oscillations of the biota within a simplified soil foodweb.

Thesis outline

This research on microbial dynamics started with a comparison of the dynamics of copiotrophic and oligotrophic bacteria (colony-forming units, CFUs) in the rhizosphere along the total length of wheat roots. Water-soluble total organic carbon (TOC) concentrations were also measured, and number of lateral roots were counted, to determine correlations with bacterial populations at various distance lags (chapter 2).

A simulation model "BACWAVE" was written to investigate the possibility to reproduce wave-like dynamics of copiotrophic bacteria in relation to readily utilizable substrate, which was defined to be only a small fraction of TOC and had to be continuously replenished (chapter 3). This model was based on the assumption that wave-like spatial distributions of bacteria along the root resulted from temporal oscillations in bacterial populations in soil after disturbance caused by the passing root tip. This assumption was supported by observed wave-like oscillations in bacterial populations along the path of a moving artificial nutrient source (van Bruggen et al., 2000). Then model "BACWAVE" was expanded to consider population dynamics of oligotrophic bacteria together with copiotrophic bacteria in order to better understand the functioning of the soil heterotrophic bacterial community (chapter 4).

The occurrence of wave-like oscillations over time was proven by harmonics analysis of populations of copiotrophic bacteria (colony-forming units) and total and active bacterial cells (direct microscopic counts) during one month after incorporation of fresh organic matter (clover-grass) into soil (chapter 5). Mineral nitrogen concentrations, redox potential, and pH were also monitored daily. A general hypothesis about the relationships between these characteristics over time was formulated. Total nematode populations and nematode community composition were determined on a daily basis to investigate if predation by nematodes could account for the microbial oscillations observed (chapter 6). To further investigate if predator-prey relations were responsible for microbial oscillations, part of the soil was defaunated. Oscillations in bacterial populations still occurred (chapter 6). To unravel the main reasons for the oscillating behavior of bacteria and their predators and the associated nitrogen release, a dynamic simulation model was formulated, in which microbial growth and death rates were dependent on available substrate content, similar to the model "BACWAVE" (chapter 7). The model simulated wave-like dynamics of three functional groups of bacteria and their predators, namely protozoa and nematodes, and was able to predict release of mineral nitrogen. The thesis will be concluded with a general discussion of the results obtained during the thesis research (chapter 8).

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Chapter 2

Moving waves of bacterial populations and total organic carbon along roots of wheat

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Abstract

To determine if spatial variation in soluble carbon sources along the root coincides with different trophic groups of bacteria, copiotrophic and oligotrophic bacteria were enumerated from bulk soil and rhizosphere samples at 2 cm intervals along wheat roots 2, 3, and 4 weeks after planting. There was a moderate rhizosphere effect in one experiment with soil rich in fresh plant debris, and a very pronounced rhizosphere effect in the second experiment with soil low in organic matter. We obtained wavelike patterns of both trophic groups of bacteria as well as water-soluble total organic carbon (TOC) along the whole root length (60 or 90 cm). TOC concentrations were maximal at the root tip and base and minimal in the middle part of the roots. Oscillations in populations of copiotrophic and oligotrophic bacteria had two maxima close to the root tip and at the root base, or three maxima close to the tip, in the middle section, and at the root base. The location and pattern of the waves in bacterial populations changed progressively from week to week and was not consistently correlated with TOC concentrations or the location of lateral root formation. Thus, the traditional view that patterns in bacterial numbers along the root directly reflect patterns in exudation and rhizodeposition from several fixed sources along the root may not be true. We attributed the observed wavelike patterns in bacterial populations to bacterial growth and death cycles (due to autolysis or grazing by predators). Considering the root tip as a moving nutrient source, temporal oscillations in bacterial populations at any location where the root tip passed would result in moving waves along the root. This change in concept about bacterial populations in the rhizosphere could have significant implications for plant growth promotion and bioremediation.

Introduction

Research on rhizosphere microbiology has had a long history (almost 100 years), but there are still many unresolved questions. One of these concerns the spatial and temporal distribution of microorganisms along the root and the mechanisms underlying this distribution. This area of research is important for various reasons, including a basic understanding of microbial dynamics as well as potential applications in agriculture and bioremediation. Insight in microbial dynamics, competition, and succession in relation to distribution of carbon sources may provide a basis for successful introduction of microorganisms in the rhizosphere, be it for biocontrol of plant pathogens or for bioremediation.

The rhizosphere is the narrow zone around the root where microbial activity is affected by root excretions (Hiltner, 1904, in Curl and Truelove, 1986). The rhizosphere effect can be measured by the ratio or difference between the number of microorganisms near the root compared with that in the bulk soil (Curl and Truelove, 1986). In most cases this ratio is positive, however, there are exceptions (Klemedtsson et al., 1987; Polyanskaya, 1996). It is generally accepted that the rhizosphere effect exists thanks to root exudation of photosynthetically derived carbon sources and sloughing off of root cap cells and older sections of the cortex (Curl and Truelove, 1986; Lynch and Whipps, 1990; Rovira, 1973). The main source of root exudates is the root tip, while emergence of lateral roots also results in carbon losses (McCully and Canny, 1985; McDougall and Rovira, 1970).

The distribution and densities of microorganisms along the root are generally assumed to reflect patterns of exudate concentrations along the root (Schippers and van Vuurde, 1978; van Vuurde and Schippers, 1980). Indeed, the number of microorganisms near the root tip is generally higher than that at other locations along the root (Scott et al., 1995). In the middle and upper regions of the rhizosphere, higher concentrations of microorganisms compared with the bulk soil is commonly attributed to exudation due to lateral root formation (Schippers and van Vuurde, 1978; van Vuurde and Schippers, 1980). Thus, patterns of microbial density along the roots seem to be fully explained by exudation patterns. However, this is a very static concept, while in reality, concentrations of exudates and microbial densities result from many interacting dynamic processes. Unfortunately, most studies on microbial populations in the rhizosphere were conducted with young seedlings disregarding spatial differences (Curl and Truelove, 1986) or distinguishing a limited number of locations in the rhizosphere (Liljeroth et al., 1991; Maloney et al., 1997; Polyanskaya, 1996; Scott et al., 1995). Similarly, data on root exudation mostly concern the total root system, either in nutrient solutions or in C^{14} balance studies (Whipps, 1990). Detailed observations on spatial distributions of microbial populations and soluble carbon compounds along the length of the root have not been published.

The width of the rhizosphere around the root has been estimated to range from 2 to 8 mm (Fritz et al., 1994; Rovira and Davey, 1974). Microbial populations were shown to decrease with distance from the rhizoplane (Rovira and Davey, 1974). The decrease in bacterial concentration away from the root has been modeled as a monotonic decline (Bazin et al., 1990; Darrah, 1991b). However, populations of *Rhizobium japonicum* introduced into the soybean rhizosphere fluctuated with increasing distance from the root surface (Kozhevin, 1989). This spatial distribution was described as a "running wave" (Kozhevin, 1989). In addition to this spatial fluctuation, daily dynamics of total numbers of microorganisms in rhizosphere soil of the total root system showed oscillations that were independent of environmental conditions (Arystovskaya, 1980). This indicates that both spatial and temporal components of rhizosphere microbial communities need to be taken into account to understand microbial distributions in the rhizosphere.

Two distinct methods have been widely used for quantification of microbial populations in the rhizosphere or rhizoplane: first, dilution plating on various media and second, less often used, direct counting using a fluorescent microscope (Norton and Firestone, 1991; Polyanskaya, 1996; van Vuurde and Schippers, 1980). However, microscopic methods lend themselves better to observations of microbial populations in the rhizosplane than to rhizosphere studies. In rhizosphere studies, carbon-rich media have generally been used for enumeration of bacterial populations. While plant roots provide significant amounts of carbon to soil environments, the rhizosphere can still be considered oligotrophic, with estimated carbon concentrations of about 100 µg per gram of dry soil (Darrah, 1991a; Swinnen et al., 1994). As a result, oligotrophic microorganisms may be important components of bacterial communities at rhizosphere sites where carbon is limiting (Maloney et al., 1997). However, only relatively recently have dilute media been employed so that oligotrophic bacteria could be quantified (de Leij et al., 1993; Liljeroth et al., 1991; Maloney et al., 1997; Sugimoto et al., 1990). Using various media, it was shown that fast-growing (mostly copiotrophic) bacteria utilizing many substrates dominated at the root tip, while slow-growing (possinly oligotrophic) bacteria with limited

nutrient-use capacity were more numerous at the root base of wheat (Liljeroth et al., 1991) or lettuce plants (Maloney et al., 1997). However, these studies were limited to few locations along the root.

The detailed distribution and dynamics of trophic groups of microorganisms in the rhizosphere in relation to carbon availability are not well known. Similarly, the relationships between distribution patterns of trophic groups of bacteria have not been investigated extensively. Thus, this research was aimed at understanding rhizosphere microbial dynamics and structure, specifically the spatial and temporal distribution of copiotrophic and oligotrophic bacteria in the rhizosphere of wheat in relation to patterns of soluble carbon concentrations.

Materials and methods

Soil

Two greenhouse experiments were conducted with different soil types. Soil used in the first experiment was collected in January 1997 from an organically managed research plot (15 cm depth) at UC Davis. The soil type is a Reiff sandy loam. The soil contained substantial amounts of corn debris from the previous summer and some plant material from a vetch-oats winter cover crop. The soil was air dried, sieved (7 mm), and stored in garbage bins for two weeks. Nine parts of soil and one part of sterilized sand were mixed with 59 mg of finely ground ammonium nitrate and 92.6 mg of finely ground super phosphate per kg of sand plus soil, corresponding to 60 kg of N/ha and 50 kg of P_2O_5/ha, in a cement mixer. Two subsamples of this soil mix were submitted for analysis at the DANR analytical lab at UC Davis. The soil mix contained 45% sand, 39% silt, and 16% clay, and had a pH (water) of 7.5. Total organic carbon content, total Kjeldahl N, available P (Olsen-P) and exchangeable K of the mix were 1.04%, 1.6%, 38 ppm, and 536 ppm. The cation exchange capacity (CEC) was 22.5 meq/100 g of soil mix.

Soil for the second experiment was collected from a nearby experimental field at UC Davis. The soil type is a coarse loamy, Thermic Mollic Xerofluvent soil. Two-thirds of the soil used had been stored for 3 years in closed garbage cans, and one third was collected from the same field in March 1997, and air-dried. Both soils were sieved (7 mm), and stored in a garbage can for one week. Eight parts of soil and two parts of sterilized sand were mixed with the same amount fertilizers as in first experiment. The soil mix consisted of 60% sand, 27% silt, and 13% clay, pH (water) 7.2. Total organic carbon content, total Kjeldahl N, available P (Olsen-P), exchangeable K, and the CEC of the soil mix were 0.68%, 0.117%, 9 ppm, 235 ppm and 18 meq/100 g, respectively. At the end of each experiment, the average bulk densities at the top and bottom were 1.06 and 1.13 g/cm^3 for the boxes in experiments 1, and 1.05 and 1.20 g/cm^3 in experiment 2.

Experimental setup

Root observation boxes were constructed from PVC irrigation pipes (diameter 19 cm) cut longitudinally into halves, with perforated PVC foam sheets on the bottom. The boxes were 60 and 90 cm tall for experiments 1 and 2, respectively. The front of each box was covered by a plexiglass sheet held in place with semi-permanent silicone glue, and wooden boards on both sides of the box, held

together with bolts and nuts.

A thin layer of autoclaved gravel was placed at the bottom of 10 boxes in the first experiment and 14 boxes in the second experiment. The boxes were then filled with the soil mix described above. Seeds of wheat (*Triticum durum* Desf., cultivar UC 1041) were pre-germinated for 17 hours on moist sterile filter paper. Two germinated seeds with equal radicle length (1 mm) were planted in each box at a depth of 1 cm. The plexiglass was covered by aluminum foil to maintain darkness, and the pots were tilted at an angle of 60^o in experiment 1 and 55^o in experiment 2 so that roots grew against the plexiglass.

The irrigation system consisted of circular drip irrigation tubing connected by transverse tubes like the runners of a ladder, with one hole in each of the sections, placed vertically in each box. Irrigation water was applied 3 times per day for one minute. Soil water potential varied from about -0.9 bar to -0.2 bar in experiment 1 and from -0.7 bar to -0.2 bar in experiment 2, increasing with depth. However, there were no significant correlations between soil moisture and bacterial populations or total water-soluble organic carbon in either experiment. The minimum and maximum temperatures in the greenhouse were 15-17 ^{o}C and 17-25 ^{o}C during experiment 1, and 12-16 ^{o}C and 20-26 ^{o}C during experiment 2. The first experiment was conducted under natural light supplemented with multivapor lamps, with a 14 hr day length. Plants in the second experiment received only natural light through whitewashed greenhouse glass, with an average day length of 13 hr.

Root growth

Root growth was monitored weekly by tracing new growth with various colored markers onto acetate sheets. The lengths of the colored tracings over the main roots which were used for sampling were measured using a map tracer. Average growth rates per box were calculated for each week.

Root and soil sampling procedures

Two, three, and four weeks after planting, the plexiglass front panels were removed from the boxes to be sampled. Two (experiment 1) or three (experiment 2) boxes were sampled per week for dilution plating, total organic carbon (TOC) analyses and soil moisture. Samples were collected starting from the root tips. Samples for dilution plating and carbon analyses were taken alternately. Two-cm long root sections together with rhizosphere soil were carefully lifted and cut off for dilution plating every 4 cm (2 cm between sections). Lateral roots were counted and removed from the main root sections. Root sections from two main roots per box at the same distance from the root tip were pooled. The rhizosphere was considered to be the root surface and adhering soil after root sections were gently shaken to remove bulk soil. Root sections (2 cm) immediately above every other section sampled for dilution plating (every 8 cm) were carefully stripped from attached soil with flamed forceps for analysis of TOC in the rhizosphere. Again, the numbers of lateral roots per root section were counted and rhizosphere samples from two roots were pooled for similar distances from the tip. Small bulk soil samples at 3-5 cm distance from the main roots and at similar distances from the root tip as the root sections were also collected for quantification of bacteria and TOC. Separate bulk soil samples were collected for determination of soil moisture content. All samples were immediately placed on ice and

transported to the lab in a cool box.

Total soluble carbon

Extraction of water-soluble total organic carbon from soil samples was carried out in a cold room (5 ^{o}C) as described by Maloney et al. (1997). The suspension was centrifuged and the supernatant filtered to remove the microbial component (Maloney et al., 1997). Extracts were frozen (-20 ^{o}C) until analyzed for dissolved C using a Shimadzu total organic carbon analyzer (Shimadzu TOC-5000/5050, Shimadzu Scientific Instruments, Inc., Columbia, MD).

Dilution plating

Rhizosphere and bulk soil samples were suspended in sterile deionized water, vortexed, sonicated for 5 minutes, diluted in 10-fold serial dilutions, plated on high and low nutrient media in triplicate, and incubated at 23 ^{o}C.

The high nutrient medium, for isolation of copiotrophic bacteria, contained 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KNO_3, 1.3 g $K_2HPO_4 \cdot 3H_2O$, 0.06 g $Ca(NO_3)_2 \cdot 4H_2O$, 2.5 g glucose, 0.2 g enzymatic casein hydrolysate, and 15.0 g Bacto Agar per liter and 100 ppm sterile cycloheximide. The total amount of carbon was estimated at 1000 mg/liter of medium. The medium for isolation of oligotrophic bacteria was similar to the one for copiotrophic bacteria, but 100-fold diluted and contained Agar Noble (Difco Laboratories, Detroit, MI) instead of Bacto agar. For the second experiment, a third medium was used that contained the same salt and casein hydrolysate concentrations as the first medium, but 25.0 g of glucose per liter. Bacteria isolated on this medium will be referred to as copiotrophic 10. Bacterial colonies on the two carbon rich media were counted after 60 h and on the carbon poor medium after 3 weeks. Actinomycetes were distinguished from other bacterial colonies based on morphology of colonies (confirmed through a compound microscope).

Statistical analyses

All data were checked for normality and log-normality, since bacterial populations had been shown to be log-normally distributed in the rhizosphere (Loper et al., 1984). When all data, including concentrations of total water-soluble organic carbon (TOC) proved to be log-normally distributed, analyses of variance were performed on log-transformed data, using week of sampling, location (rhizosphere versus bulk soil), and distance from the root tip as sources of variation (SAS Institute, Inc., Cary, NC). Next, geometric means of bacterial populations and TOC concentrations were calculated for each distance from the root tip and for all distances combined (for comparison of rhizosphere and bulk soil data). Standard deviations of geometric means were calculated from coefficients of variation based on log-transformed data (Snedecor and Cochran, 1979).

Harmonical (Fourier) analysis was performed, initially on rhizosphere and bulk soil data separately, and subsequently on differences of geometric means in the rhizosphere and bulk soil for each sampling time. Because our interest was in rhizosphere effects, only results of the last analyses will be presented. For spectral analysis, observation points need to be equidistant. Since there were some irregularities in the data set due to missing observations (less than 5%), a cubic spline interpolation

procedure was used to estimate values at these locations. There were no significant (P=0.05) differences between means and variances of the original and interpolated data sets. After comparing the original and interpolated data sets, a few statistical outliers (less than 1%) in the original data sets, were replaced by interpolated data. The data set was smoothed using a 3-point window (Shumway, 1988). Fourier analysis was conducted according to Jenkins and Watts (1968) with modifications according to Gorbenko and Kryshev (1985). Modifications consisted of changing the order of some of the calculations. Significant harmonics were detected comparing the periodogram value and total variance of the series. Harmonics were included in the results section when the calculated F value exceeded the critical F-value at 90% probability. Harmonics that were significant at the 5% level are also denoted. Finally, auto- and cross correlations between bacterial populations and TOC concentrations were calculated (Snedecor and Cochran, 1979).

Results

The growth rates of the roots visible against the plexiglass front panel were quite high. The average growth rates were 2.1 cm/day during the first week, 4.2 cm/day in the second week, 4.0 cm/day in the third week, and 3.0 cm/day in the fourth week. There were only slight differences between experiments.

Bacterial populations and total water-soluble organic carbon (TOC) were greater in the first experiment when a nutrient-rich soil mix was used than in the second experiment when a more sandy soil mix was used with less organic matter, total nitrogen and available phosphorous than in the first experiment (Table 1). TOC concentrations in the bulk soil ranged from 139 to 273 µg C/g dry soil in the first experiment and from 16 to 21 µg C/g dry soil in the second experiment. TOC concentrations were significantly higher in the rhizosphere than in the bulk soil in both experiments (P<0.01). Concentrations of TOC in the rhizosphere increased with plant age to 366 µg C/g dry soil (197 µg C/g dry soil above the bulk soil concentration) in the first experiment and 139 µg C/g dry soil (120 µg C/g dry soil above the bulk soil concentration) in the second experiment, both in week 4. At that time, the cortex of the upper 20 cm of the main roots was visibly decaying. Populations of copiotrophic bacteria (both on 1000 ppm and 10,000 ppm carbon media, called copiotrophs 1 and copiotrophs 10, respectively) were significantly (P<0.01) higher in rhizosphere samples than in bulk soil samples in both experiments. Populations of oligotrophic bacteria were higher in rhizosphere samples than in bulk soil samples only in experiment 2 (P<0.01). Differences in bacterial populations among sampling times did not reflect differences in TOC in the first experiment. In the second experiment, rhizosphere populations of all trophic groups were higher in week 4, when the TOC concentrations were highest. In particular, copiotrophic bacteria isolated on 10,000 ppm carbon medium were more numerous than the other trophic groups, indicating that these copiotrophic bacteria were dominant at that time. Actinomycetes constituted 40% of the oligotrophic bacteria in experiment 1 and 23% in experiment 2. Actinomycetes were not isolated on carbon rich media.

In both experiments, concentrations of bacteria and TOC fluctuated along the roots in relation to distance from the root tip (Fig. 1). Fourier analysis revealed significant harmonics for all trophic groups in the rhizosphere and only sporadic harmonics in the bulk soil. Because of our interest in

rhizosphere effects only differences between geometric means of rhizosphere and bulk soil populations and TOC concentrations are presented. The fluctuations were similar for both copiotrophic groups (copiotrophs 1 and copiotrophs 10), so that only data for copiotrophs 1 are presented in Fig. 1. Similarly, fluctuations in actinomycetes reflected those of all oligotrophic bacteria, and are not presented.

Table 1. Geometric means of copiotrophic and oligotrophic bacteria and of total water-soluble organic carbon (TOC) in the rhizosphere of wheat plants and corresponding bulk soil locations

Week	Location	Copiotrophs 1[a] ($1 \cdot 10^7$ CFUs per g dry soil	Copiotrophs 10[a] ($1 \cdot 10^7$ CFUs per g dry soil)	Oligotrophs ($1 \cdot 10^7$ CFUs per g dry soil)	TOC (µg C/g dry soil)
	Experiment I				
2	Rhizosphere	10.2 (4.8)[b]	-[c]	3.4 (2.2)	288.0 (311.5)
	Bulk soil	8.3 (5.0)	-	3.5 (2.3)	273.5 (225.3)
3	Rhizosphere	7.0 (1.8)	-	2.7 (3.2)	320.8 (347.8)
	Bulk soil	4.8 (1.6)	-	1.2 (1.3)	139.1 (47.8)
4	Rhizosphere	6.8 (2.1)	-	3.4 (4.1)	366.5 (297.8)
	Bulk soil	5.7 (1.8)	-	2.7 (2.8)	169.8 (88.6)
	Experiment II				
2	Rhizosphere	1.9 (1.1)	1.3 (1.1)	0.6 (0.5)	34.0 (26.4)
	Bulk soil	0.2 (0.04)	0.1 (0.04)	0.04 (0.05)	16.2 (7.1)
3	Rhizosphere	1.7 (2.2)	1.2 (1.3)	0.7 (1.0)	77.2 (73.4)
	Bulk soil	0.2 (0.07)	0.1 (0.04)	0.03 (0.04)	21.5 (13.7)
4	Rhizosphere	2.8 (1.8)	3.7 (3.2)	1.5 (1.1)	139.4 (201.9)
	Bulk soil	0.2 (0.07)	2.2 (0.7)	0.05 (0.03)	19.8 (16.9)

[a] Copiotrophic bacteria 1 and 10 are defined in the Methods section.

[b] In brackets are the standard deviations for all observations along the root.

[c] No data

In the first experiment, the second harmonics were significant for copiotrophic bacteria (copiotrophs 1) 2 and weeks after planting, and the first harmonic 4 weeks after planting (Table 2). For oligotrophic bacteria, the first and second harmonics were significant three and four weeks after planting, respectively. The minima for both trophic groups were sometimes negative, despite the overall significant rhizosphere effects. The harmonics explained between 39% and 59% of the overall variability.

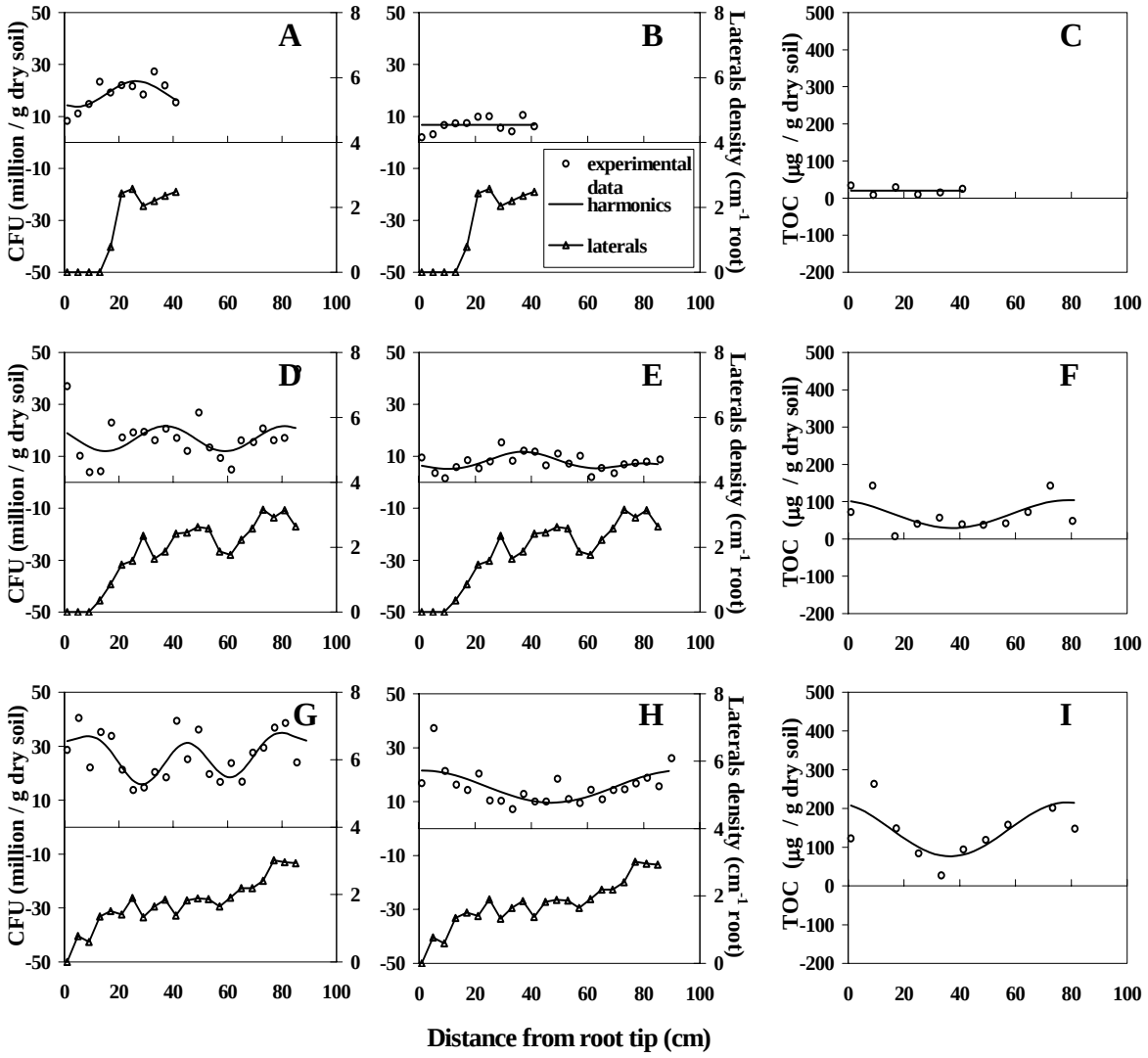

Figure 1. Colony-forming units (CFUs) of copiotrophic (A,D,G) and oligotrophic (B,E,H) bacteria, and concentration of total water-soluble organic carbon (TOC) (C,F,I) in the rhizosphere at various distances from the root tip 2 (A,B,C), 3 (D,E,F) and 4 (G,H,I) weeks after planting. Points are differences of geometric means (n = 3) between rhizosphere and bulk soil; lines are predicted values from harmonics analysis. Numbers of lateral roots per cm are indicated below the X-axis.

In the second experiment, significant harmonics were again detected for copiotrophs 1 in all observation periods, and for copiotrophs 10, 3 and 4 weeks after planting. Oscillations for oligotrophs were also significant at those sampling times. No significant harmonics were detected two weeks after planting, although a wave-like trend could be detected visually (Fig. 1B). Between 45% and 90% of the overall variance was attributed to significant oscillations for all trophic groups. Differences between rhizosphere and bulk soil populations were never negative in the second experiment.

Table 2. Characteristics of harmonics significant at 0.1 detected by Fourier analysis for distributions of copiotrophic and oligotrophic bacteria populations (CFU/g dry soil) and total water-soluble organic carbon (TOC, μg/g dry soil) along wheat roots.

Week	Variable	Harmonics Number	Amplitude	Phase cm	Period cm	Frequency cm^{-1}	Contribution to variance %	*F*-estimated	*F*-critical at 0.1 s.l.	*n*
	Experiment I									
2	Copiotrophs 1[a]	2	20,227,078	10.26	24	0.04	58.9	3.24	2.86	12
3	Copiotrophs 1	2	12,492,528	9.52	34	0.02	45.1	3.61	2.67	17
	Oligotrophs	1	17,315,062	-3.11	68	0.01	45.9	3.68	2.67	17
4	Copiotrophs 1	1	14,206,786	-3.88	64	0.01	57.3	4.30[c]	2.70	16
	Oligotrophs	2	16,675,247	18.94	32	0.03	38.6	2.89	2.70	16
	Experiment II									
2	Copiotrophs 1	1	4,933,151	29.44	44	0.02	60.4	3.02	2.92	11
3	TOC	1	36.7	0.22	88	0.01	80.4	4.02	2.92	11
	Copiotrophs 1	2	4,852,659	-3.87	44	0.02	44.5	4.67[c]	2.57	22
	Copiotrophs 10[b]	2	3,598,445	-4.81	44	0.02	47.4	4.97[c]	2.57	22
	Copiotrophs 10	3	2,699,619	-5.22	29	0.03	26.7	2.80	2.57	22
	Oligotrophs	1	2,230,463	40.7	88	0.01	44.1	4.63[c]	2.57	22
	Oligotrophs	2	1,955,039	-4.14	44	0.02	33.9	3.56[c]	2.57	22
4	TOC	1	62.1	-0.48	88	0.01	74.5	3.73	2.92	11
	Copiotrophs 1	1	5,376,058	-1.78	92	0.01	35.9	3.95[c]	2.56	23
	Copiotrophs 1	2	4,436,842	2.06	46	0.02	24.5	2.69	2.56	23
	Copiotrophs 1	3	4,928,641	17.06	31	0.03	30.2	3.32	2.56	23
	Copiotrophs 10	1	8,750,908	3.58	92	0.01	55.8	6.14[c]	2.56	23
	Oligotrophs	1	5,976,304	4.1	92	0.01	64.9	7.14[c]	2.56	23

[a] Copiotrophs 1 on 1,000 ppm C media; no isolates made on 10,000 ppm media in experiment I.

[b] Copiotrophs 10 isolated on 10,000 ppm C media.

[c] Significant at 0.05 s.l.

In both experiments, copiotrophic bacteria had a maximum close to the root tip (except after two weeks in the second experiment; Fig. 1A). Two and three weeks after planting, there was one additional maximum 40 or 60 cm from the root tip in the first experiment. There were two additional maxima 40 and 80 cm from the root tip after three weeks, and 50 and 80 cm from the root tip after four weeks in the second experiment (Fig. 1D and G). Oligotrophic bacteria oscillated more or less in a similar fashion as copiotrophs after two and three weeks (not after four weeks), but the amplitudes were smaller (Fig. 1; Table 2, amplitudes of several harmonics for the same data need to be combined). The amplitudes were generally larger in the first than in the second experiment. The periods of the harmonics were similar for all trophic groups, except for longer periods for oligotrophs 3 weeks after planting (Table 2).

TOC concentrations also fluctuated along the roots. Visually, the fluctuations were very similar in both experiments. However, the number of observations along the root was too low in experiment 1 to detect significant oscillations. Significant harmonics were detected three and four weeks after planting in experiment 2 (Fig. 1C, F, and I). Maxima in TOC (differences between rhizosphere and bulk soil) were obtained close to the root tip and at the root base in both cases, while a minimum occurred in the middle section of the root. This minimum coincided with a maximum in copiotrophic bacteria. Since concentrations in TOC must be viewed as differences between production and consumption of organic compounds, the observed patterns suggest that production outweighed consumption more at the root tip and base than in the middle section.

The number of lateral roots counted on each sampled root section increased from 0 at the root tip to maximally 5-7 laterals per section close to the root base (Fig. 1A, D, and G). The increase in laterals was not monotonous, especially in the third week of the second experiment. Sections with higher lateral root density were alternated with sections with lower root density, although the root density did not return to 0 laterals per cm in the middle and upper portions of the root. This was different for the lateral roots visible against the plexiglass wall. Areas with visible lateral roots were alternated with areas without lateral roots. Areas with visible lateral roots coincided with sections that had peaks in laterals on all sides (Fig. 1; Table 3), indicating that only a small fraction of the laterals were growing against the plexiglass wall.

Cross correlation coefficients between bacterial populations or TOC on the one hand and numbers of laterals per root section on the other were never significant at a 0 lag. Only two significant cross correlations were observed between copiotrophic bacteria (copiotrophs 1) and laterals at lags of 5 and -2 (equivalent to 20 and -8 cm), and only one significant cross correlation between oligotrophic bacteria and laterals at lag 4 (equivalent to 16 cm). The inconsistent lags for cross correlations indicates that these correlations were merely coincidental. However, when we measured the median distances from the root tip to areas with visible lateral roots against the plexiglass wall and to the maxima and minima in bacterial populations and TOC concentrations, the maxima in copiotrophs and oligotrophs seemed to coincide with the middle regions with lateral roots in the second experiment (Table 3). Maxima of TOC did not coincide with regions of visible lateral root emergence. In the first experiment, neither bacterial populations nor TOC coincided with regions of visible lateral roots.

Table 3. Average distances to the root tip of zones with visible lateral roots and to maxima and minima in CFUs/g dry soil of copiotrophic and oligotrophic bacteria and total soluble organic carbon (TOC, µg /g dry soil) along the main roots of wheat.

Week	Distance of laterals to tip, cm	Distance to tip (cm):					
		Copiotrophs		Oligotrophs		TOC	
		Max	Min	Max	Min	Max	Min
	Experiment I						
2	41.5	10.3	17.0	34.0	-	26.8	-
		36.3	41.0				
3	21.3	9.5	26.5		32.4	7.5	35.7
	43.4	43.5	60.5	64.8		71.5	
	57.0						
4	27.0		26.2	18.0	34.0	1.7	28.0
	46.0			50.0			
	55.0	60.1				57.7	
	Experiment II						
2	29.2	29.4	-	25.0	33.0	19.3	30.3
3	41.2	40.1	10.0	40.0	5.0	2.6	40.6
	72.9	84.3	62.0	80.0	61.0	78.6	
4	22.8	10.0	28.0	4.1		0	
	56.1	45.5	60.0		50.1		39.6
	80.6	79.0		96.1		81.6	

Cross correlations between copiotrophic (copiotrophs 1 and 10) and oligotrophic bacteria were often significant (P=0.05) at lag 0 (Table 4). There were no significant cross correlations between copiotrophic bacteria and TOC, except for one occasion when peaks of copiotrophic bacteria (copiotrophs 10) coincided with peaks in TOC (r=0.56) (Table 4). Cross correlations between oligotrophic bacteria and TOC were inconsistent (r=-0.71 after 3 weeks, r=0.59 after 4 weeks).

Discussion

The magnitude of the rhizosphere effect was closely related to the organic matter and inorganic nutrient contents of the soil (Table 1). The rhizosphere effect was always positive and strong in the soil low in organic matter, while the rhizosphere effect was less pronounced and at some locations negative in soil high in organic matter. The absence of a rhizosphere effect in some other studies

Table 4. Cross-correlation coefficients (CCC) between number of lateral roots per unit root length, bacteria (copiotroph and oligotroph, CFU/g dry soil) and total water-soluble organic carbon (TOC, µg/g dry soil) along the main root of wheat plants at 0.05 significance level.

Week	Variable	Variable					
		Copiotrophs 10[a]		Copiotrophs 1[b]		Oligotrophs	
		Lag[c]	CCC	Lag[c]	CCC	Lag[c]	CCC
	Experiment I						
2	Laterals	-[d]	-	-	-	-	-
	Copiotrophs	-	-	-	-	0	0.81
3	Laterals			-	-	4	0.57
	Copiotrophs	-	-	-	-	0	0.78
4	Laterals	-	-	5	0.66	-	-
	Experiment II						
2	Laterals	-	-	-2	0.71	-	-
	Copiotrophs 10	-	-	-	-	0	0.69
3	Laterals	-		-	-	-	-
	Copiotrophs1	-	-	-	-	0	0.51
	Copiotrophs 10	-	-	-	-	0	0.62
	TOC	-	-	-	-	0	-0.71
4	Laterals			-	-	-	-
	Copiotrophs1	-	-	-	-	0	0.58
	Copiotrophs10	-	-	-	-	0	0.72
	Copiotrophs10	-	-	-	-	2	0.42
	TOC	0	0.56	-	-	-	0.59

[a] Isolated on medium with 10,000 ppm C.

[b] Isolated on medium with 1,000 ppm C.

[c] 1 lag = 4 cm.

[d] Correlation not significant

(Klemedtsson et al., 1987) may be partially explained by the location along the root where samples were collected. Despite the local absence of a detectable rhizosphere effect, there likely is always a rhizosphere effect which may be temporarily masked (Gilbert et al., 1994) by relatively high microbial activity in the surrounding bulk soil in combination with growth and death phases of fast-growing microorganisms in the rhizosphere. Thus, the external manifestation of a rhizosphere effect can not always be observed, particularly in rich soil.

This interpretation is plausible in light of the observed waves in population densities of both

copiotrophic and oligotrophic bacteria along the roots. Harmonics analysis confirmed that there were significant waves in rhizosphere bacterial populations 2, 3 and 4 weeks after seeding in both soils. However, in the soil with low organic matter (experiment 2) the harmonics were often significant at 0.05 level, while in experiment 1, most harmonics were significant only at 0.1 level. So, the significance of both the overall rhizosphere effect and the spatial distribution of bacterial populations were dependent on the quality of the soil.

There are hints of wave-like patterns in the graphs of several publications depicting microbial populations along roots. For example, the distribution of microbial populations along roots (either as CFUs in the rhizosphere or direct counts on the roots) observed by Schippers and van Vuurde (1978), van Vuurde and Schippers (1980), and Kim et al. (1997) were very close to wave-like patterns but were not recognized as such. Scott et al. (1995) described a simple monotonic decline for populations of introduced bioluminescent *Pseudomonas fluorescens* from the tip to the base of wheat roots. This trend was confirmed by experimental data for young seedlings but not for older seedlings where microbial populations seemed to fluctuate along the roots. Again, this oscillation was not recognized as such by the authors, but was attributed to random variation. There are several reasons why the authors did not recognize wavelike patterns earlier. First, the depth of the containers or root observation boxes was generally limited, and thus the number of root sections investigated was also limited. Second, very limited or no statistical analysis was used to analyze the data. Third, the traditional concept that microbial population patterns were a direct reflection of exudation patterns prevented researchers from recognizing dynamic wavelike patterns. However, several other authors did recognize the presence of wave-like patterns for populations of various organisms in space (Davidson et al., 1997; Kot et al., 1996; Kozhevin, 1989). Of particular interest is Kozhevin's study (1989) where he described oscillations in *Rhizobium japonicum* populations emanating from the main roots of soybean in the direction of the bulk soil as “running waves”. Considering the horizontal waves described by Kozhevin (1989) and the vertical waves observed by us, we suggest that the development of microbial populations in the rhizosphere has a wave-lake character in both directions and may be a general phenomenon.

In our investigation we demonstrated significant oscillations for different trophic groups of bacteria along the roots of wheat. Cross correlation coefficients for these groups were sometimes quite high at lags of 0 or 4 cm. These high correlations may be partially due to the considerable overlap in the bacteria of different trophic groups able to grow on the media used (Maloney et al., 1997). Nevertheless, the phases and frequencies of the waves were slightly different for the trophic groups, in accordance with their characteristic place in succession (Hu and van Bruggen, 1997) and differences in their growth rates. Moreover, the amplitude of the waves was greater for copiotrophs than for oligotrophs, especially near root tip. Again, this difference was typical for the difference in biology between these trophic groups (Semenov et al., 1997).

Significant wave-like patterns were also detected for water-soluble total organic carbon (TOC) in the rhizosphere of wheat in both of our experiments (Fig. 1). However, there were only two peaks, one near the root tip and the other at the root base. It is well known, that the root tip and root base are

locations of high exudation and rhizodeposition of substances used as substrates by different trophic groups of microorganisms (Liljeroth et al., 1991). TOC reflects mainly residual organic compounds that were either not yet utilized or could not be metabolized. The minimum of TOC in the middle part of the root coincided with maxima of both copiotrophs and oligotrophs. This indicates that consumption of organic compounds released from the middle section of the root was equal to production. Alternatively, release of organic compounds was low in that region of the root. At the root tip and base, on the other hand, the release of organic substances was in excess of consumption by microorganisms. The reasons for this excess may have been different. At the tip, a lag in microbial growth can be expected since microorganisms in bulk soil are largely in a dormant state. At the root base, overproduction was largely due to decay of the cortex.

The distribution patterns of bacteria along the roots maintained their structure over time (from 2 to 4 weeks) but moved in relation to a fixed location, namely the root base (Fig. 2). Hence, we truly

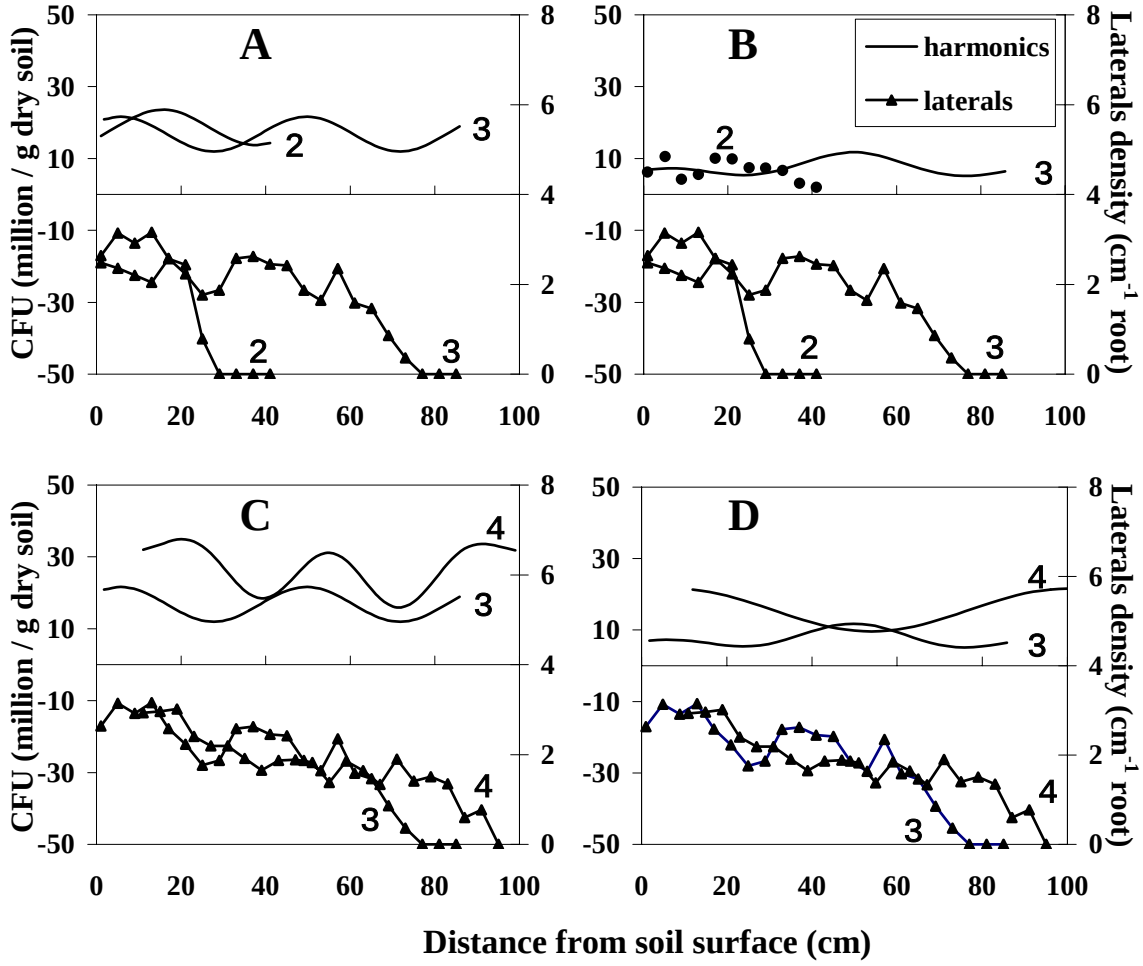

Figure 2. Predicted values of colony-forming units (CFUs) of copiotrophic (A,C) and oligotrophic (B,D) bacteria in the rhizosphere and number of lateral roots per cm at various distances from the root base 2, 3, and 4 weeks after planting. Lines are predicted values from harmonics analysis; points are experimental data when no significant harmonics were detected.

observed 'traveling waves'. Traveling waves were produced in models for fungal growth (Davidson et al., 1997) as well as density-dependent dispersion of populations (Gilligan, 1995; Maini and Sanchez-Garduno, 1993). So, wave-like development of populations of microorganisms and microbial communities may be universal in nature. There are several potential explanations for the development and maintenance of this spatial distribution in space and over time. According to the traditional concept of microbial populations in the rhizosphere, the oscillations could be a reflection of carbon release in zones at various distances from the root tip. However, we propose a very different explanation for the patterns observed. Periodic growth and death cycles after passage of a single nutrient source (root tip) could also result in the observed spatial patterns, without the need for additional nutrient sources.

First, let us explore the traditional view. The root tip, branching points, and root base are generally considered as the main sources of exudates and sloughed-off cells. Schippers and van Vuurde (1978) linked two maxima in bacterial numbers along wheat roots to exudates originating from the root elongation zone just behind the root tip and from areas with lateral branch formation. A slight shift in location of these maxima and root tip and lateral branch locations was attributed to root growth and the time needed for microbial multiplication. In our experiments, the density of lateral roots per root section correlated only occasionally with maxima in bacterial densities at those locations or at various lags (Table 4). Moreover, if locations of exudation and rhizodeposition were the sole explanation for the observed patterns in microbial distribution, the patterns would remain more or less constant over time since lateral roots do not change position (although additional roots are produced). Microbial populations would be more or less static after an initial increase. This would only be the case if we assume that overall death rates did not exceed growth rates, as was assumed by Scott et al. (1995). However, instead of constant waves, we observed moving waves over time, indicating that microbial death sometimes exceeds growth.

Moving waves can result from temporal oscillations in bacterial populations at any location in soil where a moving nutrient source (i.e., the root tip) passes through. Temporal oscillations typically occur where two or more populations interact with a negative feedback mechanism (Gilligan, 1995; Kozhevin, 1989). Bacterial growth and death cycles could be due to grazing by predators such as protozoa and nematodes. The importance of these organisms for regulation of bacterial numbers in bulk soil and in the rhizosphere was emphasized in several publications (Clarholm, 1981; Venette et al., 1997). However, grazing would result in a dramatic loss of carbon sources readily available for renewed microbial growth, and overall microbial populations would decline over time (assuming that no additional nutrient sources would be available in the middle sections of the roots after emergence of the lateral roots). We observed a decrease in copiotroph bacteria between 2 and 4 weeks after planting in the first experiment but not in the second (Fig. 1, Table 1). Moreover, considering the generation times of protozoa and nematodes and the growth rate of the wheat roots in our experiment, the periods of the waves would have been longer than observed if they had been primarily due to growth and death by predators.

A more plausible explanation for the wave-like distribution of bacterial populations along a root is the periodical growth, death, and autolysis of micro-organisms, reflecting successional events in the rhizosphere. Besides root exudates and rhizodeposits from the root tip, lateral branch points, and older root sections, there is an important additional source of substrate for bacterial growth, namely microbial necromass. We envision the following scenario. Before root tips reach a location in the bulk soil, most microorganisms are in a dormant state. Microorganisms respond to a passing root after a lag period, when the concentration of metabolizable substances in the rhizosphere exceeds a threshold concentration (in particular for copiotrophs). Exudates are absorbed and metabolized initially by copiotrophic bacteria resulting in a fast increase of these bacteria and a slower increase in oligotrophs (Hu and van Bruggen, 1997; van Bruggen et al., 1997), so that nutrients are temporarily depleted. This leads to autolysis of the copiotrophs and release of nutrients initiating a second cycle of growth and possibly death. Meanwhile, the root tip continues to extend into deeper soil layers releasing exudates along its path. The growth and death cycles of microbes at any location where the root tip passed result in a wave-like pattern of microbial populations along the root on individual sampling dates, and 'traveling waves' over time. This scenario could only be true if death rates were dependent on nutrient concentration, and if secondary points of exudation were minor compared to the root tip. The minimal concentrations of TOC in the mid-root sections (with abundant lateral roots) in these experiments support this hypothesis.

The proposed conceptual change in the understanding of the spatio-temporal distribution of microorganisms in the rhizosphere can have profound effect in various areas of agriculture and bioremediation. Introduction of microorganisms into the soil environment, and particularly inoculation of roots with useful plant-growth-promoting rhizo-bacteria (PGPR), biocontrol agents, or pollution-degrading microorganisms, has not provided appreciable results so far. One of the basic reasons may be that workers selecting and applying these beneficial organisms have not taken into account the cyclic development of both introduced and endemic communities, including, for example, plant pathogens. For successful application of beneficial microorganisms it is necessary to know not only the biological cycles of the endemic and introduced organisms, but also the spatio-temporal cycles of their interaction or their ‘phase condition’. Selection of beneficial microorganisms needs to be geared towards organisms that multiply at the right location and the right time to be effective. These findings provide a foundation for selection of microorganisms that are active at certain locations in the rhizosphere at a certain time after seeding. The optimal location and time for introduction of selected microorganisms could also be predicted so that introduced organisms have a chance to compete with the endemic microbial communities, for example, at a certain depth below the seed line, so that competition is minimized.

The three potential explanations for the occurrence of wave-like patterns in the rhizosphere need not be mutually exclusive. Neither root growth, nor nutrient release, nor microbial or faunal populations in the rhizosphere are static, and various combinations of factors could result in the wavelike patterns we described. Further investigations of the dynamic processes in the rhizosphere are needed to elucidate the complex mechanisms leading to the observed moving waves. We are currently investigating the role of lateral roots in creation and maintenance of maxima in bacterial populations among the main

roots in controlled experiments. We are also working on a rhizosphere simulation model where exudation patterns and variable growth and death rates of different trophic groups of microorganisms are taken into account.

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Chapter 3

"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 wave-like 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 wave-like 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, which 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 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 impulse 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 wave-like 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 (Dandurand et al., 1997; Kim et al., 1997; Polyanskaya, 1996; Schippers and van Vuurde, 1978; Scott et al., 1995; Semenov et al., 1999; van Vuurde and Schippers, 1980). High microbial densities have generally been observed close to the root tip and in middle and upper sections of the roots (Schippers and van Vuurde, 1978; Semenov et al., 1999; van Vuurde and Schippers, 1980). Similarly, distribution patterns of photosynthetically derived carbon compounds in the rhizosphere have been widely studied, usually using $^{14}CO_2$ (Norton et al., 1990; Rovira, 1973; Whipps, 1990). 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 (McCully and Canny, 1985; McDougall and Rovira, 1970). Thus, patterns in microbial density have generally been attributed to corresponding points of exudation, taking root growth into account (Lynch and Whipps, 1990; Rovira, 1973; Schippers and van Vuurde, 1978).

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 (Semenov et al., 1999). 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 (Semenov et al., 1999). Based on spatial shifts in the phases of the waves as the plants grew older, we considered the wave-like patterns as moving waves along the roots. The patterns observed by van Vuurde and Schippers (1979; 1980) could also be interpreted as waves.

We hypothesized the following mechanism underlying the observed wave-like 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 (Semenov, 1991) a second cycle of growth and death is initiated. Meanwhile the root tip continues to extend into deeper soil layers releasing exudates along its path. The growth and death cycles of microbes at any location where the root tip passes result in a wave-like 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 bacterial CFUs (Maloney et al., 1997; Semenov et al., 1999) supports this hypothesis.

One of the means to test the potential of this proposed hypothesis to explain the observed wave-like patterns is the construction of a simulation model that incorporates the hypothetical mechanism sketched above. Several models with increasing complexity have been constructed to simulate microbial growth in the rhizosphere. Newman and Watson (1977) 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 (Newman and Watson, 1977). Darrah (1991a) 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 (Darrah, 1991b). Growth of microbial biomass was dependent on nutrient concentrations. Live microbial biomass was converted into nonsoluble necromass (Darrah, 1991a) instead of into soluble carbon instantaneously (Newman and Watson, 1977). However, death rates were again not specified (Darrah, 1991a). Scott et al. (1995) also included root growth through

layers of soil. Root exudation was larger for one-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 wave-like patterns with depth (Scott et al., 1995).

Wave-like patterns have been analyzed and modeled in many scientific disciplines. Running or traveling waves have been documented for growth of fungal colonies (Davidson et al., 1997), distribution of interacting organisms in two-dimensional space (Gilligan, 1995), and invasion of an organism in an area (Ermentrout and Lewis, 1997; Kot et al., 1996). Reaction-diffusion models have been employed to simulate these situations (Gilligan, 1995). Using sets of interconnected nonlinear equations, Gilligan (1995) 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 wave-like 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 wave-like 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 wave-like 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, in particular readily utilizable substrate in soil.

Materials and methods

Experimental data

For this research we used data on dynamics of colony-forming units of copiotrophic bacteria and water-soluble total organic carbon (TOC) along wheat roots two, three, and four weeks after seeding (Semenov et al., 1999). The data used were geometric means of 3 replicates of 2-cm long root sections at 4-cm intervals along the total root length. CFUs/g dry soil were transformed into biomass carbon per 1 cm^3 of soil according to a factor $4.76 \cdot 10^{-8}$ [$\mu g\ C \cdot g\ ds/CFU\ cm^3$]. This value was calculated from previously measured cell sizes for copiotrophic bacteria in a cover crop amended soil (0.61 um^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 $9.15 \cdot 10^{-8}$ μg, which is similar to values used by Atlas and Bartha (1998).

Transformation of spatial to temporal dimension

In the experiment mentioned above (Semenov et al., 1999), 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 1 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 due to 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 off root cells, constant background exudation, and bacterial necromass. Exudation of photosynthetically derived carbon substances takes place primarily at the lowest 1 cm above the root tip, resulting in a short-term pulse of substrate input 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 (Curl and Truelove, 1986; Whipps, 1990), 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 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 (Brussaard et al., 1990). 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 Figure 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).

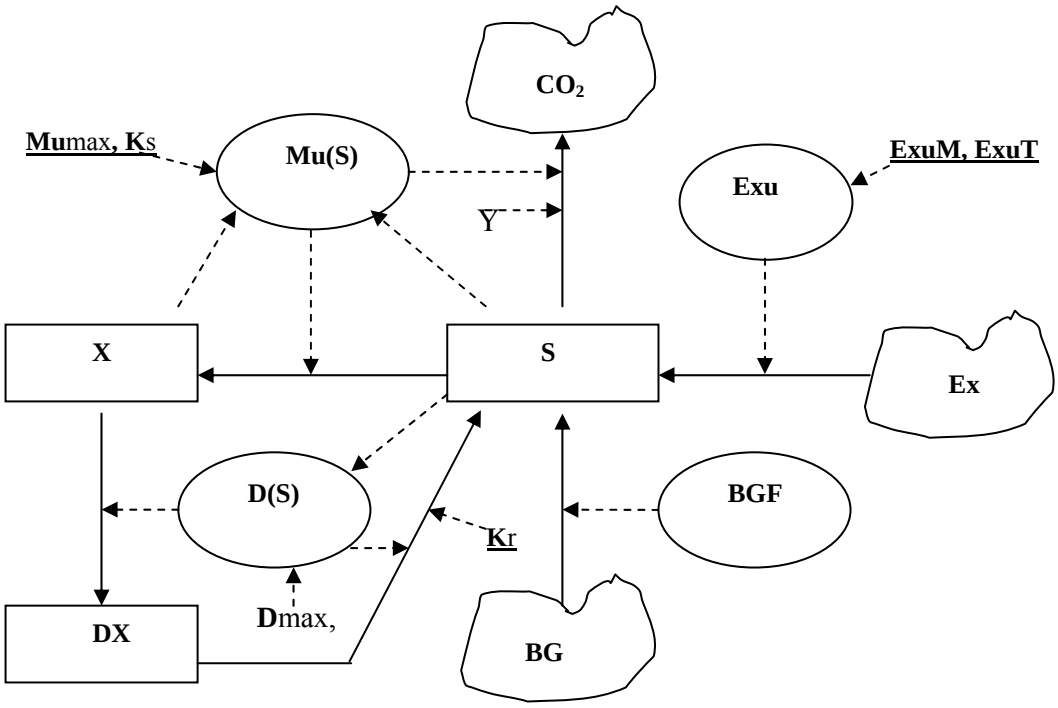

Figure 1. Diagram of the simulation model BACWAVE with the state variables X (biomass of bacteria, μg C / cm^3 soil), S (substrate concentration, μg C / cm^3 soil), DX (dead biomass of bacteria, μg C / cm^3 soil), Ex (exudate concentration, μg C / cm^3 soil), BG (background carbon, μg C / cm^3 soil)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

The set of equations is presented below:

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

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

Where

$\mu(S) = \mu_{max} \cdot S/(Ks \cdot \theta + S)$,

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

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

and

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

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 wave-like 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 4th-order method with a constant time step of 0.01 hour (Forsythe et al., 1977). 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 (Semenov et al., 1999), 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 hours. The yield coefficient and recyclable fraction of biomass were set at 0.44 and 0.4, respectively (Pirt, 1975; Pirt, 1982). 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 (Semenov et al., 1999). Parameter values were compared with literature data in as far as they were available. Finally, the model was run for various run 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 another root observation box experiment with wheat grown in soil rich in organic matter (Semenov et al., 1999). For validation, the same parameter values were used while only the background substrate influx was increased.

Sensitivity analysis

Since the cross-over 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 check 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 wave-like 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

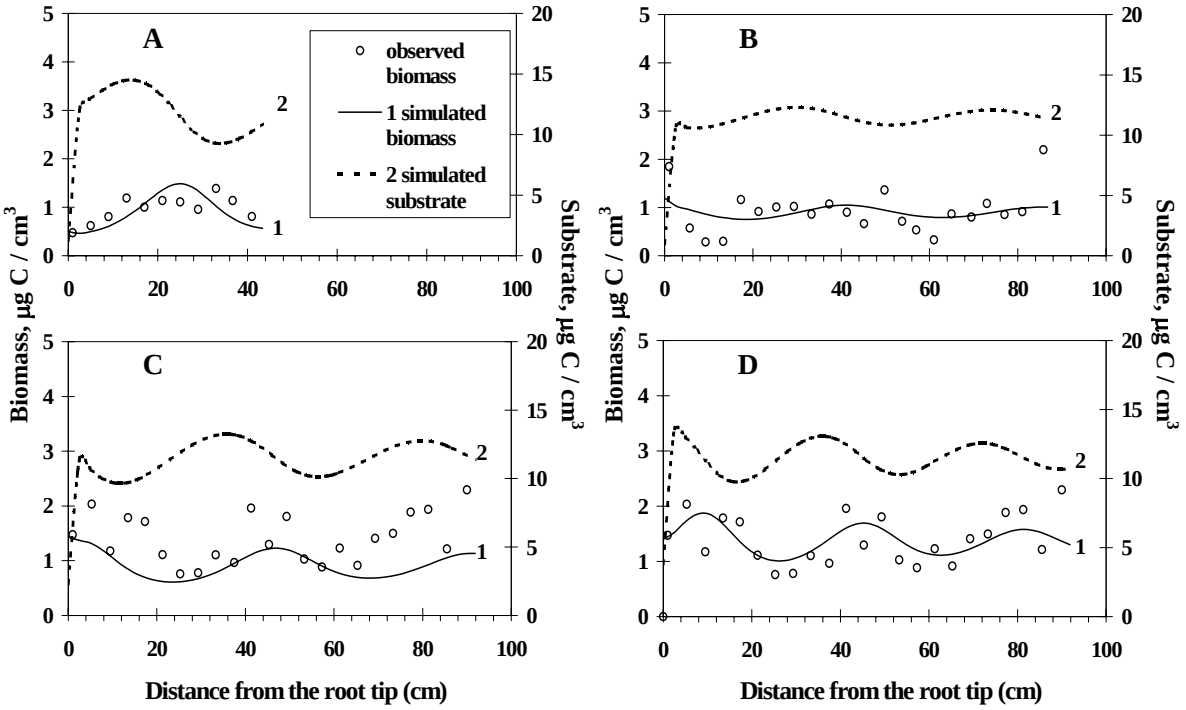

Figure 2. Simulated (solid line) and observed (open circles) bacterial biomass and simulated (dashed line) substrate concentrations at increasing distances from the tip of the seminal root of wheat, (A) two, (B) three, and (C) four weeks after planting using the same parameter values in Table 1 including BGF=0.1 µg C/ cm^3 h, and (D) same as (C) but BGF=0.15 µg C/ cm^3 h. Experimental data are from the second root observation experiment with soil low in organic matter as described in Semenov et al. (1999).

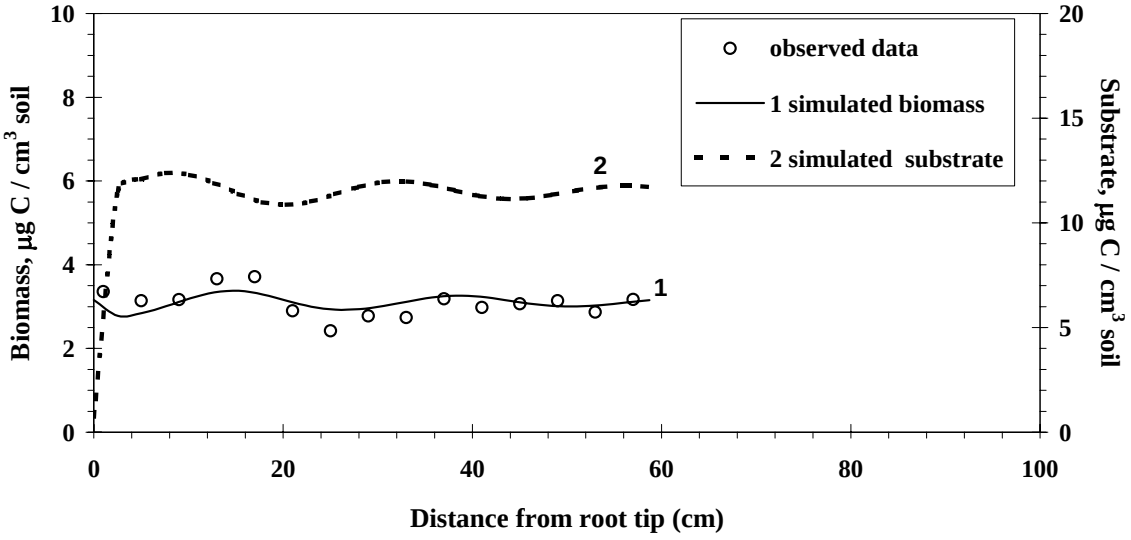

Figure 3. Simulated (solid line) and observed (open circles) bacterial biomass and simulated (dashed line) substrate concentrations at increasing distances from the tip of the seminal root of wheat three weeks after planting, using the same parameter values as in Fig. 2, but BGF=0.35 µg C/ cm^3 h. Experimental data are from the first root observation experiment with soil high in fresh organic matter as described in Semenov et al. (1999).

experimental points four weeks after seeding (Fig. 2C). However, by increasing the background influx from 0.10 to 0.15 μg C/cm^3 soil/h, 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/h) resulted also 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%). As 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 0.5-1.5 μg C/cm^3 for initial biomass, 1.0-4.0 μg C/cm^3 for initial substrate, and 0.1-0.15 μg C/cm^3/h for background flux (Table 1). The maximum relative growth rate

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. (1999).

PARAMETER	SYMBOL	UNIT	VALUE
INTERNAL			
Maximal relative growth rate	μmax	[1/h]	0.063
Substrate constant	Ks	[μg C/ml]	3.0
Maximal relative death rate	Dmax	[1/h]	0.26
Substrate death constant	Kd	[μg C/ml]	14.5
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 h]	0.10-0.15
Maximal exudation rate	ExuM	[μg C/cm^3 h]	8.0
Exudation time constant	ExuT	[1/h]	0.8
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

of copiotrophic bacteria was 0.063 h^{-1} and the substrate constant for growth was 0.68 μg C/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 h^{-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 h^{-1} for the basic variant of the model (Fig. 5A).

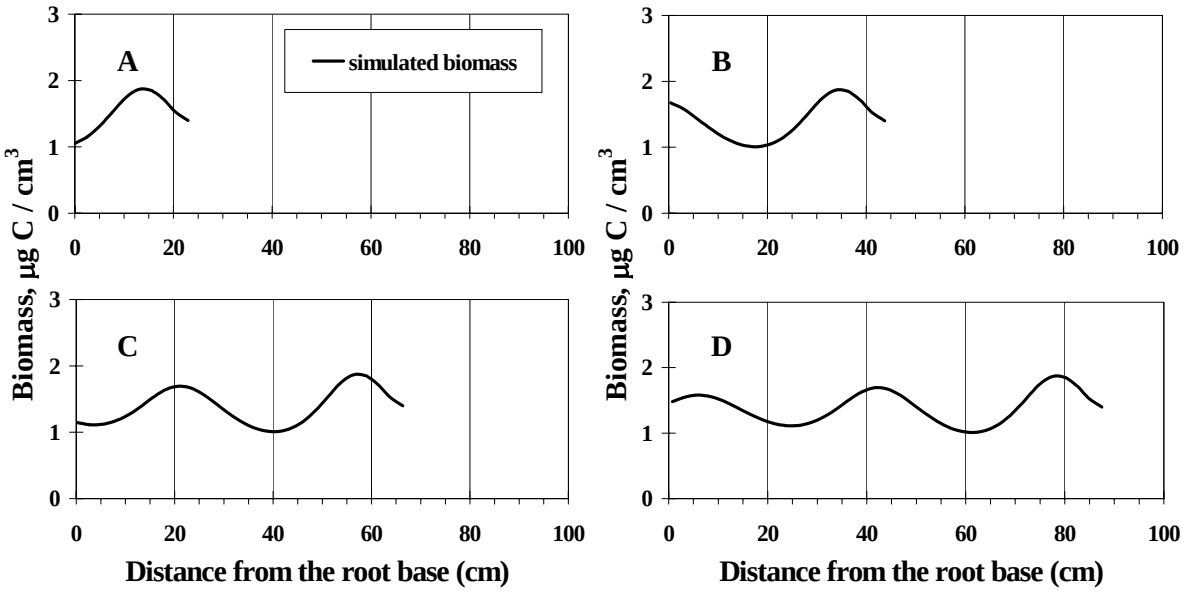

Figure 4. Simulated running waves of bacterial biomass (μg C/cm^3 soil) from the root base to the tip after runnning BACWAVE for (A) 150 h, (B) 300 h, (C) 450 h, and (D) 600 h. Standard parameter values were used for these runs (see Table 1).

When biomass was plotted in relation to a fixed point in soil (the root base) for different run-times, the waves were extended in space, appearing as 'running waves' over time (Fig. 4). This simulated behavior was similar to the dynamic character of the wave-like 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 (Semenov et al., 1999).

Variations in growth and death curves in relation to substrate content showed that the substrate concentration at the cross-over 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). 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

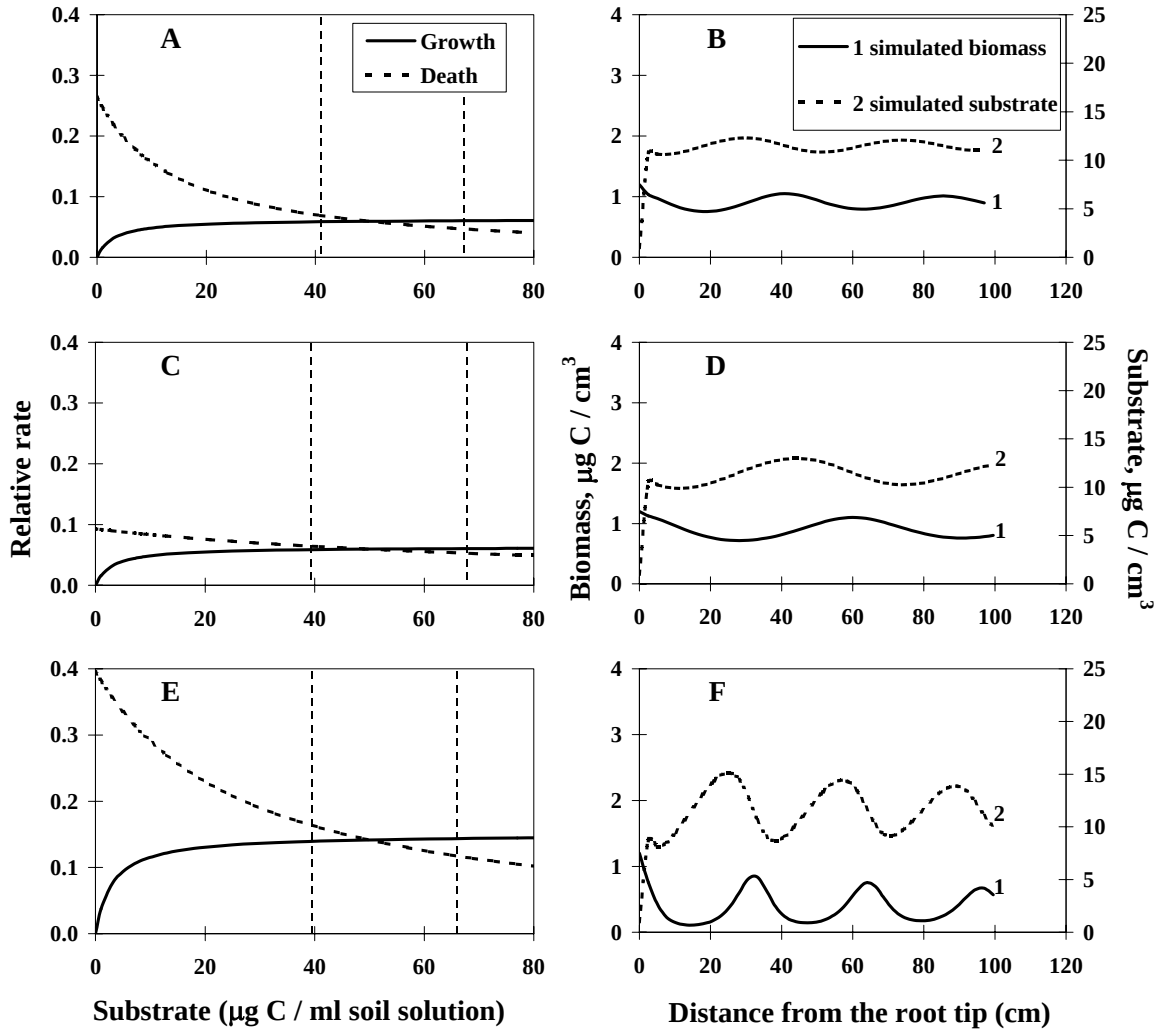

Figure 5. Effects of varying relationships of relative growth and death rates of bacteria (A, C, and E) with substrate concentrations on fluctuations of simulated bacterial biomass over time as translated into one-dimensional space (B,D, and F). Note that the cross-over points of growth and death rates are at the same substrate concentration (50 µg C/ml solution), while the angles between the curves at the cross-over points vary.

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

Graph	Growth		Death	
	µmax [h^{-1}]	Ks [µg C / ml]	Dmax [h^{-1}]	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

between growth and death curves (Fig. 5C) resulted in an intermediate amplitude of biomass oscillations (Fig. 5D), while intermediate and large angles in growth and death curves (Fig. 5A and E) resulted in small and large biomass amplitudes, respectively (Fig, 5B and F).

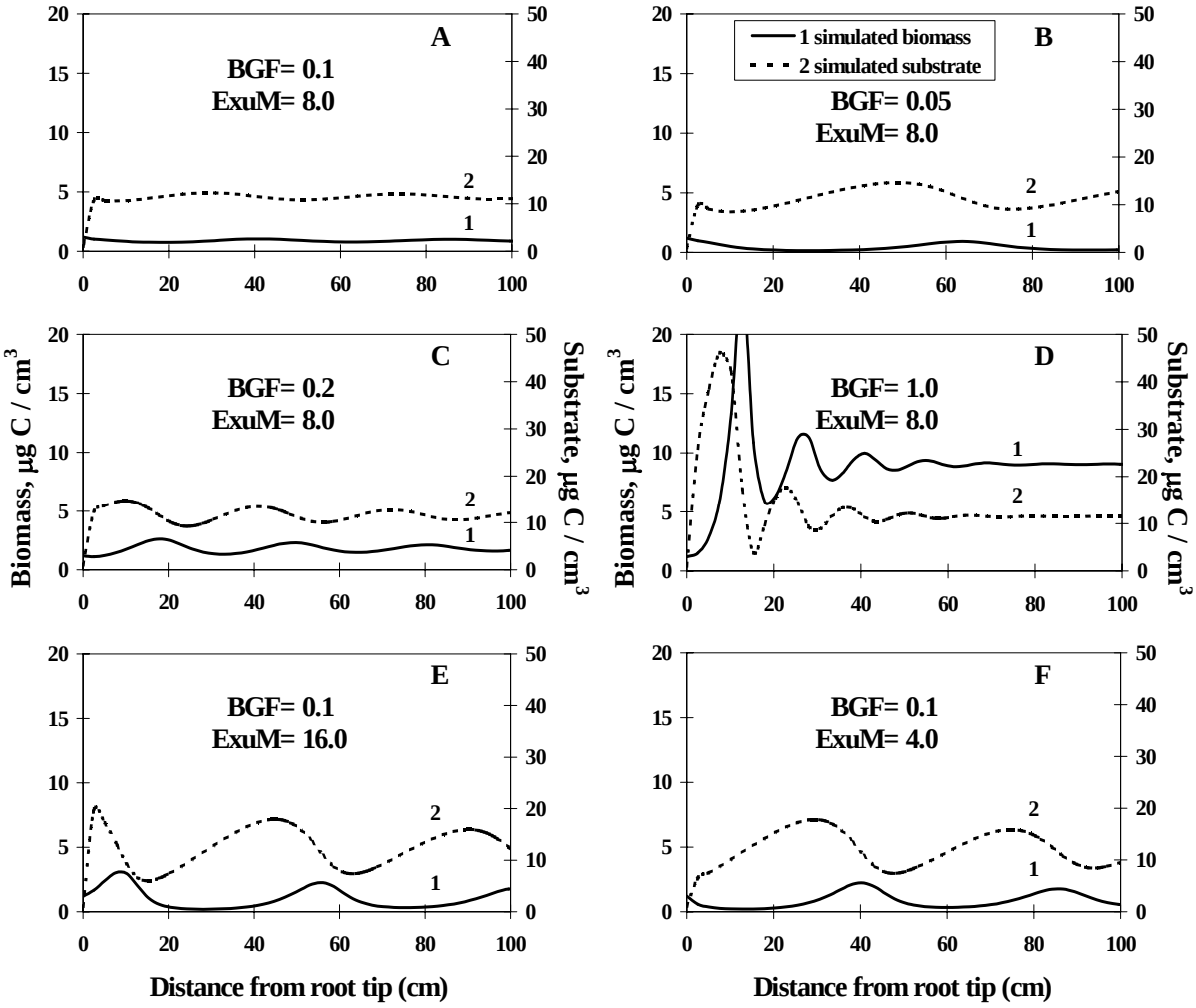

Figure 6. Effects of varying substrate input from a moving source (ExuM=maximum exudation rate in µg C/cm^3/h) and background substrate flux (BGF in µg C/cm^3/h) on fluctuations of simulated bacterial biomass (solid line) and substrate concentration (dashed line) over time as translated into one-dimensional 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 hours is 10 µg C/cm^3 for ExuM=8.0 µg C/cm^3/h, and from background flux is 0.6 µg/cm^3 for BGF=0.1 µg C/cm^3/h.

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 higher levels of background substrate flux. The value of this stationary biomass level also depends on the yield coefficient, 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 is. 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 create 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 wave-like patterns of bacterial biomass over time. These temporal wave-like patterns can be translated into a spatial wave-like distribution along a root at a particular moment since the start of root growth, similar to the wave-like distribution patterns observed previously for wheat (Semenov et al., 1999). Relative to a fixed location (the root base), simulated fluctuations in bacterial populations appear as moving waves in the spatio-temporal domain, again similar to the shifting wave-like distributions described for wheat roots (Semenov et al., 1999). 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 (Darrah, 1991a, 1991b; Newman and Watson, 1977; Scott et al., 1995) 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 influence biomass behavior considerably (Pirt, 1975). 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. (1995) 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 wave-like 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 wave-like patterns with our model thanks to the cross-over 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, 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 (Badalucco et al., 1996; Ramirez and Alexander, 1980; Sundin et al., 1990) or host-parasite interactions (Bohannan and Lenski, 1999) are not a necessary prerequisite for wave-like 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 like 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 non-recyclable 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 (Semenov et al., 1999). Thus, the microbial peaks along the middle and upper sections of the roots (Semenov et al., 1999) 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 (Harvey et al., 1998; Hernawan and Fleet, 1995; Ronchel et al, 1998).

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 (Semenov et al., 1999). 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 (Newman and Watson, 1977; Scott et al., 1995). The maximum relative growth rate was relatively low (0.063 h^{-1}) for copiotrophic bacteria, considering that the estimated doubling time would be 11 hours. In continuous flow culture with an easily utilizable substrate like glucose, the maximum relative growth rate for copiotrophs would be almost 10 times as high (Matin and Veldcamp, 1978; Semenov, 1991). However, root exudates consist of a mixture of compounds with different decomposition rates (Schilling et al., 1998). Moreover, our experimental data consisted of relatively large rhizosphere samples compared to the micro-scale 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 (Button, 1985; Pirt, 1975; Semenov, 1991). The death rate varied from 0.05 to 0.07 h^{-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. (1995).

The estimated initial biomass of copiotrophic bacteria at the root tip corresponded to $1\text{-}3 \cdot 10^7$ CFUs/g of dry soil. These are reasonable numbers for the rhizosphere, although they are higher than those

commonly found in bulk soil (Semenov et al., 1999). 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 (Darrah, 1991b). 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 reasonable compared to concentrations estimated for bulk soil (Darrah, 1991a). However, the maximal simulated substrate concentration (about 15 μg C/cm^3 soil) was much lower than the measured concentrations of water-soluble TOC (Semenov et al., 1999). Moreover, the fluctuations in simulated substrate concentrations did not correspond to changes in water-soluble TOC along wheat roots (Semenov et al., 1999). The high concentrations of TOC at the root tip and root base as presented earlier (Semenov et al., 1999) must represent residual compounds that are not easily utilized by microbes. Carbon compounds in the rhizosphere, including TOC, are quite complex (Hodge et al., 1996; Rovira, 1969, 1973; Schilling et al., 1998; Whipps and Lynch, 1983), and readily utilizable substrates naturally constitute only a small part of these substances. For example, the percentage of simple sugars in released soluble organic matter is often low, varying from 1-5% for various crops (Kobayashi et al., 1996). A large part of the rhizodeposits that can be extracted by water are polymers, phenolics and glycosides, which are more difficult to metabolize (Kobayashi et al., 1996). 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 non-sterile soil, RUS is absorbed by microorganisms as soon as the concentration passes the uptake threshold (Semenov, 1991). 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. Newman and Watson (1977) came to a similar conclusion, namely that the substrate concentration is generally low, while the microbial concentration may vary greatly.

In conclusion, we created a simulation model describing a new concept about microbial population dynamics and resulting wave-like distributions in the rhizosphere. With this model we showed that wave-like 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 wave-like 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 (Celenza et al., 1995) and by studying microbial distribution patterns along the path of a moving artificial root without lateral exudation points (van Bruggen et al., 2000).

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Chapter 4

Modeling wave-like dynamics of oligotrophic and copiotrophic bacteria along wheat roots in response to nutrient input from a growing root tip

Adapted from:
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Abstract

A spatial-temporal model "BACWAVE" for wave-like dynamics of copiotrophic bacteria (CB) was extended to include dynamics of oligotrophic bacteria (OB). BACWAVE simulates wave-like distributions of bacteria along wheat roots from their temporal dynamics with growth and death rates depending on readily utilizable substrate (RUS) concentrations, consisting of a pulse of substrate from a passing root tip and constant substrate background flux (BGF) from decaying root cortex, dead bacterial cells, and soil organic matter. CFUs of OB and CB along wheat roots (24 samples) in a low C soil (0.7% C) were transformed to temporal biomass taking root growth rate and cell sizes into account. Growth rates of both groups of bacteria increased with substrate according to Monod equations, but each with their own characteristic parameter values. Death rate of CB decreased monotonically with substrate concentration, while death rates of OB first decreased and then increased with substrate concentration. Model parameters were estimated with an iterative optimization method. Initial biomass and kinetic parameters were lower for OB than for CB, and fell in the range of values in the literature. The model was validated with an independent data set of bacteria along wheat roots in relatively C-rich soil (1% C), so that BGF and initial microbial populations were higher, but other model parameters were the same for both data sets. A satisfactory fit was obtained between experimental and modeled data.

Keywords: Fluctuations, Microbial dynamics, Oscillations, Rhizosphere, Simulation model

Introduction

Oligotrophic bacteria (OB) are characterized by low maximum relative growth rates and half saturation constants with respect to carbon sources (Semenov, 1991). They can be isolated after 2 to 4 weeks of incubation on low carbon media (1 – 100 mg C/L), although many copiotrophic bacteria (CB) and bacteria with a wide reaction range can also be isolated on such media (Semenov, 1991; Suwa and Hattori, 1984). Nevertheless, bacteria isolated on low carbon media are commonly referred to as oligotrophic (Maloney et al., 1997; Suwa and Hattori, 1984). True OB can not be isolated on nutrient-rich media (higher than approximately1000 mg C/L), and die at high carbon concentrations (Button, 1985; Button, 1991). OB are generally smaller than CB, with diameters less than 0.5 μm (van Bruggen and Semenov, 2001).

OB are generally more numerous than CB in bulk soil, but are temporarily outnumbered by CB after a disturbance, such as addition of fresh organic matter or root exudates to soil (Hu et al., 1999; Maloney et al., 1997). While plant roots provide significant amounts of carbon to soil environments, parts of the rhizosphere can still be considered oligotrophic, with estimated soluble organic carbon concentrations fluctuating around 10-100 μg per gram of dry soil (Darrah, 1991a; Semenov et al., 1999; Swinnen et al., 1994). As a result, OB can be important components of bacterial communities at rhizosphere sites where carbon is limiting (Maloney et al., 1997; Semenov et al., 1999). However, OB have been quantified in relatively few rhizosphere studies (De Leij et al., 1993; Maloney et al., 1997; Semenov et

al., 1999; Sugimoto et al., 1990). When different locations were distinguished along the root, fast-growing (copiotrophic) bacteria utilizing many substrates dominated at the root tip, while slow-growing (oligotrophic) bacteria with limited nutrient-use capacity were more numerous at the root base (Liljeroth et al., 1991; Maloney et al., 1997). According to De Leij et al. (1993) the rhizosphere microbial community changes from a community dominated by r-strategists to one dominated by K-strategists with age of the root. Thus, they considered the rhizosphere of a young root excreting readily utilizable substrates an early-successional ecosystem, while that of an aged root can be considered a late-successional ecosystem.

Recently, however, we demonstrated that the situation is more complex than a simple gradual shift from an early-successional to a late-successional community from the root tip to the root base. We described wave-like fluctuations of copiotrophic and oligotrophic CFUs along the length of wheat roots (Semenov et al., 1999). The periods of the waves of OB populations were slightly longer and the phase was slightly shifted upwards compared to those of CB populations, in accordance with the successional position of these functional groups (Semenov et al., 1999). The waves moved with the growing root tip, and the hypothesis was put forward that bacterial growth and death cycles were generated at any location in soil where a growing root tip passed, resulting in running waves along the length of a growing root. This hypothesis was supported by the occurrence of wave-like patterns of bacterial populations along the path of a moving artificial root (van Bruggen et al., 2000). The period of the waves, when transformed from space into time, was independent of the moving rate of the root, but was probably related to the growth and death rates of the bacterial populations.

The reasons for death of the bacteria were unknown, but could be related to nutrient exhaustion rather than predator-prey relationships, as no protozoa or nematodes were observed in the experiment with artificial root systems (van Bruggen et al., 2000). Accordingly, a simulation model (BACWAVE) was developed in which fluctuations in bacterial populations were generated by bacterial growth and death solely in relation to substrate concentrations (Zelenev et al., 2000). In previous rhizosphere models neither wave-like fluctuations nor different functional groups of bacteria were taken into consideration (Newman and Watson, 1977; Darrah, 1991b; Scott et al., 1995; Zelenev et al., 2000).

For controlled conditions (batch- and continuous flow cultures), growth curve models were developed for CB and OB separately (Button, 1985 and 1991). When a fast- and slow-growing species (*Pseudomonas* and *Spirillum* sp.) were studied in mixed continuous flow culture, *Spirillum* was dominant at low flow rates while *Pseudomonas* dominated at high flow rates (Matin and Veldkamp, 1978). However, in the rhizosphere, homogeneous mixing of nutrients would not occur, and we observed different trophic groups simultaneously at similar locations along wheat roots, although peaks in OB occurred slightly higher up the roots than peaks in CB. With the model BACWAVE we simulated growth and death of CB satisfactorily. However, no attempt was made to model fluctuations of the OB populations.

The objectives of this research were:

(1) to adapt the model "BACWAVE" to simulate wave-like distributions of CB together with OB in the rhizosphere along a wheat root;
(2) to check if the model adequately describes distributions of CFUs isolated on carbon rich and poor media along a wheat root;
(3) to check if peaks in simulated oligotrophic CFUs would coincide with those of copiotrophic CFUs as observed experimentally along wheat roots.

Materials and methods

Model description

In this research similar assumptions and approaches to modeling were used as described by Zelenev et al. (2000). Here we present only a brief description of the model BACWAVE that was developed to simulate the dynamics of only one functional group of bacteria, namely CB. In this model bacterial biomass carbon and substrate carbon concentrations were simulated in time for any location in space after an impulse of nutrients from a passing root tip. Additional nutrient input consisted of a so-called background substrate flux that was constant in time (reflecting decomposing soil organic matter). Temporal dynamics were transformed into spatial distributions along a root, taking root growth rate into account.

In this investigation the model was expanded to include the dynamics of both CB and OB simultaneously (Fig. 1). The basic relations between state variables for CB and OB were the same as presented previously (Zelenev et al., 2000). Both trophic groups were assumed to utilize common substrate S. However, the equations describing the relationships between relative growth and death rates [$\mu(S)$ and $D(S)$] and substrate concentration were different for each trophic group. In particular, the substrate constant K_S and the maximum growth rate μ_{max} were lower for OB than for CB (see under parameter estimation below). More importantly, the equation for the relative death rate of OB in relation to substrate concentration was modified to reflect not only a decrease in death rate between 0 and low substrate concentrations but also an increase in death rate at higher substrate concentrations (Fig. 2).

Dead biomass of both trophic groups was divided into two parts: a proportion K_r consisting of easily decomposable compounds going directly to the substrate compartment and the fraction $(1-K_{rC})$ consisting of slowly decomposing compounds accumulating in a stable organic residue pools X_{DC} and X_{DO} (sinks external to the model).

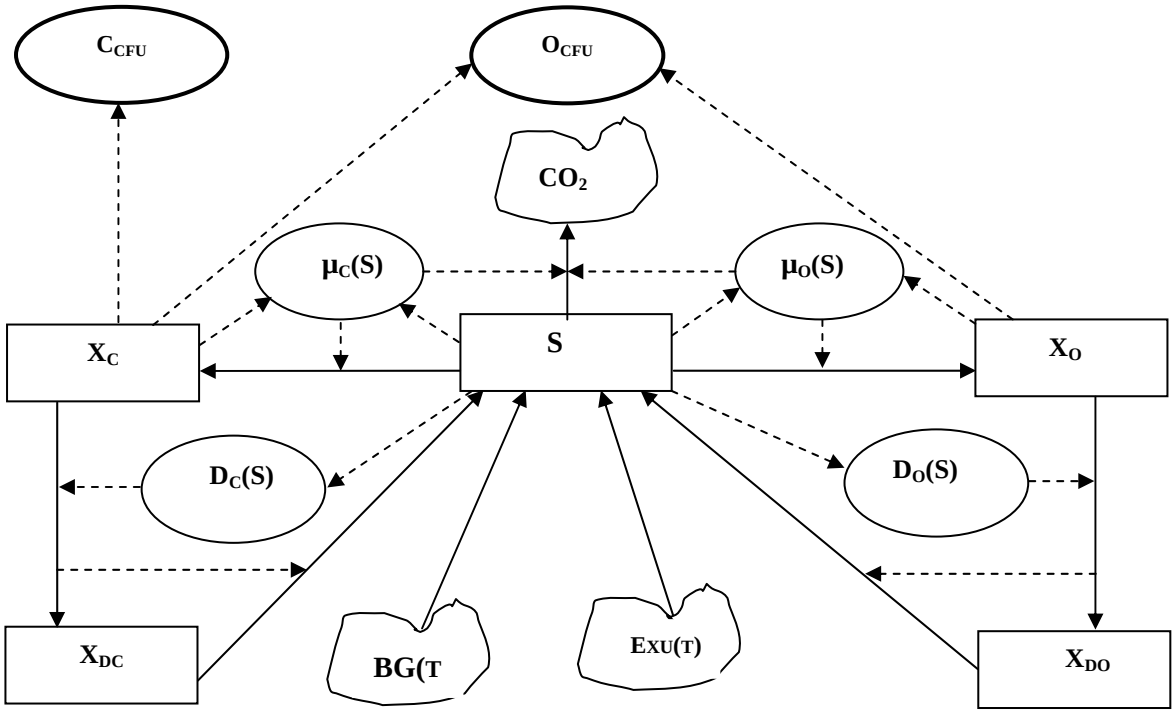

Figure 1. Model diagram. C_{CFU} and O_{CFU} - the number of CFUs isolated on C-rich and C-poor media, respectively. The state variables: X_C and X_O (biomass of copiotrophic and oligotrophic bacteria, μg C/cm^3 soil), S (substrate content, μg C/cm^3 soil), X_{DC} and X_{DO} (dead biomass of copiotrophic and oligotrophic bacteria, μg C/cm^3 soil); the sources: BG(t) (rate of substrate background influx, μg C/ h cm^3 soil) and Exu(t) (root exudation rate, μg C/h cm^3); the sink: CO_2 (amount of carbon dioxide respired by bacteria, μg C/cm^3 soil); governing functions: $\mu_C(S)$ and $\mu_O(S)$ (relative growth rate of copiotrophic and oligotrophic bacteria, h^{-1}), $D_C(S)$ and $D_O(S)$ (relative death rate of copiotrophic and oligotrophic bacteria, h^{-1}). Solid lines represent carbon flows, and dashed lines information flows.

The set of differential equations, relationships and parameter descriptions for CB and OB are presented below:

$dX_C/dt = (\mu_C(S) - D_C(S)) \cdot X_C;$

$dX_O/dt = (\mu_O(S) - D_O(S)) \cdot X_O;$

$dS/dt = -X_C \cdot \mu_C(S)/Y_C - X_O \cdot \mu_O(S)/Y_O + K_{rC} \cdot X_C \cdot D_C(S) + K_{rO} \cdot X_O \cdot D_O(S) + BG(t) + Exu(t);$

$dX_{DC}/dt = (1 - K_{rC}) \cdot D_C(S) \cdot X_C;$

$dX_{DO}/dt = (1 - K_{rO}) \cdot D_O(S) \cdot X_O;$

$C_{CFU} = Cc \cdot X_C/Bc$

$O_{CFU} = Co \cdot X_C/Bc + Oo \cdot X_O/Bo$

$\mu_C(S) = \mu_{maxC} \cdot S/(K_{SC} \cdot \theta + S);$

$\mu_O(S) = \mu_{maxO} \cdot S/(K_{SO} \cdot \theta + S);$

$D_C(S) = D_{maxC} \cdot K_{dC}/(K_{dC} + S/\theta);$

$D_O(S) = D_{maxO} \cdot K_{dO} /(K_{dO} + S/\theta) + K_L \cdot S/\theta$;

$BG(t)=BGF$;

$Exu(t)=ExuM \cdot \exp(-ExuT \cdot t)$.

Where

t = time [h];

X_C = biomass of copiotrophic bacteria [µg C/cm^3 soil];

X_O = biomass of oligotrophic bacteria [µg C/cm^3 soil];

S = substrate content [µg C/cm^3 soil];

X_{DC} = stable fraction of dead copiotrophic bacteria biomass [µg C/cm^3 soil];

X_{DO} = stable fraction of dead oligotrophic bacteria biomass [µg C/cm^3 soil];

$\mu_C(S)$ = specific growth rate of copiotrophic bacteria dependent on substrate content [h^{-1}];

$\mu_O(S)$ = specific growth rate of oligotrophic bacteria dependent on substrate content [h^{-1}];

μ_{maxC} = maximal specific growth rate of copiotrophic bacteria [h^{-1}];

μ_{maxO} = maximal specific growth rate of oligotrophic bacteria [h^{-1}];

K_{SC} = substrate constant for growth of copiotrophic bacteria [µg C/ml soil solution];

K_{SO} = substrate constant for growth of oligotrophic bacteria [µg C/ml soil solution];

$D_C(S)$ = specific death rate of copiotrophic bacteria dependent on substrate content [h^{-1}];

$D_O(S)$ = specific death rate of oligotrophic bacteria dependent on substrate content [h^{-1}];

D_{maxC} = maximal death rate of copiotrophic bacteria [h^{-1}];

D_{maxO} = maximal death rate of oligotrophic bacteria [h^{-1}];

K_{dC} = substrate constant for death of copiotrophic bacteria [µg C/ml soil solution];

K_{dO} = substrate constant for death of oligotrophic bacteria [µg C/ml soil solution];

K_L = constant for death increase with substrate [ml soil solution/h · µg C];

BG(t)= current background substrate influx rate [µg C/(h · cm^3 soil)];

BGF = initial value of substrate background influx [µg C/(h · cm^3 soil)];

Y_C = yield coefficient for copiotrophic bacteria [µg C/µg C];

Y_O = yield coefficient for oligotrophic bacteria [µg C/µg C];

K_{rC} = fraction of dead copiotrophic biomass recycling to substrate [-];

K_{rO} = fraction of dead oligotrophic biomass recycling to substrate [-];

θ = soil water content [ml solution/cm^3 soil];

ExuM = maximal exudation rate [µg C/(h · cm^3 soil)];

ExuT = time constant for exudation, responsible for duration of exudation [h^{-1}];

Bc= factor to transform copiotrophic CFUs per g dry soil into microbial biomass C per cm^3 soil [µg C · g ds/CFU · cm^3];

Bo= factor to transform oligotrophic CFUs per g dry soil into microbial biomass C per cm^3 soil [µg C · g ds/CFU · cm^3];

Cc= fraction of total copiotropic bacteria that grow on a rich medium [-];

Co= fraction of total copiotropic bacteria that grow on a poor medium [-];

Oo= fraction of total oligotropic bacteria that grow on a poor medium [-].

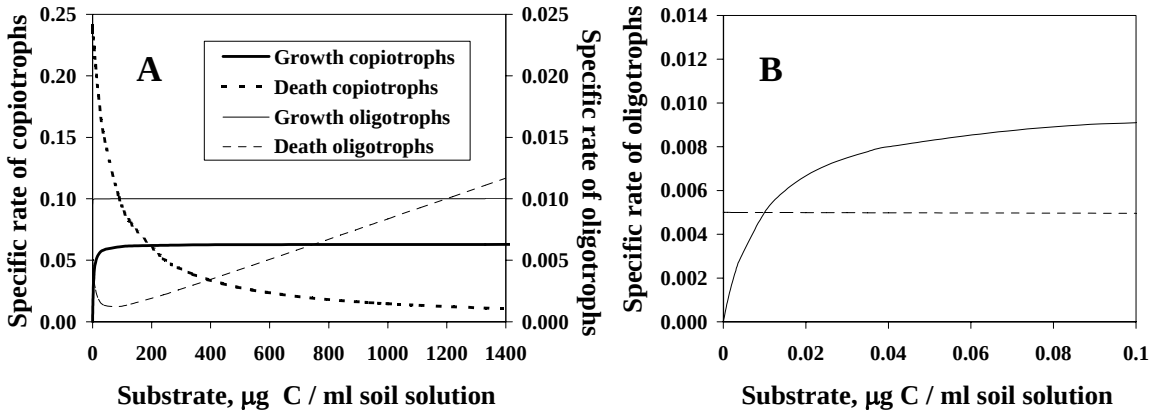

Figure 2. Specific growth and death rate for copiotrophic and oligotrophic bacteria as dependent on substrate concentration in soil solution, on a scale from 0 to 1400 μg C/ ml solution (A) and from 0 to 0.1 μg C / ml solution (B).

The model was run in the program 'Model Maker' (Cherwell Scientific Publ. Ltd., Oxford, UK), where the Runge-Kutta method of integration was selected with a variable time step. For adjustment and optimization of parameter values and for model goodness of fit calculations the respective facilities of the program were used.

Experimental data

The input and validation data for development of the simulation model for wavelike distributions of CB and OB along roots were obtained from two greenhouse experiments with wheat growing in root observation boxes (Semenov et al., 1999). The first experiment was conducted with a sandy loam soil relatively high in organic matter (1.0% C) and the second with poor sandy loam soil (0.68% C). Details of the experimental design and techniques used were presented in Semenov et al.(1999). Soil moisture content varied only slightly (Semenov et al., 1999) and was considered as constant for modeling purposes: $\theta = 0.23$ [ml/cm^3]. The average root extension rate was 3.3 cm/day (Semenov et al., 1999; Zelenev et al., 2000).

The distributions of bacterial CFUs along wheat roots were obtained 3 and 4 weeks after seeding on rich (1000 ppm C) and poor (10 ppm C) media in each experiment. Geometric means were calculated for 2 or 3 replicates of 2-cm long root sections at 4-cm intervals along the total root length.

The experimental data were smoothed by a 3-point window, a common preliminary procedure for time series analysis to reduce noise and to highlight periodical components (Shumway, 1988; Semenov et al., 1999). Thus, smoothed geometric means of CFUs in different weeks of each experiment were used as experimental data sets for calibration and validation of the model.

Transformation of CFUs on rich and poor media to bacterial biomass.

CFU/g dry soil was transformed to carbon of cultivable biomass per 1 cm^3 of soil by multiplication with a factor $Bc = 4.76 \cdot 10^{-8}$ [μg C · g ds/CFU cm^3] for CB and $Bo = 1.01 \cdot 10^{-8}$ [μg C · g ds/CFU cm^3] for OB. These values were calculated from the sizes of large plus medium and small FITC-stained cells,

respectively, as determined microscopically (0.61 μm^3 and 0.13 μm^3 per cell, respectively) (van Bruggen and Semenov, 2001), estimated cell dry matter (15%), carbon content of dry bacterial biomass (40%), and soil density (1.3 g/cm^3). Thus, the dry mass of CB and OB cells was estimated to be $9.15 \cdot 10^{-8}$ µg and $1.95 \cdot 10^{-8}$ µg per cell, respectively, which was similar to the range of values used by Atlas and Bartha (1998). Cell carbon contents were estimated as $36.6 \cdot 10^{-9}$ µg and $7.8 \cdot 10^{-9}$ µg for CB and OB, respectively, which fell within the range given by Kroer (1994).

The proportion of copiotrophic CFUs to the numbers of large plus medium cells was around 0.35 (van Bruggen and Semenov, 2001). This value (parameter Cc) was used to calculate the modeled copiotrophic biomass from total number of copiotrophic CFUs and vice versa. Most colonies on C-poor media (10 mg C/L) were copiotrophic or broad range bacteria, whereas true oligotrophs (not able to grow on a medium with 1000 mg C/L) comprised only about 5% of the total CFU numbers grown on a poor medium. To calculate the numbers of CFUs on a C-poor medium from modeled biomass, we assumed that the fraction Oo of true OB (Oo=0.05) and a fraction Co of CB (to be found by optimization) could be isolated on a poor medium.

Parameter estimation and optimization

Basic growth parameters for CB μ_{maxC}, K_{SC}, Y_C were taken as reported in the literature: μ_{maxC} =0.063 [h^{-1}], K_{SC}=3.0 [µg C/ml] (Scott et al., 1995; Zelenev et al., 2000),Y_C=0.45 [µg C/µg C] (Pirt, 1975; Semenov,1991; Semenov and Staley, 1992). Basic growth parameters, μ_{maxO}, K_{SO}, Y, and K_r, were assigned values typical of oligotrophic microorganisms in the literature: μ_{maxO} =0.01 [h^{-1}], K_{SO}=0.01 [µg C/ml], Y_O=0.6 [µg C/µg C] (Poindexter, 1981; Button, 1985; Fry, 1990; Semenov, 1991; Semenov and Staley, 1992; Pirt, 1975). Parameter $K_L=8.3 \cdot 10^{-6}$ was chosen so that the upper limiting concentration for growth of OB was 1200 ppm of C (Sato et al., 1993; Klimova and Nikitin, 1994).

Parameters K_{dC} and K_{dO} are the values of substrate concentration which bring the specific death rate of each group of bacteria to half of its maximal value (D_{max}). They were chosen so that K_{dC} was higher than K_{dO} , namely K_{dC} =65[µg C/ml soil solution] (Zelenev et al., 2000) and K_{dO}=10 [µg C/ml soil solution]. Parameter D_{maxO} was adjusted to get a cross-over point of specific growth and death rates of OB at a substrate concentration around 0.001 ppm C, which was chosen 10 times less than the K_{SO} value used in the model (Fig 2B). D_{maxC} was estimated during the calibrating optimization procedure. K_{rC} for CB was set to the value 0.4 (Pirt, 1982). Estimation of this parameter for OB was done on the basis of cell circumference to volume ratios for OB relative to CB, resulting in a value 0.1 for the parameter K_{rO}.

Total initial substrate amount originating from the root tip was estimated as two times the first rhizosphere biomass peak of CB (as OB contributed relatively little to the initial biomass peak). From this substrate amount the initial maximal exudation rate (ExuM=8 [µg C/h·cm^3 soil]) and exudation time constant (ExuT= 0.8 [h^{-1}]) were estimated to provide 10 µg C/cm^3 soil exuded from the root tip for the first 6 hours (Zelenev et al., 2000).

Model calibration.

The model was calibrated using the CFU data on poor and rich media in week 3 and 4 of the second experiment where wheat was grown in soil with a lower organic matter content (Semenov et al., 1999). During initial (calibrating) optimization, all parameter values given above were kept constant, except the initial biomass X_O and X_C, initial substrate value Si, background substrate flux BGF and maximal death rate of CB D_{maxC}. Next, the estimated parameters and optimized value D_{maxC} were kept the same, while the initial biomass X_O and X_C, initial substrate value Si, background substrate flux BGF were optimized for each individual data set. Adjusted parameter values were compared with literature data as far as they were available.

For each data set used for optimization, the contribution of the model to the experimental variance was calculated and compared with the contribution of significant harmonics to the experimental variance as determined previously (Semenov et al., 1999).

Model validation

The model was validated with the CFU data on poor and rich media in week 3 of the first experiment where wheat was grown in soil with higher organic matter content (Semenov et al., 1999). For validation the same set of internal (i.e. responsible for growth and death of CB and OB) model parameter values was used while initial values of biomass, substrate and background substrate influx were adjusted to take into account higher microbial biomass and soil organic matter content.

Results

The model produced distributions of bacterial CFUs along wheat roots similar to the distributions of CFUs on rich and poor carbon media in the second experiment (calibration data set). Both the simulated and experimental CFUs revealed oscillating patterns along roots (Fig. 3). The fit was similar for populations on rich and poor media, except after 4 weeks of plant growth (Fig. 3B) when CFUs on poor media were underestimated by the model close to the root tip. Model contribution to total variance of the calibration data set was higher (54%) than the contribution of significant harmonics to the variance for the same data set detected with Fourier analysis (39% on average), indicating sufficient quality of the model.

Calculated CFUs from modeled biomass data fit very well to the experimental CFUs of the validation data set (Fig. 3C). The contribution of the model to total variance of the validation data set was also higher (48%) than the contribution of significant harmonics to the variance of the same data set (45%) (Semenov et al., 1999).

Biomass of CB fluctuated over time, but biomass of true OB (5% of all CFUs on C-poor media) did not fluctuate at the substrate concentrations calculated (Fig. 4). Biomass of CB fluctuated within the range 1.7-3.0 [μg C/cm^3 soil] for the 3rd week and 3.0-5.3 [μg C/cm^3 soil] for the 4th week of the second experiment, while biomass of OB grew monotonically from 0.002 up to 0.65 [μg C/cm^3 soil]

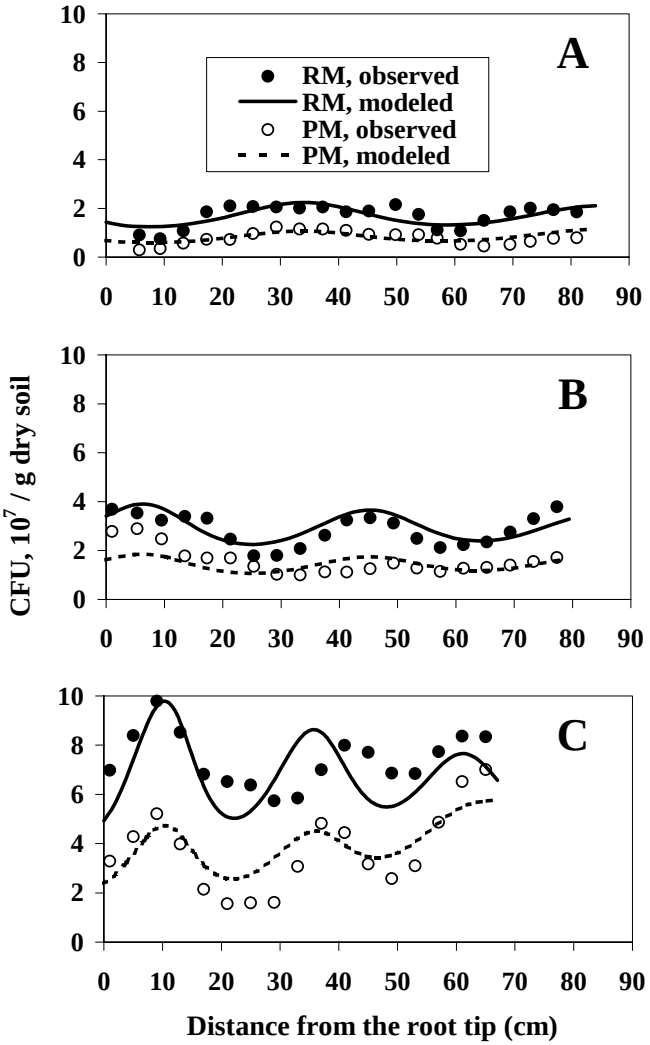

Figure 3. Distributions of modeled (lines) and experimental (circles) CFUs on C-rich (closed circles) and C-poor (open circles) media (RM and PM, respectively) along wheat roots for the calibration data set (3 weeks of plant growth in soil low in organic matter) (A), and two validation data sets (4 and 3 weeks of plant growth in soil low (B) and higher (C) in organic matter). Root observation boxes were 90 cm long in the experiment with low organic matter (A and B) and 70 cm long in the experiment higher in organic matter (C)

during each of the runs (Fig. 4A and B). Total bacterial biomass fluctuated similarly to CB biomass (not shown). Simulated substrate concentrations revealed similar periodical behavior as total bacterial biomass, but in slightly shifted opposite phase. Substrate concentrations fluctuated between 38 and 48 [µg C/cm^3 soil], equivalent to 190-240 [µg C/ml soil solution], in both the 3rd and 4th week of the second experiment. For the validation data set (3rd week of the first experiment), biomass of CB fluctuated within the range 6.7-13.4 [µg C/cm^3 soil], whereas substrate fluctuated between 32 and 58 [µg C/cm^3 soil], equivalent to 160-290 [µg C/ml soil solution]. Biomass of OB grew again monotonically from 0.1 to 6.0 [µg C/cm^3 soil] (Fig. 4C).

The initial optimization procedure with the data set of the 3rd week of the second experiment yielded besides the initial biomass X_C and X_O, initial substrate value Si, background substrate flux BGF, a

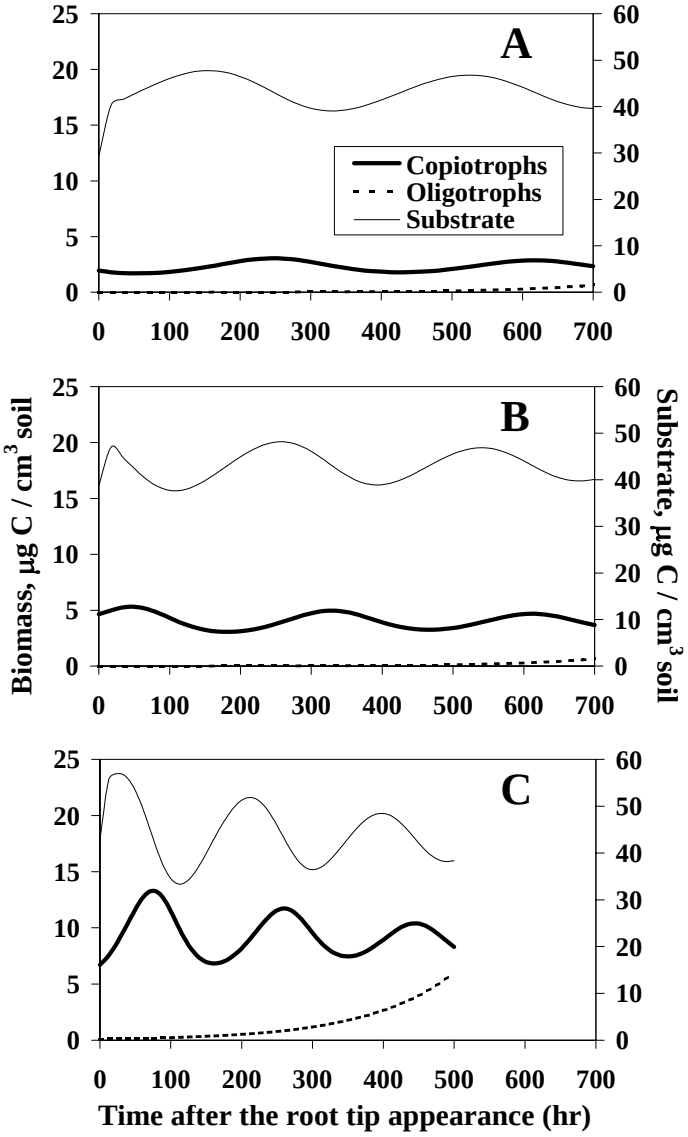

Figure 4. Dynamics of modeled biomass of copiotrophic and oligotrophic bacteria and substrate at a certain point of the rhizosphere over time since appearance of the root tip. A, B and C correspond to A, B and C in Figure 3.

value of maximal death rate of CB D_{maxC}. Thus, a complete set of parameter values for microbial kinetics was deduced from this data set (Table 1). For following applications of the model to various experimental data this set of parameter values was kept constant. Nevertheless, application of the model to other experimental series required adjustment of the values for initial microbial biomass X_C and X_O, initial substrate value Si and background substrate flux BGF, since each of the series related to an object with different initial and environmental conditions (Table 2). For the 4th week of the second experiment, the initial values of CB biomass and substrate as well as BGF needed to be higher than for the 3rd week (Table 2), probably due to more pronounced decaying of the cortex of the older root. Initial values of OB biomass were the same for the 3rd and 4th week, namely 0.002 [µg C/cm^3 soil]. The initial value of OB biomass for the validation experiment with carbon-rich soil was 0.1 [µg C/cm^3 soil], much higher than the initial value of OB biomass for the experiment with soil with low

Table 1. Constant model parameters for BACWAVE, a dynamic simulation model for copiotrophic and oligotrophic bacteria in soil.

PARAMETER	SYMBOL	UNIT	VALUE
COPIOTROPHS			
Maximal relative growth rate	μ_{maxC}	$[h^{-1}]$	0.063
Substrate constant	K_{SC}	[μg C / ml]	3.0
Maximal relative death rate	D_{maxC}	$[h^{-1}]$	0.24
Substrate death constant	K_{dC}	[μg C / ml]	65.0
Yield coefficient	Y_C	[μg C / μg C]	0.45
Recyclable fraction of biomass	K_{rC}	[]	0.4
OLIGOTROPHS			
Maximal relative growth rate	μ_{maxO}	$[h^{-1}]$	0.01
Substrate constant	K_{SO}	[μg C / ml]	0.01
Maximal relative death rate	D_{maxO}	$[h^{-1}]$	0.005
Substrate death constant	K_{dO}	[μg C / ml]	10.0
Constant of relative death rate increase with substrate	K_L	[ml / h μg C]	$8.3 \cdot 10^{-6}$
Yield coefficient	Y_O	[μg C / μg C]	0.60
Recyclable fraction of biomass	K_{rO}	[]	0.1
EXTERNAL			
Maximal exudation rate	ExuM	[μg C / cm^3 h]	8
Exudation time constant	ExuT	$[h^{-1}]$	0.8
Moisture of soil	θ	[ml / cm^3]	0.23
AUXILIARY			
Factor to transform COPIOTROPHIC CFUs per g dry soil into microbial biomass C per cm^3 soil	Bc	[μg C g / CFU cm^3]	$4.8 \cdot 10^{-8}$
Factor to transform OLIGOTROPHIC CFUs per g dry soil into microbial biomass C per cm^3 soil	Bo	[μg C g / CFU cm^3]	$1.0 \cdot 10^{-8}$
Fraction of total COPIOTROPHIC bacteria that grow on a RICH medium	Cc	[]	0.35
Fraction of total COPIOTROPHIC bacteria that grow on a POOR medium	Co	[]	0.17
Fraction of total OLIGOTROPHIC bacteria that grow on a POOR medium	Oo	[]	0.05

organic matter content. Similarly, the BGF value was considerably higher (1.06 [μg C/(cm^3 soil h)]) for the validation experiment than for the calibration experiment, probably because the organic matter content was higher. The total amount of substrate released as background flux was 175 and 296 [μg C/cm^3 soil] for the 3rd week (500 hours model run time) and 4th week (655 hours model run),

Table 2. Variable model parameters for BACWAVE, a dynamic simulation model for copiotrophic and oligotrophic bacteria in soil.

PARAMETER	SYMBOL	UNIT	VALUE		
			Week 3[a]	Week 4[a]	Week 3[b]
INITIAL VALUES					
Biomass of copiotrophs	X_{Ci}	[μg C/cm^3]	1.9	4.6	6.7
Biomass of oligotrophs	X_{Oi}	[μg C/cm^3]	0.002	0.002	0.102
Substrate	S_i	[μg C/cm^3]	29.4	38.8	42.9
EXTERNAL					
Background substrate influx	BGF	[μg C/cm^3 h]	0.26	0.45	1.06

[a] – Experiment I
[b] – Experiment II

respectively, of the second experiment, and 546 [μg C/cm^3 soil] for the 3rd week (500 hours model run) of the first experiment.

Parameter D_{maxO}, representing maximal death rate of CB, was 0.24 [h^{-1}], very close to the value of this parameter reported before as 0.26 [h^{-1}] (Zelenev et al., 2000). Parameters responsible for the death rate of OB, D_{maxO} and K_{dO} , were 0.005 [h^{-1}] and 10 [μg C/ml], respectively. The actual range of the specific death rate fluctuations during model runs were 0.0014-0.0022 [h^{-1}] for OB, and 0.05-0.08 [h^{-1}] for CB.

Generalized apparent specific growth rate (difference between net specific growth and death rates; negative value implies apparent specific death rate) of OB fell in the range 0.0078-0.0087 [h^{-1}], and had always positive values at substrate content fluctuations within the range 127-247 [μg C/ml soil solution] (29-57 [μg C/cm^3 soil]). As for CB, the range of generalized apparent specific growth rate fluctuations was between -0.019 and +0.011 [h^{-1}] showing considerable bias to death at substrate content oscillations within the same range. The substrate stationary point value of CB (substrate concentration at which net death of bacteria is equal to net growth) was 184 [μg C/ml soil solution] (42.3 [μg C/cm^3 soil]), while for OB the value was 0.01 [μg C/ml soil solution] (0.0023 [μg C/cm^3 soil]) (Fig. 2A). Thus, OB are tolerant to low substrate concentrations and grow up in conditions, which are unfavorable for CB growth. At the same time CB are subject to rapid extinction when substrate level is slightly lower than the stationary point value. CB keep substrate concentration mostly below their stationary point value and hardly allow substrate concentration to exceed the value, but for OB this substrate concentration is sufficient for growth, since their stationary point values are far away from the range of substrate fluctuations which CB maintain (Fig. 2A and 2B).

Discussion

The model "BACWAVE" adapted for description of growth and death of OB and CB dependent on substrate content accurately simulated temporal dynamics and spatial distribution of bacterial CFUs isolated on C-rich and C-poor media along wheat roots. The model explained the dynamics of the variables as accurately as, or better than, the sum of significant harmonics did. This model successfully simulated bacterial dynamics in a number of cases differing in root age as well as soil quality. This was accomplished with a constant set of parameters responsible for bacterial growth and death, while only initial values of biomass and substrate, and only one external parameter - the rate of background substrate influx - were varied.

Simulation of an impulse of substrate from the root tip combined with a certain background flux resulted in wave-like behavior of CB in the rhizosphere along the root, whereas OB grew monotonously since the substrate concentration was sufficient for sustained growth. Darrah (1991a) compared a model with uniform exudation along a root with one with exudation and rhizo-deposition primarily at the root tip. He found significant differences between these models in microbial dynamics and soluble carbon concentrations (Darrah, 1991b), but variations in microbial population densities along the root were not explicitly studied and wave-like patterns were not visible from the figures (Zelenev et al., 2000). Scott et al.(1995) included root growth in their model and considered effects of matrix potential and diffusion of exudates between soil layers. Bacterial populations were modeled along the length of the root, starting with lux-marked *Pseudomonas fluorescens* bacteria applied on the seed. Simulated populations of *P. fluorescens* decreased monotonically with depth, while experimental populations fluctuated with depth (Scott et al., 1995). Another model for dynamics of bacteria in the rhizosphere presented by Toal et al. (2000) did not show any fluctuations either in bacterial biomass or in substrate, despite very similar equations for substrate supply and durations of runs as we used (Fig. 4). Models of bacterial dynamics in bulk soil did not result in wave-like dynamics either. Blagodatsky and Richter (1998) and Blagodatsky et al. (1998) modeled bacterial dynamics in soil including turnover of nitrogen through bacterial biomass. Their model did not result in fluctuations in any of the variables, although experimental data on microbial nitrogen dynamics in soil, which Blagodatsky et al. (1998) presented for model validation, showed evident fluctuations within 15 days.

The reason why fluctuations in bacterial biomass were not reproduced by the range of models presented in the literature could be, on the one hand, the use of a generalized set of model parameter values without distinction between trophic groups, whereas fluctuations can be revealed with parameter values specific for copiotrophic bacteria. On the other hand, differences in the equations used for growth and death of bacteria can be responsible for the differences in dynamics and spatial patterns of simulated populations. Darrah (1991a and b), Scott et al. (1995) and Toal et al. (2000) used constant bacterial relative death rates. Blagodatsky and Richter (1998) and Blagodatsky et al. (1998) applied a hyperbolic equation for a substrate dependent relative death rate of bacteria in soil. Nevertheless, none of these models simulated the experimentally observed fluctuations. The key issue is the relationship between growth and death curves in dependence of the substrate concentration. In

BACWAVE, there are cross-over points between growth and death curves at realistic substrate concentrations.

In the current work, bacterial biomass was satisfactorily transformed to CFUs isolated on rich and poor media when limitations of the plating technique to culture soil bacteria were taken into account. True OB did not fluctuate, but total bacteria on poor media (often incorrectly called oligotrophic in the literature) did oscillate. Dynamics of modeled total CFUs on poor medium largely coincided with those on rich medium. This observation is supported by the significant correlation coefficients (0.51-0.81) between experimental CFUs on rich and poor media (Semenov et al., 1999). Nevertheless, OB made up a significant proportion of the CFUs on poor medium towards the end of some model runs, corresponding to the root base area. This is in agreement with experimental results of Liljeroth et al. (1991) and Maloney et al.(1997), who showed that OB were more numerous than CB at the root base.

Modeled CB biomass fluctuations had a maximal amplitude of 6 [μg C/cm^3 soil], which decreased slightly in the course of 500-700 hours of model run. For the soil with relatively high organic matter content dampening of fluctuations was more obvious and fluctuations had greater frequency. This resulted from higher value of BGF influencing both these characteristics. Faster dampening of fluctuations indicates a greater resilience of microbial communities in a soil with higher organic matter content. This can be interpreted as a sign of a more 'healthy' soil (van Bruggen and Semenov, 2000).

For the soil with lower organic matter content (second experiment), the range of fluctuations in cell numbers was $3.8 \cdot 10^7$-$1.7 \cdot 10^8$, which is quite low for a soil. However, we actually modeled dynamics and distribution of active (cultivable) bacteria. Active bacteria comprise around 5% of total bacterial populations as determined by DAPI staining (Christensen et al., 1999). These authors applied a fluorescence *in situ* hybridization technique and demonstrated that only $4.8 \cdot 10^8$ active bacteria per g dry soil were discovered in bulk soil incubated for 2 days with 0.3% glucose, and even less in the same soil when air-dried and then rewetted.

In the same experiment with low-organic-matter soil, the range of changes in modeled microbial biomass was 1.3-4.0 μg C per g dry soil, assuming that 90% of microbial biomass in arable soils is bacterial biomass (Bloem et al., 1994). Assuming that microbial biomass carbon constituted 1.25% of the total soil organic matter content (Alvarez and Alvarez, 2000), the average microbial biomass was estimated as 85 μg C per g dry soil. Thus, our modeled microbial biomass comprised around 3% of this estimate. Again, this is close to the percentage active bacterial cells (Christensen et al., 1999). Similar proportions between modeled bacterial biomass and total amount of bacterial biomass in soil were found for the first experiment with a soil with higher organic matter content. Soil microbial biomass was estimated as 125 μg C/g dry soil according to Alvarez and Alvarez (2000). The range of modeled bacterial biomass fluctuations was 5.2-11.0 μg C/g dry soil, which was on average 6.5% indicating a higher proportion of active bacterial biomass in this soil.

Initial background flux rate (parameter BGF) needed to be adjusted for each experiment, probably due to unique properties of each of the experimental units and aging of roots. Soil with higher organic

matter content and older roots had a higher BGF. The BGF value for the C-richer soil was estimated as 1.06 [μg C/(h·cm^3 soil)], while this value was 0.26 to 0.45 [μg C/(h·cm^3 soil)] for the younger and older root in the poorer soil. The values of BGF for the low-carbon soil were of the same order of magnitude as the value (0.168 [μg C/(h·cm^3 soil)]) which was used by Toal et al. (2000) as a rate of continuous substrate input.

Since all internal parameters could be kept unchanged for all data sets, they can be considered as kinetic values, characterizing properties of these bacterial populations, even though the populations behaved differently depending on external conditions. So differences in initial and mean biomass values, numbers of peaks, amplitudes and periods of biomass oscillations could be attributed to external conditions. Thus, the model allows estimation of invariant properties of active copiotropic and oligotrophic microbial populations and ranges of substrate concentrations that are realistic for comparable soils and that govern bacterial population dynamics.

As the parameters μ_{max}, K_S, K_d and Y were predetermined from the literature (e.g. Semenov, 1991), we restrict the discussion to the values of the death rate parameters obtained by optimization (Table 1). Parameter D_{maxC} =0.24 [h^{-1}] was found to be approximately the same as in the previously developed model BACWAVE (0.26 [h^{-1}]) (Zelenev et al., 2000), but about 5 time higher than estimated by Blagodatsky and Richter (1998) and Blagodatsky et al. (1998), D_{maxC} =1.309 [d^{-1}] (0.055 [h^{-1}]). Nevertheless, values of $D_C(S)$ which were really achieved in our model runs were around 0.06 [h^{-1}]. The value of maximal relative death rate for OB was considerably lower: D_{maxO} =0.05 [h^{-1}]. Since there was no distinction between CB and OB in the model of Blagodatsky et al. (1998), the weighed average of parameters D_{max} of these two trophic groups of bacteria used in our model is similar to the value reported in that paper.

In conclusion, with a simple model with acceptable parameter values we succeeded to generate wave-like distributions of active fractions of bacterial biomass in the rhizosphere along wheat roots. We could also explain why total bacterial CFUs isolated on low-carbon media fluctuate in tandem with CFUs on high-carbon media (copiotrophs), even though the true oligotrophic bacterial biomass did not fluctuate, but increased monotonically from root tip to root base. The BACWAVE model is the first model to successfully simulate short-term bacterial fluctuations in soil. It could be used as the basis for more extensive models for microbial dynamics in soil that encompass nitrogen as well as carbon flows, and could constitute an improvement over existing models (Blagodatsky and Richter, 1998; Blagodatsky et al., 1998).

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Chapter 5

Short-term wave-like dynamics of bacterial populations in response to nutrient input from fresh plant residues

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Abstract

The objectives of the research were to investigate short-term dynamics of bacterial populations in soil after a disturbance in the form of fresh organic matter incorporation and to investigate how these dynamics are linked to those of some environmental parameters. To reach these objectives, soil bacterial populations, mineral nitrogen, pH, and redox potential (ROP) were monitored daily for one month after incorporation of clover-grass (CG) plant material in microcosm experiments. Colony-forming units (CFUs) and direct microscopic counts of FDA-stained and FITC-stained bacteria increased immediately after incorporation of the plant material, dropped within 2 days, and fluctuated thereafter. Harmonics analysis demonstrated that there were significant wave-like fluctuations with three or four significant peaks within one month after incorporation of clover-grass material. Peaks in CFUs were 1-2 days ahead of those in direct counts. Ammonium (NH_4^+) concentrations increased from the start of the experiments until nitrification commenced. Nitrate (NO_3^-) concentrations dropped immediately after plant incorporation, and then rose monotonically until the end of the experiments. There were no wave-like fluctuations in NH_4^+ and NO_3^- concentrations, so that bacterial fluctuations could not be attributed to alternating mineral N shortages and sufficiencies. pH levels rose and declined with NH_4^+ levels. ROP dropped shortly before NH_4^+ concentrations rose, and increased before NH_4^+ concentrations decreased; there were no regular fluctuations in ROP, so that temporary oxygen shortages may not have been responsible for the observed fluctuations in bacterial populations. Thus, for the first time, regular wave-like dynamics were demonstrated for bacterial populations after perturbation by addition of fresh organic matter to soil, and several potential reasons for the death phase of the fluctuations could be excluded from further consideration.

Introduction

Decomposition of fresh organic matter in soil has been studied extensively, and many models have been constructed to describe the decomposition process (Hassink, 1994; Probert et al., 1996; van Veen et al., 1984). The role of the soil microflora and -fauna in decomposition of fresh organic matter and release of NH_4^+ (and NO_3^- after nitrification) have also been documented extensively (Kuikman and van Veen, 1989; Verhoeff and Brussaard, 1990). However, detailed and frequent observations immediately after a disturbance like tillage or incorporation of fresh organic matter have rarely been made (Calderón et al., 2000, 2001; Lundquist et al., 1999). Microbial response can be very rapid and can lead to considerable mineralization of nitrogen during the first week following tillage or incorporation of low C/N plant material into soil (Jackson, 2000; Lundquist et al., 1999; van Scholl et al., 1997). Fast increases in free NH_4^+ can lead to nitrogen losses from soil after denitrification (Calderón et al., 2001), especially if a burst in microbial activity would exhaust the oxygen supply locally. In recent years, losses of nitrogen in the form of the greenhouse gas nitrous oxide have attracted attention (Priemé and Christensen, 2001). N_2O emissions are enhanced immediately after perturbations such as drying-wetting or freezing-thawing (Priemé and Christensen, 2001). Thus, it is important to study short-term effects of disturbances such as drying-wetting or incorporation of fresh organic matter into soil.

The above-mentioned disturbances are accompanied by an impulse of available organic matter into the

system, and may lead to fluctuations in populations of soil biota. Fluctuations in microbial processes or populations have been observed and graphically displayed, but seldom analyzed statistically or mathematically. For example, N_2O and CO_2 fluxes seemed to fluctuate with decreasing amplitudes within a period of about 5 days after a single perturbation (rewetting or thawing) (Priemé and Christensen, 2001). Similarly, microbial biomass carbon and total phospholipid fatty acids fluctuated during the first 14 days after (simulated) tillage (Calderón et al., 2000, 2001). No special attention was paid to these fluctuations. Amendment of various soils with grass resulted in two peaks in O_2 consumption within 2-3 days (Griffith et al., 2001). Although functional stability and resilience were the central theme of this paper, the fluctuations were not considered for characterization of stability or resilience as suggested previously (van Bruggen and Semenov, 1999, 2000). Finally, no attention was paid to potential fluctuations in microbial biomass in simulation models for decomposition of fresh organic matter in soil. These models resulted in single peaks in calculated CO_2 evolution and microbial biomass carbon and nitrogen, while the observed data showed at least two peaks within a period of several days or weeks, depending on the experimental resolution (Grant et al., 1993a,b).

Previously, we demonstrated that there were wave-like distributions of microbial populations along roots of wheat using harmonics analysis. The fluctuations in space and over time were shown to be moving waves (Semenov et al., 1999). The occurrence of these waves was hypothesized to be due to growth and death phases in response to an impulse of nutrients from a passing root tip. Death of microbes was considered to be a consequence of depletion of substrate, followed by regrowth on recycled carbon from bacterial necromass and additionally decomposing soil organic matter. The potential existence of growth and death cycles was substantiated by results from experiments with a moving artificial nutrient source in soil (van Bruggen et al., 2000). However, temporal wave-like dynamics were deduced from observed spatial wave-like distributions (van Bruggen et al., 2000; Zelenev et al., 2000). Temporal wave-like dynamics have seldom been analyzed by harmonics analysis (Gorbenko and Panikov, 1989), and not at all after incorporation of fresh organic matter into soil.

In preliminary experiments on short-term temporal dynamics of microbial populations and soluble carbon and nitrogen after incorporation of cover crop debris into soil there appeared to be fluctuations in bacterial populations and total soluble organic carbon concentrations over time (Hu et al., 1997; Semenov et al., 2002; van Bruggen and Semenov, 2000). However, the number of observations was too small to prove statistically (for example by harmonics analysis) that the observed fluctuations in microbial populations and soluble carbon and nitrogen formed significant waves (van Bruggen and Semenov, 2000). In these experiments, the decrease in microbial populations after the first peak seemed to coincide with an increase in NH_4^+ concentration, leading to the hypothesis that the drop in microbial populations may be associated with a temporary oxygen reduction (Priemé and Christensen, 2001). However, oxygen concentrations or ROP were not measured.

The overall aim of this research was to arrive at a better understanding of short-term microbial dynamics and associated nitrogen release from decomposing organic matter under controlled environmental conditions. Specific objectives of this research were: (1) to verify wave-like dynamics of bacteria after incorporation of clover-grass (CG) biomass into soil, (2) to relate release of mineral nitrogen to short-

term fluctuations in bacterial populations after incorporation of CG biomass in soil, and (3) to test the hypothesis that temporary nitrogen or oxygen shortages or low pH levels may lead to temporary declines and subsequent fluctuations in bacterial populations in response to addition of CG to soil.

Materials and methods

Soil

For all experiments reported here sandy soil was collected from a conventional arable farm located at Noordberg in Heelsum, the Netherlands, in March of 2000 and September of 2001. Barley (*Hordeum vulgare* L.) had been harvested from the field in the autumn of 1999 and barley residues were still visible when soil was collected in Spring of 2000. Barley was also planted in 2001, and barley stubbles were present when soil was sampled in September of that year. The soil was collected with a shovel to a depth of 20 cm along over a distance of 4 m at the border of a field, for a total of about 0.5 m^3 of soil. After collection, the soil was slightly dried, sieved through a screen with 2-mm mesh and stored until the start of the experiments. Field moisture content of the soil was determined immediately on sub-samples collected from the field. Air-dried samples were analyzed for general soil characteristics. The sand, silt and clay contents were 91.4%, 5.6% and 3.0% respectively, and the total organic carbon content was 3.0%. The total N and P contents were 933 and 732 mg/kg, and the total available N, P, and K contents were 19.7, 6.2, and 125.0 mg/kg, respectively. The soil pH (KCL) value was 6.8. Three days before the start of each experiment a calculated amount of water was added to the soil to adjust its moisture to 60% of field capacity value (11.6% of wet weight basis). Moistened soil was put into a plastic basin and covered with a plastic film to prevent desiccation.

Experimental setup

The experiments were carried out three times, in the Spring and Fall of 2000, and in the Fall of 2001. Each experiment consisted of two series of plastic pots containing soil without amendment (control series) or soil amended with a mixture of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). There were 3 pots for each sampling time and treatment in each experiment. The pots were randomized in two incubators (25ºC) and incubated for time periods of 26, 30 or 34 days depending on experiment. A day before the start of each experiment, grass and clover were cut from a field experiment at Wageningen University. Immediately after collection the material was cut into pieces so that they could pass through 2-cm mesh. The plant material was kept in a cold room (5ºC) till incorporation into the soil. At the start of each experiment, a mixture of grass and clover was made (1 : 1 ratio for fresh weight, and 1.27 : 1 to 1.79 : 1 for dry weight). The carbon and nitrogen contents of the plant material was determined by an EA 1110 element analyzer (CE instruments, Milano, Italy) according to the principle of Dumas in the Soil Analytical lab at Wageningen University on 3 plant samples with 3 sub-samples each. The average carbon and nitrogen contents based on dry weights were 43.9% and 2.8% for grass, and 44.6% and 4.4% for clover, respectively. The C:N ratio of the plant mixture was on average 12.8. This plant mixture was subsequently mixed with soil at a ratio of plant mixture : soil of 0.05 : 1 (wet : wet weight) or 0.01 : 1 (dry : dry weight), corresponding to 29.4 t/ha dry weight (assuming a soil bulk density of 1.37 g/cm3 and plough depth of 20 cm). The final C:N ratio of the plant-soil mixture ranged from 26.1 to 27.5, and it's moisture content ranged from 14.5 to

15.5%. Square plastic pots of 11x11x12 cm size were filled with this mixture or with unamended moistened soil (1 kg per pot). To limit drying of the soil, plastic film was placed on the bottom of each pot before filling the pot. After being filled every pot was covered with a thin polyethylene film to allow for air-exchange while minimizing water-vapor losses. During the course of the experiments, the soil moisture dropped between 0.06% and 0.17% per day.

Sub-sampling

Every day 3 pots of each series were randomly taken from both incubators for destructive sampling. To minimize potential edge effects, soil samples were taken with a brass corer (1.5 cm diameter) from the surface to the bottom in the center of a pot. Each soil core was placed into a plastic flask and thoroughly mixed. Sub-samples of the mixed samples were taken separately for determination of bacterial populations (CFUs and direct counts), soil moisture, pH, and mineral nitrogen content.

Plate-counting of bacteria

CFUs were determined in experiments 1 and 2. Ten g of soil was added to 90 ml of sterile deionized water, and shaken on a rotary shaker for 15 min at 300 rotation per min. The suspension was sonicated in an ultra-sonic device for 5 min (Branson 5200, Branson Ultrasonic Corporation, Danbury CT, USA). Ten-fold serial dilutions were made. One hundred µl of the 10^{-5} and 10^{-6} suspensions were plated on triplicate1 plates with agar medium for quantification of copiotrophic bacteria as described in Semenov et al. (1999). Agar medium contained 0.5 g $MgSO_4*7H_2O$, 0.5 g KNO_3, 1.3 g $K_2HPO_4*3H_2O$, 0.06 g $Ca(NO_3)_2*4H_2O$, 2.5 g glucose, 0.2 g enzymatic casein hydrolysate, and 15.0 g Bacto Agar per liter and 100 ppm sterile cycloheximide. The total amount of carbon was estimated at of medium. Bacterial colonies were counted after 60 hours of incubation at 25 ^oC.

Microscopic counting of bacteria

Direct counts of bacterial cells were made in experiments 1 and 3. The same soil suspensions were used as prepared for plate-counting. Sonicated soil suspensions were shaken vigorously for 10-15 sec by hand and left still for 5 min. The necessary numbers of serial dilutions (mostly 3 dilutions) were made to get appropriate counts afterwards. Concentrations of both active and total soil bacteria were determined after staining with Fluorescein Diacetate (FDA) (F-7378, Sigma Chemical CO, PO Box 14508, St. Louis MO 63178, USA) and Fluorescein Isothiocyanate (FITC) mixed isomers (F-3651, Sigma Chemical CO), respectively. Cell suspensions were stained with FDA according to Bottomley (1994), and FITC according to Babiuk and Paul (1970). The stained samples were filtered through a 0.2 um membrane Nucleopore filter (Nucleopore® (PC) Polycarbonate, Corning Costar Corporation, Acton, MA 01720, USA) according to Bloem (1995). Filters were rinsed according to Bloem (1995) and mounted onto a microscopic slide with a drop of immersion oil. Bacteria on filters treated with either of the two stains were counted under an epifluorescence microscope (ZEISS Axioskop 20 with a HBO 50 mercury lamp for fluorescence-illumination, Carl Zeiss Jena GmbH, D-07740 Jena, Germany) using an oil immersion lens at 1000× total magnification and a fluorescein filter set (Blue 450-490 nm; excitation filter: BP 450-490 (44 77 22); chromatic beam splitter: FT 510 (44 64 34); barrier filter: LP520 (44 77 37)). Every sample filter was examined according to a Z-route and as a rule 25-30 fields were counted along the way.

Soil mineral nitrogen concentrations

Mineral nitrogen concentrations were determined for each experiment. Twenty gram of moist soil was placed into a plastic flask, 50 ml of 1M KCl was added into the flask, and then it was shaken for 2 h on a linear shaker at 120 movements/min. Immediately after shaking, 2 ml of suspension was placed into an Eppendorff tube and centrifuged for 10 min at 15,000 rotations per minute. The supernatant was transferred into a clean Eppendorff tube and analyzed immediately or kept frozen (-86 ºC) until it was analyzed. Determination of NO_3^- and NH_4^+ content in the supernatant was carried out on an automatic inorganic nitrogen analyzer, an AutoAnalyzer™ II System (TECHNICON™ Industrial Systems, Technicon Instruments Corporation, International Division Headquarters, 6-10 Quai de Seine, 93200 St. Dennis, France).

Redox potential and pH measurements

In experiments 2 and 3, the ROP of the soil was measured with platinum electrodes and a glass calomel reference electrode in each pot as described previously (Blok et al., 2000). ROPs were expressed as E_h values by adding 247 mV (potential of a calomel electrode against a standard electrode) to the measured potentials. After taking samples of soil suspension for plating and staining, the pH of the suspension was measured with a pH-meter (inoLab pH Level 1, Wissenschaftlich Techische Werkstätten D-82362, Weilheim, Germany).

Statistical analyses

The General Linear Models (GLM) procedure of SAS (SAS Institute Inc., Box 8000, Cary, NC 27511-8000, USA) was used to evaluate effects of treatments (control and CG amendment) and dates on the behavior of the measured variables. Geometric means and their standard errors were calculated of the three replicates at each sampling time according to Snedecor and Cochran (1979). Assuming log-normal distributions (Semenov et al., 1999), the standard error was calculated as follows:

$$SE_{(log\text{-}normal)} = M_G * (10^{(SE(\log 10(bi))} - 1)$$

Where$SE_{(log\text{-}normal)}$ = standard error of log-normally distributed data

M_G = geometric means of the original data set

$SE(\log 10(b_i))$ = regular standard error of decimal logarithms of the original data.

To detect the presence of significant wave-like fluctuations, harmonics analysis was conducted on the geometric means according to Jenkins and Watts (1968) with modifications according to Gorbenko and Kryshev (1985) and Semenov et al. (1999). Before harmonics analyses, the data were smoothed using Hann's 3-point window (Shumway, 1988) and detrended using regression analysis. The predicted values from harmonics analysis were back-transformed before plotting them versus time. Cross-correlation functions (Jenkins and Watts (1968) were calculated for all variables (non-smoothed) with the software Statistica for Windows (Release 4 B, StatSoft Inc., 1993).

Results

Bacterial populations

CFUs increased immediately after incorporation of the plant material with a first peak after 2 days,

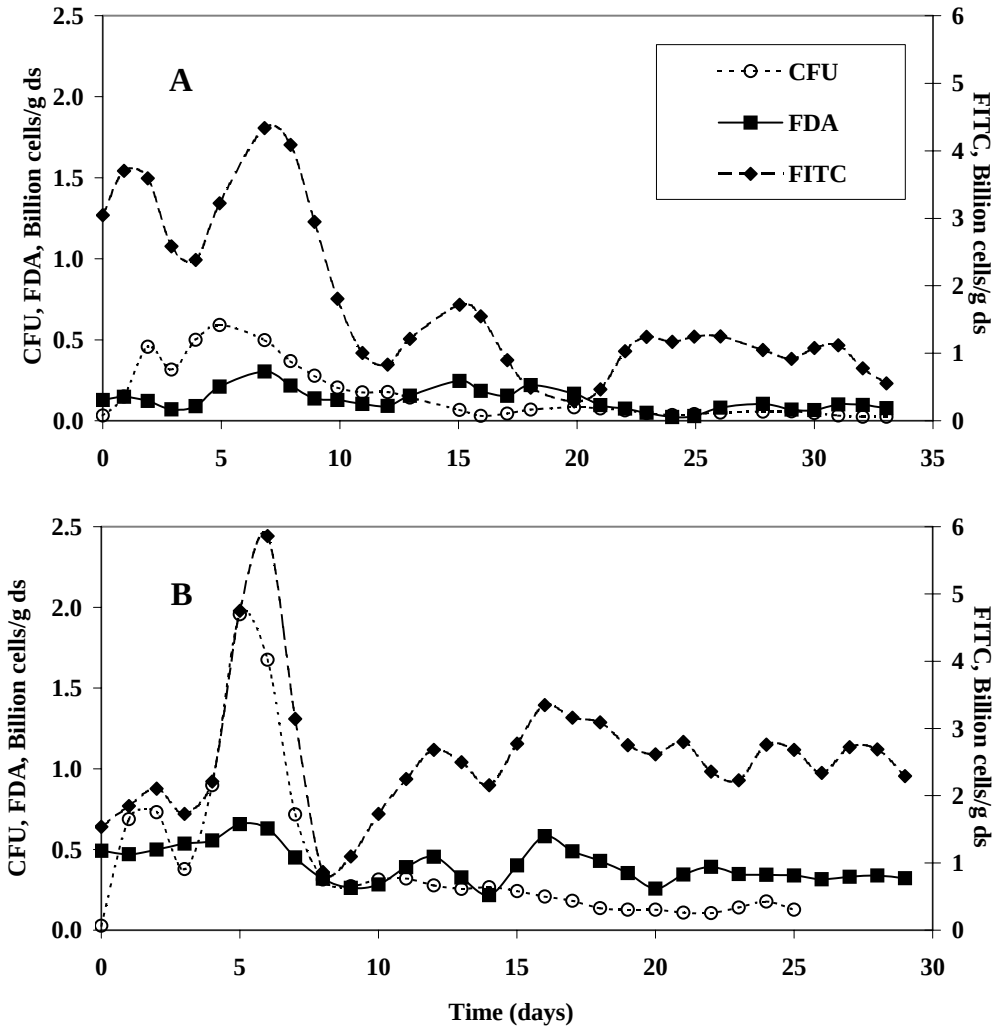

Figure 1. Fluctuations in geometric means (n=3) of colony forming units (CFU), FDA- and FITC-stained cells over time in days since incorporation of clover-grass material into soil in three pot experiments in the Spring of 2000 (A) and in the Fall of 2000 (CFU) or 2001 (direct counts) (B). Mean standard errors among replicates were 6.5×10^6, 1.4×10^6, 1.4×10^9 for CFU, FDA and FITC per g dry soil in the Spring experiment; and 29×10^6, 7.5×10^6 and 0.1×10^9 per g dry soil in the Fall experiments.

dropped and rose again with a second peak after 5 days (Fig. 1). This timing was consistent among experiments (Fig. 1 A and B). The first peak was always smaller than the second peak. These two peaks were followed by weak but significant fluctuations ($P<0.05$). The average numbers of CFUs in amended soils were significantly (10-40 times; $P<0.01$) higher than in unamended soils (data not shown); the fluctuations were also much larger in amended soils. The standard errors among replicates of the CG treatments were small compared to the fluctuations in time. Harmonics analysis of CFUs in CG amended soil resulted in three and two significant harmonics in the first and second experiment, respectively (Fig. 2; Table 1). Together, these harmonics contributed 63.5% and 43.3% to the observed variation in CFUs. The phases and periods varied among experiments, because only the third

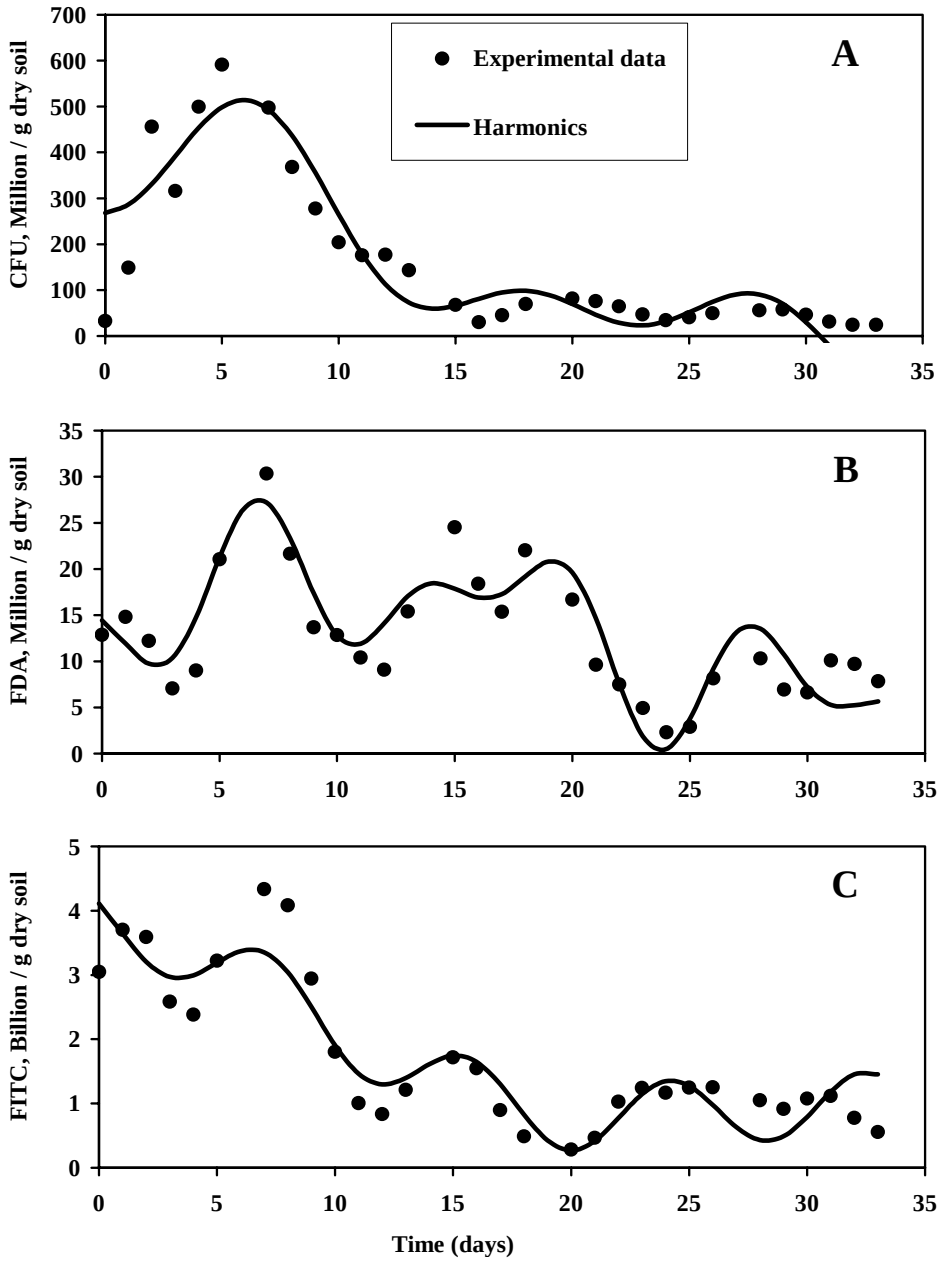

Figure 2. Harmonics curves and geometric means (n=3) of CFU (A), FDA-stained cells (B) and FITC-stained cells (C) over time in days since incorporation of clover-grass material into soil in a pot experiment in the Spring of 2000.

and fourth harmonics were significant in the second experiment, while the first three harmonics were significant in the first experiment. The amplitudes were much greater in the second than in the first experiment, probably because the clover-grass was collected in the Fall for the second experiment and in the Spring for the first experiment. The first brief peak was not detected by harmonics analysis because its contribution to the variance of the whole series was relatively small (Fig. 1 and 2).

Table 1. Characteristics of significant harmonics (harmonic number, amplitude, phase, period, frequency, contribution to variance, calculated F-value) detected by harmonics analysis for dynamics of bacteria populations (CFUs and FDA- or FITC-stained direct cell counts / g dry soil) after clover-grass biomass incorporation into soil. nnumber of observations in each series.

Experiment	Variable	No. Harmonics	Amplitude *	Phase (days)	Period (days)	Frequency ($days^{-1}$)	Contribution to variance (%)	F calculated	n
1	CFU	1	7.90E+07	3.55	34.0	0.03	20.3	3.35 ***	34
		2	8.75E+07	6.93	17.0	0.06	24.4	4.03 ***	34
		3	7.67E+07	6.19	11.3	0.09	18.8	3.10 **	34
1	FDA	1	3.85E+07	12.94	34.0	0.03	17.6	2.90 **	34
		3	5.61E+07	6.54	11.3	0.09	37.2	6.14 ***	34
		5	3.99E+07	-0.19	6.8	0.15	18.9	3.11 **	34
1	FITC	1	6.73E+08	0.71	34.0	0.03	36.9	6.08 ***	34
		4	5.40E+08	-1.37	8.5	0.12	23.8	3.92 ***	34
2	CFU	3	2.65E+08	4.68	8.7	0.12	22.5	2.81 **	26
		4	2.55E+08	-2.09	6.5	0.15	20.8	2.60 **	26
3	FDA	2	5.71E+07	3.20	15.0	0.07	18.5	2.68 **	30
		3	6.13E+07	5.38	10.0	0.10	21.2	3.08 **	30
		5	6.99E+07	-0.96	6.0	0.17	27.7	4.01 ***	30
3	FITC	3	6.69E+08	5.61	10.0	0.10	24.7	3.59 ***	30

* (CFU or cells/ g dry soil)
** = significant at 0.1 level
*** = significant at 0.05 level

Direct microscopic counts of FDA-stained and FITC-stained bacteria showed also two peaks within 10 days after clover-grass incorporation into soil (Fig. 1). These peaks in direct counts lagged one to two days behind the first two peaks in CFUs. Direct counts continued to fluctuate after the initial two larger peaks (Fig. 1). The average numbers of FDA-stained cells in amended soils were significantly (20-30 times; $P<0.01$) higher than in unamended soils (data not shown), with greater fluctuations in amended soils. However, the average numbers of FITC-stained cells were only 1.3–1.7 times lower in control soils than in amended soils (data not shown); these differences were not significant. Harmonics analysis demonstrated that there were significant wave-like fluctuations with three and two significant harmonics for FDA and FITC, respectively, after incorporation of clover-grass material (Fig. 2; Table 1). In the second experiment only the third harmonic was significant for FITC. The harmonics for FDA and FITC data accounted for 73.7% and 60.7% of the variation in the first experiment and 67.4 and 24.7% of the variation in the second experiment. Fluctuations in FDA-stained cells were most consistent.

Table 2. Significant (P<0.05) cross-correlation functions (CCF) among measured variables at different lags (in days) in three experiments, in which and redox potential (ROP), pH, NO_3^-, NH_4^+, bacterial CFU, FDA-stained bacteria, and FITC-stained bacteria were measured daily after incorporation of clover-grass into soil. ROP was not measured in the first experiment.

Variable	Experiment	pH		NO_3^-		NH_4^+		CFU		FDA		FITC	
		CCF	Lag*	CCF	Lag*	CCF	Lag*	CCF	Lag*	CCF	Lag*	CCF	Lag*
ROP	2	-0.9	0	0.98	0	-0.9	1	-0.5	-1	nd		nd	
	3	-0.6	2	0.97	0	-0.8	0	nd		-0.44	0	ns	
pH	1			-0.94	-1	0.92	0	0.92	0	0.44	1	0.80	1
	2			-0.90	-1	0.98	0	0.62	-1	ns		ns	
	3			-0.62	-2	0.81	0	nd		0.45	0	0.45	1
NO_3^-	1					-0.9	2	-0.9	2	ns		-0.8	1
	2					-0.8	1	-0.5	0	nd		nd	
	3					-0.8	1	nd		-0.42	0	ns	
NH_4^+	1							0.95	0	0.42	1	0.69	2
	2							0.64	-1	nd		nd	
	3									0.50	1	ns	
CFU	1									0.41	1	0.68	2
	2											nd	
	3											nd	
FDA	1											0.51	0
	2											nd	
	3											0.52	0

nd = not determined
ns = not significant
* - POSITIVE (NEGATIVE) lag values mean that the variable in the first cell of the row LEADS (FOLLOWS) the variable in the top cell of the column with a |LAG| number of days

There were significant (P<0.05) positive correlations between FDA- and FITC-stained bacteria at lag 0 in both experiments where direct counts were made (Table 2). CFUs of bacteria on agar plates were positively correlated with FDA- and FITC-stained cells at a lag of 1 or 2 days in the first experiment. Thus, CFUs were indeed ahead of direct counts by 1 or 2 days, as observed visually. CFUs and FDA-stained cells (but not FITC-stained cells) were negatively correlated with ROP (at lag 1 and 0, respectively). CFUs were positively correlated with pH and NH_4^+ and negatively with NO_3^-. FDA was weakly positively correlated with pH and NH_4^+, and in only one experiment negatively with NO_3^-. FITC was positviely correlated with pH and NH_4^+ and negatively with NO_3^- in the first experiment, but not in the third experiment. The soil chemical variables were correlated among each other as expected (Table 2).

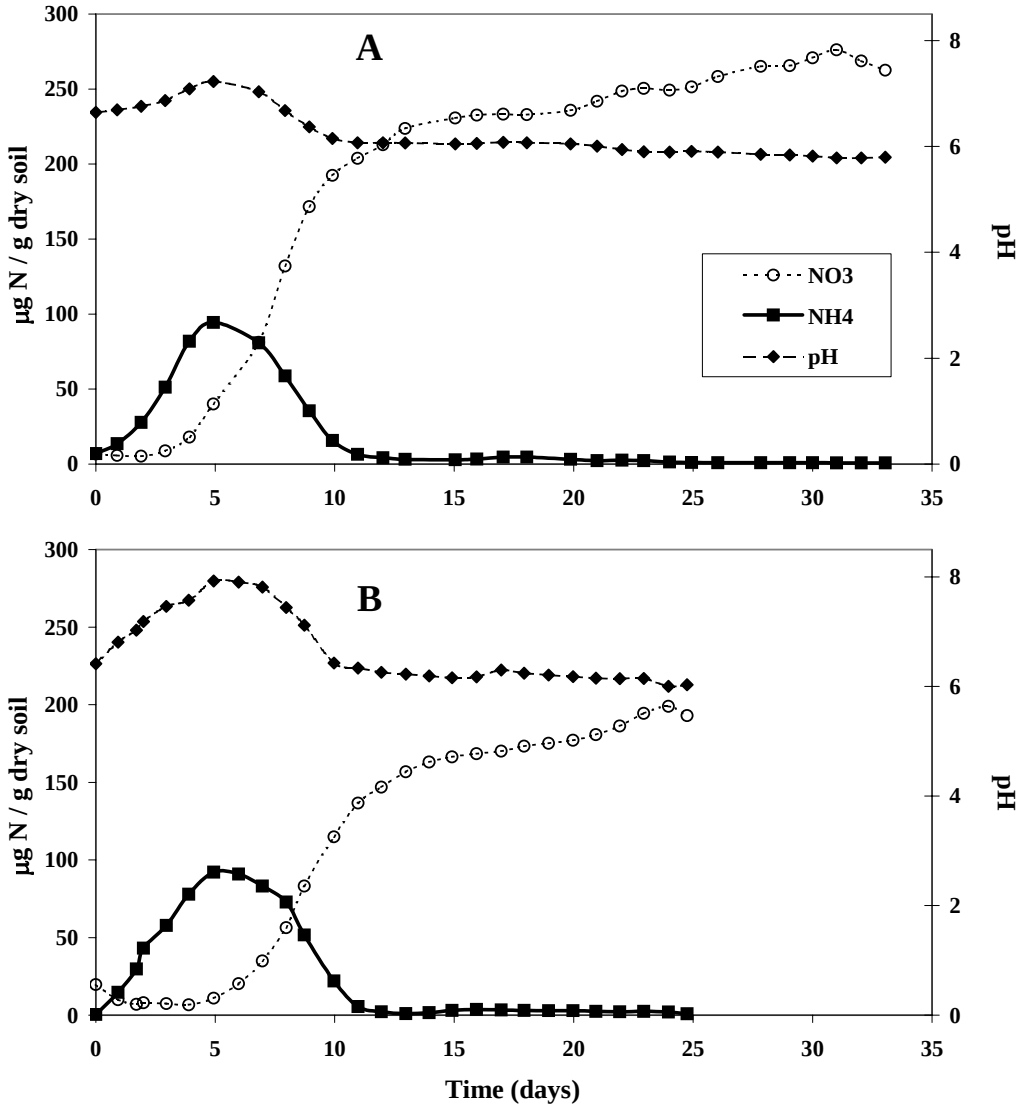

Figure 3. Mean NO_3 and NH_4 concentrations (n=3) and pH values (n=3) in soil since incorporation of clover-grass material into soil in two pot experiments in the Spring of 2000 (A) and Fall of 2000 (B). Mean standard errors among replicates were 1.9 and 0.7 µg N per g dry soil for NO_3 and NH_4 in experiment 1; and 2.7 and and 1.2 µg N per g dry soil for NO_3 and NH_4 in experiment 2. Standard errors for pH were 0.01 and 0.02 in experiment 1 and 2, respectively.

Soil nitrate and ammonium

There were significant ($P<0.01$) interactions between the CG treatment and time with respect to NO_3^- and NH_4^+ concentrations. The NO_3^- concentrations dropped from 14 µg/g dry soil in the control treatment to 4 µg/g dry soil after clover-grass incorporation as soon as the bacterial populations increased. NH_4^+ concentrations increased immediately (Fig. 3), and continued to increase as bacterial populations started to decline. The NH_4^+ content declined when the bacterial populations declined precipitously after the second peak (Fig. 1). The declines in NH_4^+ and bacterial populations were preceded by an increase in ROP (Fig. 4). When NH_4^+ concentrations started to drop, NO_3^- concentrations started to increase due to nitrification, and there were significant negative correlations

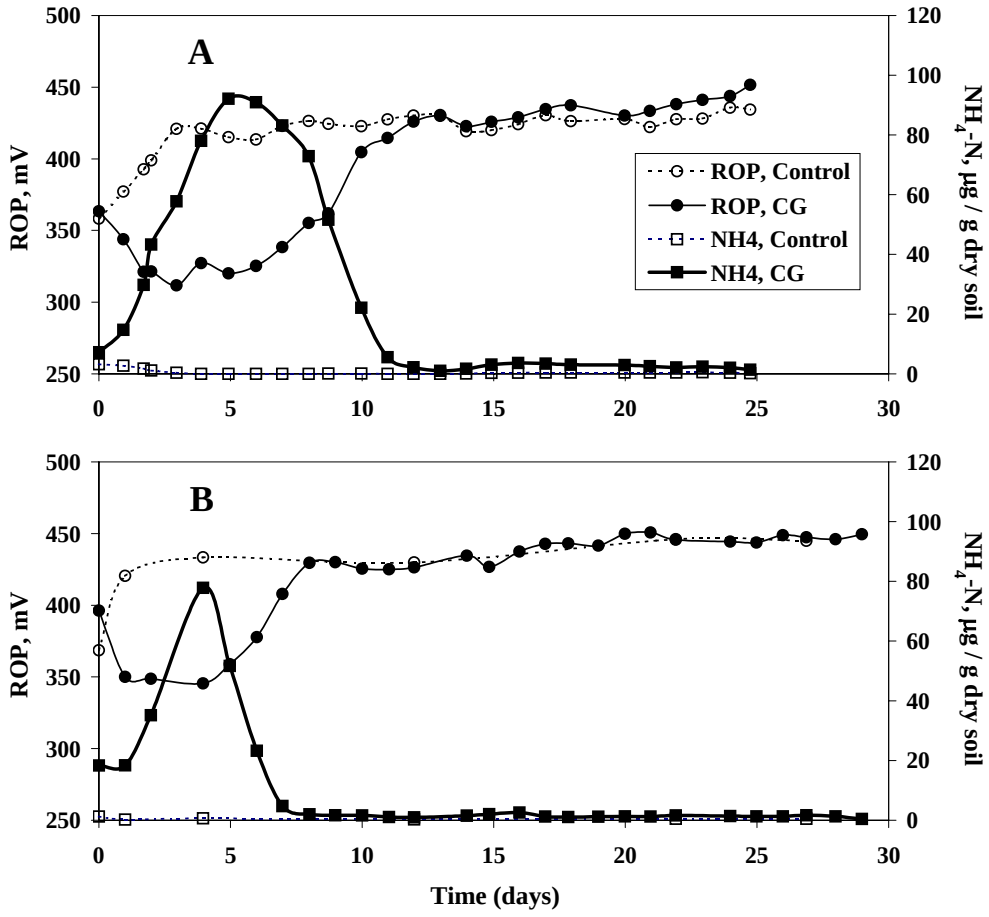

Figure 4. Mean redox potential (n=3) and NH_4 concentration (n=3) in clover-grass amended soil (CG) and control soil (C) over time in days in the Fall of 2000 (A) and 2001 (B). Mean standard errors among replicates in CG treatments were 1.5 mV and 1.8 mV for redox potential and 1.2 and 0.2 µg NH_4-N per g dry soil in the two experiments.

between NH_4^+ and NO_3^- concentrations at lags of 1-2 days (Table 2). This means that the minimum in NO_3^- took place 1-2 days earlier than the maximum in NH_4^+. After this minimum, NO_3^- content continued to increase monotonically until the end of the experiments (Fig. 3). There were no wave-like fluctuations in NH_4^+ and NO_3^- concentrations after the first peak or increase, so that bacterial fluctuations could not be attributed to alternating N shortages and sufficiency's. There were comparatively small changes in these variables in the control treatments over time: generally for all the experiments NH_4^+ changed within the ranges 0-3.5 in control versus 0.4-100 ug N/g dry soil in experimental series, in the case of NO_3^- ranges of changes for control series were 12-44 and for amended series 5-287 ug N/g dry soil.

pH and redox potential

There were significant ($P<0.05$) interactions between CG treatment and time with respect to pH and ROP. pH levels rose and declined with NH_4^+ concentrations (Fig. 3). ROP dropped significantly in the CG treatment as NH_4^+ concentrations rose (Fig. 4), and increased before NH_4^+ concentrations

decreased. Thus, there were significant negative correlations between ROP and NH_4^+ concentrations (Table 2). ROP was also negatively correlated with pH, and the change in ROP took place at the same time or 2 days earlier than that in pH (Table 2). After the initial peak in pH and trough in ROP, there were no additional fluctuations in these variables. There were considerably lower changes in these variables in the control treatments: the range of pH changes was 4-20 times, and the range of E_h changes twice lower than for amended treatment.

Discussion

For the first time, we demonstrated that there were significant wave-like dynamics in bacterial populations when daily observations were made after a disturbance, namely incorporation of fresh plant material into soil. Peaks in CFUs could be detected before peaks in direct bacterial cell counts were registered. This that fast-growing culturable bacteria increase in soil before the majority of bacteria increase. FDA- and FITC-stained bacterial cells also fluctuated, and did this almost simultaneously (FITC-stained cells were slightly behind FDA-stained cells). Thus, regular waves were detected by all three estimates of bacterial populations after a perturbation by which nutrients were added to soil, although, as could be expected, the fluctuations in total (FITC-stained) cells were less pronounced than those of active (FDA-stained) cells and CFUs. Similar to our observations of earlier peaks in CFUs than direct counts, active *gfp-lux* marked cells (quantified by luminometry) peaked earlier than total cells (quantified by fluorescence counts), but this was not particularly noted by the authors (Unge et al., 1999).

Irregular fluctuations and regular oscillations in bacterial populations have been known for a long time (Zvyagintsev and Golimbet, 1983), but the wave-like nature has seldom been proven statistically Gorbenko and Panikov, 1989). Thus, Gorbenko and Panikov (1989) demonstrated long- and short-wave dynamics (22-44 and 2-15 days, respectively), the last dynamics being related to numbers of protozoa and concentrations of dissolved organic substances. However, the frequencies of these measurements did not coincide, and measurements were not made in relation to a particular disturbance.

The population dynamics of other organisms has been described as periodic, wave-like or chaotic using deterministic analytical models, in particular when host-parasite and predator-prey interactions were studied (Gilligan, 1995; Sherratt et al., 1995). Spatially chaotic patterns can revert to regular, wave-like patterns as a result of a perturbation, resulting in synchronization (Doebeli and Ruxton, 1998). This holds for both multiple-species and single-species models (Doebeli and Ruxton, 1998). For example, chaotic distributions of gipsy moth populations simulated by non-linear reaction diffusion equations could change to homogeneous travelling wave patterns with a constant velocity, depending on the time, location, and strength of the perturbation (Christie et al., 1995). There seemed to be a threshold for the strength of a perturbation above which the wave pattern became regular (Christie et al., 1995). In our experiments, significant regular patterns were observed after a large perturbation (addition of organic amendment), while small less regular fluctuations were observed in the unamended control treatments (data not shown).

The reasons for the wave-like dynamics, in particular for cell death, are still not clear. However, available nitrogen was probably not a factor involved in wave-like fluctuations of bacteria in these experiments (where an N-rich amendment was used), because only one peak in NH_4^+ was detected, and NO_3^- was increasing monotonously. Even in the unamended control treatments, small fluctuations in NH_4^+ concentrations were irregular, and not related to small microbial population fluctuations (data not shown). Thus, only the limitation by carbon after a burst of microbial biomass remains the main cause for bacterial death among nutritive factors. So dependence of bacterial dynamics on carbon level is the most important reason for the oscillating pattern of the dynamics.

Similar to the single peak in NH_4^+ concentration, there was only one clear trough in ROP. ROP values did not drop below 300 mV during the first 7-10 days. This value falls in the range of 'slightly reducing' conditions (Orlov, 1985). After 7-10 days, the ROP values rose to >400 mV, in the range of 'slightly oxidizing' conditions (Orlov, 1985). However, a ROP electrode measures average values at a coarse scale. At micro-scales, there could have been considerable oxygen shortages, justifying additional small-scale oxygen measurements to investigate potential effects of locally anaerobic conditions on bacterial cell death.

Temporary oxygen shortages (due to a fast increase in microbial populations) could be partially responsible for the observed fluctuations in bacterial populations during the first days after CG incorporation. This is supported by the fact that short-term peaks in N_2O emission after freezing-thawing and drying-rewetting were partially due to denitrification within 1-2 days after the thawing or rewetting event (Priemé and Christensen, 2001). From the graphs presented by Priemé and Christensen (2001) it appears that N_2O peaks occurred at the same time as CO_2 dropped. Jackson (2000) also attributed short-term losses of nitrogen from a ^{15}N-labeled cover crop to denitrification as a result of high microbial activity; and Calderón et al. (2001) showed that there was a peak in denitrification four days after tillage.

In our experiments, the drop in ROP values in the amended series suggests at least partial oxygen depletion during the first week after plant residue incorporation (Fig 4A and B). Although the ROP values were only moderately low on average, there could have been anaerobic micro-niches in the soil, possibly leading to death of bacteria a few days after CGs incorporation. A role of oxygen depletion in the wave-like bacterial dynamics can also be deduced from the absence of regular oscillations in respiration rate in respirometer tubes where a constant air flow was led over small samples of CG amended soil so that anaerobic conditions could not take place (Zelenev, unpublished).

Changes in soil chemical variables were clearly related to microbial populations (Table 2). Stronger positive correlations of NH_4^+ concentrations with CFU's (at lags 0 to 1) than with direct counts (at lags -1 to -2) suggest that culturable bacteria play the main role in ammonification, while nitrifyers develop when active and total bacterial counts increase. This is supported by the remarkably high positive correlations ($r=0.97$ and 0.98) between ROP and NO_3^- content, indicating that both are strongly dependent on oxygen concentration and availability in soil. Although ROPs were negatively correlated

with NH_4^+ content, these correlations were not as strong as those with NO_3^- content, indicating that ROP and NH_4^+ dynamics are not as tightly linked, and are partially governed by independent processes. In particular, release of NH_4^+ is not so strictly determined by oxygen content in soil as ROP values.

Populations of potential predators were not examined in this investigation. Regular oscillations in bacterial populations have since a long time been ascribed to predator-prey or host parasite-relationships in microcosm studies (Habte and Alexander, 1975). Individual gfp marked species also fluctuated after introduction into field soil (Unge et al., 1999). Protozoa can respond quickly to increases in bacteria and reach a peak a few days after the first peak in fast-growing culturable bacteria (Thirup et al., 2000). However, it is unlikely that predator-prey relations would be responsible for the first two peaks within one week after the start of our experiments, because the soil had been air-dried before the experiments, and populations of protozoa and nematodes were probably still quite low. In the literature, maxima in protozoa populations do not always coincide with minima in bacterial populations (Clarholm, 1981; Thirup et al., 2000), or protozoa accounted for only part of the decline in microbial biomass (Clarholm, 1981). Thus, it is still questionable if fluctuations in prey populations are governed by predators, or if fluctuations in prey populations are initiated by other factors and predator population fluctuations follow those of the prey.

Another potential reason for wave-like fluctuations may be a discontinuous succession in microbial communities as the substrate changes over time. This possibility was investigated in a preliminary experiment using substrate utilization profiles, phospholipid fatty acid analyses, and denaturing gradient gel electrophoresis on 16rDNA. However, no changes in community characteristics could be detected among successive peaks. Instead, the communities during the growth phases differed from those during the death phases, but were similar from peak to peak, based on substrate utilization profiles (van Diepeningen et al., 2003; de Vos and van Bruggen, 2001). Thus, a simple explanation like discontinuous succession is also unlikely. The most likely remaining factor is competition for easily available substrate. We were able to simulate such a scenario in a simulation model for bacterial growth and death in the rhizosphere (Zelenev et al., 2000), where wave-like distributions of bacteria along wheat roots were derived from temporal dynamics of bacteria at each point in soil after a root tip passed, taking only bacteria-substrate interactions into account.

Our observations on wave-like dynamics of bacterial populations after perturbation by addition of fresh organic matter to soil create the opportunity to use the periods and amplitudes of microbial resistance and resilience of microbial communities to a disturbance, which can be used as indicators for soil health (van Bruggen and Semenov, 1999, 2000).

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Chapter 6

Daily changes in bacteria-feeding nematode populations oscillate with similar periods as bacterial populations after a nutrient impulse in soil

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Abstract

Previously, we showed that bacterial populations oscillated in a regular manner in response to a nutrient impulse in soil. For this paper we investigated if the wave-like fluctuations in bacterial populations could be explained by their interactions with populations of bacterial-feeding nematodes (BFN). In two microcosm experiments, soil bacterial populations - colony forming units (CFU) and microscopic counts of stained bacteria and nematode populations in 22 families were monitored daily for 25 or 30 days after incorporation of clover+grass (CG) plant material into soil. In another microcosm experiment, dynamics of bacteria and nematode populations were monitored in response to gamma-irradiated plant material added to gamma-irradiated soil mixed with filtered bacterial suspensions and in non-irradiated soil. In the first experiment, soil bacterial populations fluctuated significantly after incorporation of the plant material with 2 peaks within the first week and 3 or 4 smaller peaks thereafter. Populations of total nematodes and BFN started to increase in the second week after CG incorporation, but the proportion of BFN increased within one week. Inactive juvenile BFN (dauerlarvae) seemed to be activated after two days (as the percentage of Rhabditidae increased and dauerlarvae decreased), followed by step-wise increases in dauerlarvae every four days, indicating that there was a new generation every four days. There were significant wave-like fluctuations in daily population changes of BFN, but not for those of total nematode communities, over the duration of these experiments. These fluctuations had similar periods (5 days) as those of bacterial populations, but were shifted about 3 days relative to the bacterial fluctuations. Gamma-irradiation of soil significantly increased the periods and amplitudes of bacterial oscillations. Nematode populations were decimated in gamma-irradiated soils, but small numbers of protozoa were accidentally introduced in the irradiated soil, and may have been partially responsible for the delayed regulation of bacterial growth. We conclude that fluctuations in bacterial populations were not directly related to similar fluctuations in populations of BFN, as expected from classical Lotka-Volterra equations for predator-prey relationships, but were related to changes in growth rates of BFN. An alternation in active and inactive stages in a synchronized predator community after a disturbance could allow periods of bacterial growth alternated with periods of death. Fluctuations in bacterial populations were dampened after a much longer period when the soil fauna was largely eliminated.

Keywords: Bacteria, disturbance, nematode community, oscillations, predator-prey relations, waves

Introduction

Cyclical dynamics of soil bacteria and their predators are usually not apparent since they frequently occur out of phase in different microniches in soil (Clarholm, 1981). However, strong disturbances that act on all microniches may temporarily synchronize biological activities throughout the soil, resulting in clearly distinguishable oscillations in various organisms (Clarholm, 1981). Examples of such disturbances are drying-rewetting, freezing-thawing, tillage, and incorporation of crop residues. These are all accompanied by an input of carbon sources into the system leading to fluctuations in populations of soil biota.

Although fluctuations in microbial populations and processes can be gleaned from the figures in several papers, they have seldom received attention from the authors. For example, microbial biomass carbon and total phospholipid fatty acids fluctuated during the first 14 days after simulated tillage (Calderón et al., 2000 and 2001). Similarly, amendment of various soils with fresh plant materials resulted in at least two peaks in microbial biomass carbon and nitrogen, CO_2 evolution, and O_2 consumption within a period of several days or weeks, depending on the experimental resolution (Grant et al., 1993a and b, Griffiths et al., 2001). N_2O and CO_2 fluxes fluctuated with decreasing amplitudes within about a week after rewetting or thawing of soil (Priemé and Christensen, 2001). None of these apparent fluctuations were analyzed statistically, sometimes because the series of observations were not long enough, at other times because the observations were not frequent enough.

Recently, we showed by harmonics analysis that bacterial populations (colony-forming units – CFUs - and direct microscopic counts) oscillated in a regular manner in response to a nutrient impulse in soil (Semenov et al., 1999; van Bruggen et al., 2000; Zelenev et al., 2000; Zelenev et al., 2003). Oscillations in microbial populations were observed both over time (Zelenev et al., 2003) and in space (Semenov et al., 1999, van Bruggen and Semenov, 2000). These oscillations were hypothesized to reflect growth and death phases in response to a nutrient impulse in soil. Death of microbes was considered to be a consequence of depletion of substrate or temporary oxygen reduction (Priemé and Christensen, 2001), followed by regrowth on recycled carbon from bacterial necromass and additional decomposing soil organic matter (Zelenev et al., 2000). Death by predation was considered (Semenov et al., 1999) but has not yet been investigated using the harmonics analysis approach.

The importance of predators for regulation of bacterial numbers in soil has been emphasized in many publications (Clarholm, 1981; Cutler et al., 1922; Darbyshire et al., 1993; Darbyshire, 1994; Griffiths, 1994; Pussard et al., 1994; Thirup et al., 2000; Venette et al., 1997). Cutler et al. (1922) were probably the first authors who described cyclic oscillations in populations of bacteria and protozoa under constant and varying environmental conditions. Since then, fluctuations in bacterial and protozoan and/or nematode populations were apparent (but not always noted by the authors) in many publications both for liquid cultures and soil (for example: Danso and Alexander, 1975; Habte and Alexander, 1978; Rønn et al., 1996). Protozoa populations usually peaked a few days after bacterial populations (Ekelund et al., 2002; Steinberg et al., 1987; Thirup et al., 2000), while nematode populations peaked about three weeks after bacterial populations (Ekelund et al., 2002; Ferris et al., 1996; van Bruggen and Semenov, 1999 and 2000). In most publications the number of observations were insufficient to prove statistically that there were significant oscillations in predator populations following similar oscillations in prey populations (Bouwman and Zwart, 1994; Griffiths and Caul, 1993; Griffiths et al., 1993; Griffiths et al., 1995; Mamilov et al., 2001; Rønn et al., 1996; Thirup et al., 2000).

Although there are examples of regulation of bacterial oscillations by protozoan predators, especially in microcosms, careful inspection of published data on population dynamics of bacteria and their predators often reveal incongruities. Increases in protozoa do not always closely follow increases in bacterial populations. For example, bacterial and protozoan populations fluctuated synchronously and

at the same distances from a root mat on a gauze (Badalucco et al., 1996). Even Cutler et al. (1922) showed sometimes simultaneous increases in soil bacteria and protozoa but did not pay attention to these anomalies. Moreover, protozoa generally did not exhaust their prey in culture nor in soil, and were only partially responsible for the observed decline in bacterial populations (Clarholm, 1981).

Thus, even though protozoa seem to be prime candidates for regulation of bacterial populations, because peaks in protozoa frequently follow peaks in bacterial populations within a few days (Ekelund et al., 2002), regulation of bacterial populations seems to be more complex, and various mechanisms may be involved. Although peaks in bacterial-feeding nematodes (BFN) were often observed only three weeks after the first peak in bacteria (Ekelund et al., 2002; Ferris et al., 1996; van Bruggen and Semenov, 1999 and 2000), more detailed observations on the dynamics of individual groups of BFN in relation to early bacterial dynamics after addition of a nutrient source seemed to be warranted.

The overall aim of this research was to arrive at a better understanding of short-term dynamics of bacteria and their nematode predators in response to addition of fresh plant material to soil under controlled environmental conditions. Specific objectives of this research were: (1) to relate wave-like dynamics of bacteria to dynamics of BFN after incorporation of clover+grass mixture (CG) biomass into soil, and (2) to compare bacterial dynamics in response to CG addition to soil in gamma-irradiated versus non-irradiated soil.

Materials and methods

Soil

The experiments were conducted with sandy soil collected from two neighboring fields at Noordberg in Heelsum, the Netherlands, in the Spring of 2000 and Autumn of 2001. One of the fields was used for barley (*Hordeum vulgare* L.) production (named further as 'barley field'), while the other field had recently planted with Triticale (*Triticale hexaploide* Lart.) (named further as 'Triticale field'). Soil (0.25 m^3) was taken to a depth of 20 cm over a distance of 4 m at the border of each field. Field moisture content of the soils was measured immediately in sub-samples collected from the field. The soil was sieved through a screen with 2-mm mesh, and stored under dry conditions at 20-25 ˚C for six months in case of the first experiment and was used immediately after sieving in the second experiment. General soil characteristics were determined on air-dried sub-samples (Table 1). Before the start of each experiment soil was pre-incubated for three days after addition of a pre-determined amount of water to reach 60% of field moisture value (14%, wet basis). Moistened soil was pre-incubated in a plastic basin covered with a plastic film to prevent water losses.

Experiments with natural field soil

The experiments were carried out from September 25 to October 20 in 2000, and from September 21 to October 20 in 2001. Both experiments consisted of two series of plastic pots containing soil without amendment (control series) or soil amended with a mixture of white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) (CG treatment). There were 3 pots for each sampling time. The first experiment had 26 sampling times for both the CG amended and control series; the second experiment 30 sampling times for the CG treatment and 6 for the control treatment. Every sampling time pots were taken randomly from a controlled-temperature room (25 ºC). One day before the start of each

Table 1. Soil characteristics of neighbouring fields at Noordberg in Heelsum, the Netherlands, with barley stubble ('Barley soil') or young Triticale plants ('Triticale soil').

Soil characteristics	Barley soil Spring 2000	Triticale soil Fall 2001
Sand content (%)	90.1	91.2
Silt content (%)	7.1	5.0
Clay content (%)	2.8	3.8
Organic matter content (%)	3.0	2.7
Total N content (mg/kg)	933	833
Total P content (mg/kg)	732	683
Mineral N content (mg/kg)	19.7	16.4
Available P content (mg/kg)	6.2	6.2
Available K content (mg/kg)	125.0	34.6
pH (KCl)	6.8	5.7

experiment, grass and clover were cut from an experimental field at Wageningen University. The plant material collected was cut into pieces, passed through a 2-cm mesh sieve, and kept in a cold room (5 ^{o}C) till incorporation into the soil. Clover and grass were mixed in a 1 : 1 ratio for fresh weight (1.3 : 1 to 1.8 : 1 for dry weight). The carbon and nitrogen contents of the plant material were determined by an EA 1110 element analyzer (CE instruments, Milano, Italy) according to the principle of Dumas in the Soil Analytical laboratory at Wageningen University (Buurman et al., 1996).

The average carbon and nitrogen contents based on dry weights were 42.6% and 4.4% for clover, and 40.6% and 2.9% for grass, respectively. The C:N ratio of the mixture was on average 11.6. This plant mixture was subsequently mixed with soil at a ratio of CG : soil of 0.05 (wet : wet weight) or 0.01 (dry : dry weight), corresponding to 29.4 t/ha dry weight (assuming a soil bulk density of 1.37 g/cm^3 and plough depth of 20 cm). The C:N ratio of the amended soil ranged from 26.1 to 27.5, and it's initial moisture content was 18.9% in the first and 16.9% in the second experiment. Square plastic pots of 11x11x12 cm were lined with plastic film and filled with the CG-soil mixture or with unamended moistened soil (1 kg per pot), and covered with thin polyethylene film to allow for air-exchange while minimizing water-vapor loss. During the course of the experiments, the soil moisture dropped to 14.9% and 15.0% in the first and second experiment, respectively.

Experiment with gamma-irradiated soil

This experiment was carried out from November 14 to December 13 in 2001 with soil from the Triticale field. Half of the soil was gamma-irradiated at 2.5 Mrad, and the other half remained non-irradiated. Filtered soil extract was prepared from non-irradiated soil by adding 9 parts of demineralized water to 1 part of soil (fresh weight) and shaking for 12 hours on a rotary shaker at 200 rpm. The suspension was sonicated in a sonication bath (Branson 5200, Branson Ultrasonic

Corporation, Danbury CT, USA) for 5 min with about 8 drops of Tween-80 per 2 L of suspension and then centrifuged for 15 min at 5000 rpm. Supernatant was filtered through 0.8 μm membrane filter. Filtered soil extract was added to the irradiated soil to reach a soil moisture content of 12% (wet basis). After addition of the soil extract irradiated soil was pre-incubated for 9 days before the start of the experiment. Clover and ryegrass were collected, cut, and gamma-irradiated at 2.5 Mrad. The rest of the experiment was carried out in the same manner as described for the experiment with natural field soil. The C:N ratio of the CG mixture was on average 11.5. The C:N ratio of the amended soil was 25.88, and its initial moisture content was 14.5-16.0% on a wet weight basis. The experiment had four series of plastic pots containing irradiated or non-irradiated soil without amendment (control series) or the same soils amended with a mixture of irradiated or freshly collected clover and ryegrass, respectively (CG treatments). There were 30 sampling times for the CG treatment and 5 sampling times for the control treatment, with 3 pots for each treatment and sampling time. All pots were kept in a controlled-temperature room (25 ºC). At the end of the experiment, the soil moisture ranged from 13.3% to 14.1% (wet basis).

Sub-sampling

Every day, 3 pots of each series were randomly taken from the controlled-temperature room for destructive sampling. Soil samples were taken with a brass corer (1.5 cm diameter) from the surface to the bottom in the center of a pot (7-12% of total amount soil in a pot), put in a plastic flask, thoroughly mixed, and sub-samples were taken for determination of bacterial populations and soil moisture. Additional soil samples (50 g per pot) were collected with a brass corer for determination of nematode samples (see below).

Determination of bacterial populations

Ten g of soil was added to 90 ml of sterile deionized water, and shaken on a rotary shaker for 15 min at 300 rotations per min. The suspension was sonicated for 5 min in a sonication bath as described above. Ten-fold serial dilutions were made. In the first experiment, appropriate dilutions were plated onto S-medium for copiotrophic bacteria to determine colony forming units (CFUs) per g dry soil as described previously (Semenov et al., 1999; Zelenev et al., 2003). Numbers of cells per g dry soil were estimated from CFUs, using a regression equation between CFUs and Fluorescein Isothiocyanate (FITC) stained cells with a lag of two days as determined from a similar experiment with the same soil (Zelenev et al., 2003). In the second experiment, FITC stained cells were determined directly. Ten-fold diluted soil suspensions were shaken vigorously for 10-15 sec by hand and left still for 5 min. Three dilutions were made to get appropriate counts afterwards. Concentrations of total soil bacteria were determined after staining with Fluorescein Isothiocyanate (FITC) mixed isomers (F-3651, Sigma Chemical Co, St. Louis, MO) according to Babiuk and Paul (1970). The stained samples were filtered through a 0.2 μm membrane Nucleopore filter (Nucleopore® (PC) Polycarbonate, Corning Costar Corporation, Acton, MA 01720, USA), rinsed, according to Bloem (1995), and mounted onto a microscopic slide with a drop of immersion oil. Bacteria on filters were counted under an epifluorescence microscope (ZEISS Axioskop 20 with a HBO 50 mercury lamp for fluorescence-illumination, Carl Zeiss Jena GmbH, D-07740 Jena, Germany) using an oil immersion lens at 1000× total magnification and a fluorescein filter set (Blue 450-490 nm; excitation filter: BP 450-490 (44 77

22); chromatic beam splitter: FT 510 (44 64 34); barrier filter: LP520 (44 77 37)). Every sample filter was examined according to a Z-route and 25-30 fields were counted along the way.

Nematode populations

Every day (except for day 20), one sample of 50 g of fresh soil was taken at random from each control and each CG treated pot. There were 2 (treatments) X 3 (replications) X 25 (days) samples for nematode analyses. The samples were preserved in 50 ml of 4% formaldehyde, so that nematodes could be isolated, counted and identified later. The soil samples were too large to use the "centrifuge-float" method (van Bezooijen, 1999) directly. A decanting method was used to remove most soil, while retaining most of the nematodes (99% in a preliminary test). Each sample was suspended in 90 ml water, shaken thoroughly, and then allowed to settle for ten seconds. The supernatant was poured into a centrifuge tube. This decanting procedure was repeated 3 more times with the left-over soil. After decanting the nematodes were extracted with the centrifugation method (s'Jacob and van Bezooijen, 1971). The centrifuge tubes were centrifuged for four minutes at 1800g with a spoon tip of kaolin (for the forming of a firm pellet). The supernatant was discarded and the pellet was brought back into a solution of saturated magnesium sulphate. The tubes were then centrifuged for one minute at 1800g and put through a filter of 25 µm. This final step was repeated one more time. The nematodes were counted and the nematode samples were stored in a 4% formaldehyde solution.

The family composition of nematodes was determined for all amended samples and one control sample per sampling period. Nematodes were identified under a microscope at 400x magnification. Usually one hundred nematodes were identified per sample following Bongers (1988). Otherwise, if a sample contained a low number of individuals, 50 nematodes were identified instead. All nematodes were identified at the family level according to the key of Bongers (1988). Larvae were considered dormant (dauerlarvae) when the head or tail (or either) of their body was still firmly locked in the double epidermis. Dauerlarvae can belong to a number of families (a.o. Rhabditidae and Diplogasteridae), but cannot be identified at the family level with the light microscope due to lack of details. If both head and tail were freed from the epidermis then the nematode was 'promoted' to the correct family (here in most cases Rhabditidae).

Statistical analyses

Geometric means and their standard errors were calculated for the bacterial counts (3 replicates at each sampling time) according to Snedecor and Cochran (1979). Assuming log-normal distributions (Semenov et al., 1999), the standard error was calculated as follows:

$$SE_{(log\text{-}normal)} = M_G \cdot (10^{(SE(\log 10(bi))} - 1),$$

where

$SE_{(log\text{-}normal)}$ = standard error of log-normally distributed data,

M_G = geometric means of the original data set,

$SE(\log 10(b_i))$ = regular standard error of decimal logarithms of the original data.

To compare the means of two series of data, paired t-tests were conducted on the geometric means of each of the series using MS Excel. To detect the presence of significant wave-like fluctuations within each series, harmonics analysis was conducted on the geometric means according to Jenkins and Watts

(1968) with modifications according to Gorbenko and Kryshev (Gorbenko and Kryshev, 1985; Semenov et al., 1999). Before harmonics analyses, the data were filtered by the first difference filter and smoothed using Hann's 3-point window (Shumway, 1988). Harmonics were considered significant at P=0.1 (Shumway, 1988).

Results

Experiments with natural soil

In the control series, the populations of total bacteria and nematodes decreased exponentially, with a rapid decline during the first 3 days and a more gradual decline thereafter (Fig. 1A). There were slight oscillations in both populations with the same periods of detected harmonics, which in the case of bacteria had peaks approximately 2 days earlier than in the case of nematodes (Table 2). The numbers of bacteria in CG amended soils were significantly higher than in unamended soils (paired t-test; P<0.0001). In the CG series, bacterial populations increased immediately after incorporation of the plant material with a first peak after 3 days and a second peak after 7 days (Fig. 1B). These two peaks were followed by weak fluctuations. In the second experiment, bacterial populations also had two large peaks within 7 days after CG incorporation into soil, and continued to fluctuate after the initial two peaks, but with larger amplitudes than in the first experiment (data not shown).

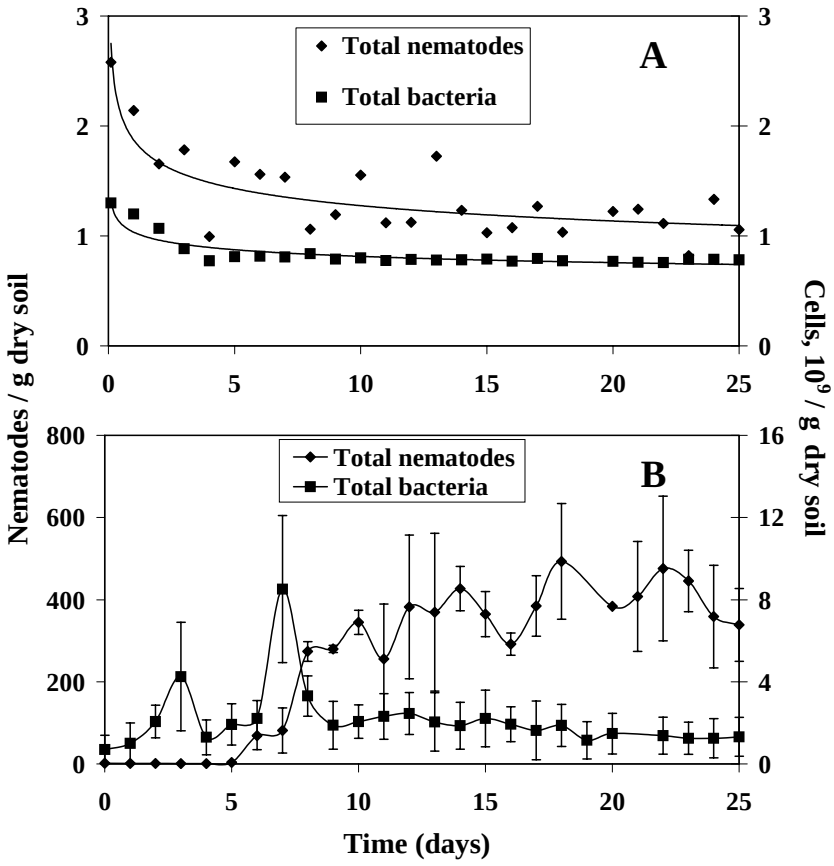

Figure 1. Dynamics of total bacterial cells and total nematodes (numbers per g dry soil) in microcosm experiment 1, control series (A) and clover+grass (CG) treatment (B). Bars represent standard errors.

Table 2. Characteristics of significant harmonics for filtered (first difference filter) and smoothed data of total bacteria and bacterial-feeding nematodes (BFN) in two microcosm experiments with natural field soil amended with clover and grass (Experiments I and II) and of total bacteria in one microcosm experiment with irradiated and non-irradiated field soil amended with clover and grass (Experiment III).

Variable	Harmonics number	Amplitude (g^{-1} soil day^{-1})	Phase (day)	Period (day)	Frequency (day^{-1})	Contribution to variance (%)	F-estimated	F-critical at 0.1 s.l.	n
Experiment I	**Control**								
Total bacteria	1	3.60E+07	14.20	25.0	0.0400	28.7	3.45 [a]	2.54	25
	2	3.35E+07	7.74	12.5	0.0800	24.9	2.98	2.54	25
	3	3.44E+07	-2.78	8.3	0.1200	26.3	3.16	2.54	25
Bacterial-feeding	2	0.024	-3.05	12.5	0.0800	31.6	3.79 [a]	2.54	25
nematodes	3	0.023	-0.54	8.3	0.1200	29.2	3.51 [a]	2.54	25
Experiment I	**CG treatment**								
Total bacteria	5	6.50E+08	0.43	5.0	0.2000	31.4	3.77 [a]	2.54	25
	6	5.73E+08	1.33	4.2	0.2400	24.4	2.93	2.54	25
Bacterial-feeding nematodes	5	20.3	1.21	5.0	0.2000	28.4	3.41 [a]	2.54	25
Experiment II	**CG treatment**								
Total bacteria	5	6.77E+07	-0.87	5.8	0.1724	25.5	3.56 [a]	2.50	29
	7	6.73E+07	1.00	4.1	0.2414	25.2	3.52 [a]	2.50	29
Bacterial-feeding	1	5.2	7.78	29.0	0.0345	26.0	3.64 [a]	2.50	29
nematodes	4	4.7	-1.06	7.3	0.1379	21.2	2.96	2.50	29
Rhabditidae	3	4.8	5.43	9.7	0.1034	30.4	4.25 [a]	2.50	29
Diplogasteridae	1	5.2	10.69	29.0	0.0345	39.4	5.51 [a]	2.50	29
	6	3.9	0.96	4.8	0.2069	21.4	3.00	2.50	29
Dauerlarve	1	4.4	13.86	29.0	0.0345	28.5	3.99 [a]	2.50	29
	8	3.6	1.29	3.6	0.2759	18.9	2.65	2.50	29
Experiment III	**CG treatment**								
Total bacteria	5	1.00E+08	-0.89	5.8	0.1724	34.5	4.83 [a]	2.50	29
(non-irradiated soil)	6	7.51E+07	0.74	4.8	0.2069	19.3	2.71	2.50	29
	7	7.42E+07	1.14	4.1	0.2414	18.8	2.64	2.50	29
Total bacteria	4	3.01E+08	-1.18	7.3	0.1379	43.2	6.05 [a]	2.50	29
(irradiated soil)	5	2.22E+08	1.49	5.8	0.1724	23.6	3.30 [a]	2.50	29

[a] - significant at 0.05 s.l.

The total nematode populations in the CG treated soil increased significantly (P<0.0001) above the populations in the control treatment. In the first experiment, total nematode populations increased faster than in the second experiment (data not shown). In the first experiment, the population size

Table 3. Percentages of nematodes (out of 100 nematodes identified) divided over different nematode families, averaged over the period from 1–25 days for control microcosms with sandy loam soil, and over periods from 1-4, 5-15, 16-26 days (experiment 1) and from 1-4, 5-20, 20-30 days (experiment 2) for microcosms with sandy loam soil mixed with clover+grass.

Nematode Functional Groups*	Nematode families	Experiment 1				Experiment 2		
		Control	Clover+grass			Clover+grass		
		% days 1-25	% days 1-4	% days 5-15	% days 16-26	% days 1-4	% days 5-20	% days 21-30
Plant feeding	Tylenchidae	6.7	3.2	0.9	0	1.2	1.3	1.3
	Dolichodoridae	5.1	4.5	0.3	0	7	2.4	1.6
	Hoplolaimidae	3.9	2.1	0.1	0	4.8	1.7	1
	Pratylenchidae	65.6	48.8	6.5	0.1	43.3	5.9	2.7
	Meloidogynidae	0.3	0	0	0	1	0	0
Hyphal feeding	Anguinidae	0.5	1.6	0.4	0	0	0	0
	Aphelenchidae	0	1.1	0.2	0.1	1	1	1
	Aphelenchoididae	0.2	7.9	1.9	3.2	1.6	1.8	1.9
	Leptonchidae	0	0	0	0	1	1.3	1
Bacterial feeding	Rhabditidae	2.1	5.8	53.9	52	17.2	42	25.5
	Diploscapteridae	0	0	0.5	0.2	2	4.9	5.7
	Cephalobidae	2.6	6.3	1.1	0.2	3.4	2.3	1.8
	Panagrolaimidae	0	2.4	0.5	0	1.3	1.6	2.3
	Plectidae	0.7	0	0.1	0	2.7	1.5	1.5
	Diplogasteridae	0.2	0	0.4	2.1	3.8	23.7	26.5
	Neodiplogasteridae	0	0	0	0	1	0	0
Animal predators	Nygolaimidae	0.2	0	0	0	0	1	0
	Discolaimidae	0	0.3	0	0	0	0	0
	Mononchidae	0	0	0	0	2.5	1	1
Omnivorous	Thornenematidae	1	1.6	0	0	1.5	1.4	1.6
	Qudsianematidae	1.4	2.6	0.3	0	6.3	2	1.8
	Aporcelaimidae	0.5	0	0	0	0	0	0
Dauerlarvae		8.9	11.9	32.9	42.2	7.3	16	33.8

* - according to Yeates et al., 1993.

increased from roughly 2 to 70 nematodes per g soil on day seven (Fig. 1B), and in the second experiment from roughly 20 to 60 nematodes per g soil. In the following days the population continued to grow until the highest population reached 493 nematodes per g soil on day 19 in the first experiment and 236 nematodes per g soil on day 21 in the second experiment.

There were no significant differences in the composition of the families over time in the control treatment of the first experiment. Pratylenchidae was the most abundant family in the controls (Table 3). In the CG treatment, the Rhabditidae became the most abundant nematode group about a week

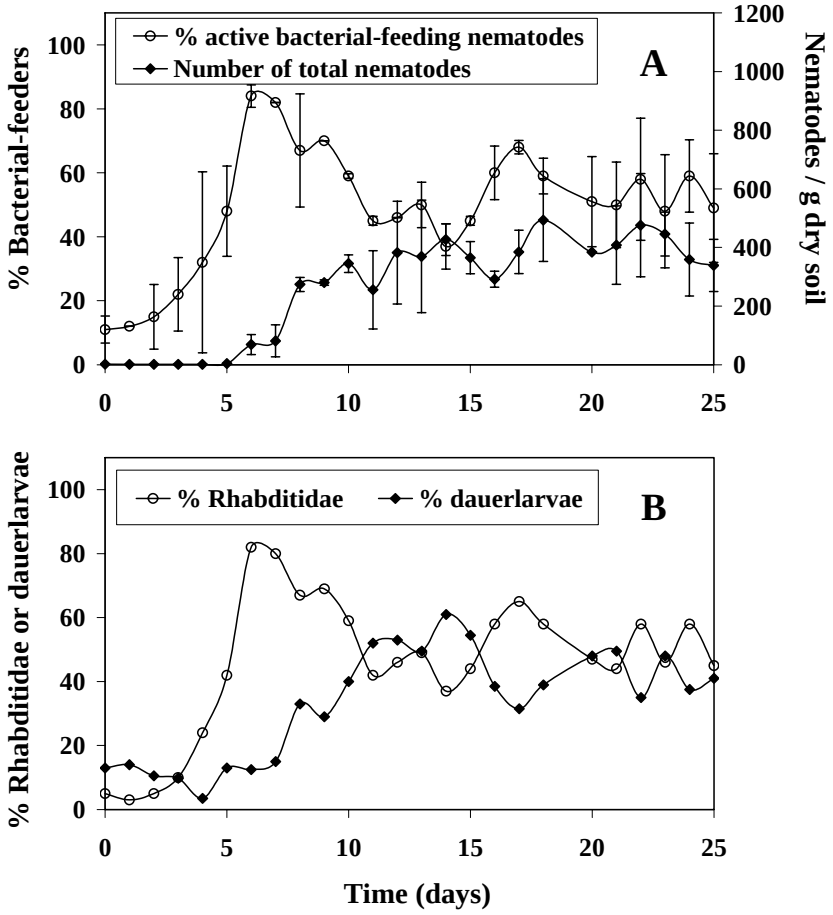

Figure 2. Changes in fraction of active bacterial-feeding nematodes versus total nematodes (per g dry soil) (A), and in percentages Rhabditidae versus inactive juveniles of bacterial-feeding nematodes (dauerlarvae) of total nematodes numbers (B), both during microcosm experiment 1.

after incorporation of plant material into soil. A few days later, the nematode community was almost a monoculture of Rhabditidae and dauerlarvae. In the second experiment, Diplogasteridae also became abundant next to the Rhabditidae and dauerlarvae (Table 3). In the CG-amended soil samples the diversity became less as the experiment advanced. Most families were probably still there but in such low quantities that they did not reach the detection threshold.

The rise in the percentage of Rhabditidae and total BFN was 4-5 days ahead of the rise in the total nematode population (Fig. 2A). The percentage Rhabditidae fluctuated after the initial increase (Fig. 2B). The percentage dauerlarvae initially decreased, as the percentage Rhabditidae increased. Subsequently, the percentage dauerlarvae increased stepwise approximately every four days until day 12 (experiment 1) or 15 (experiment 2) and then fluctuated counter to the percentage Rhabditidae (Fig. 2B).

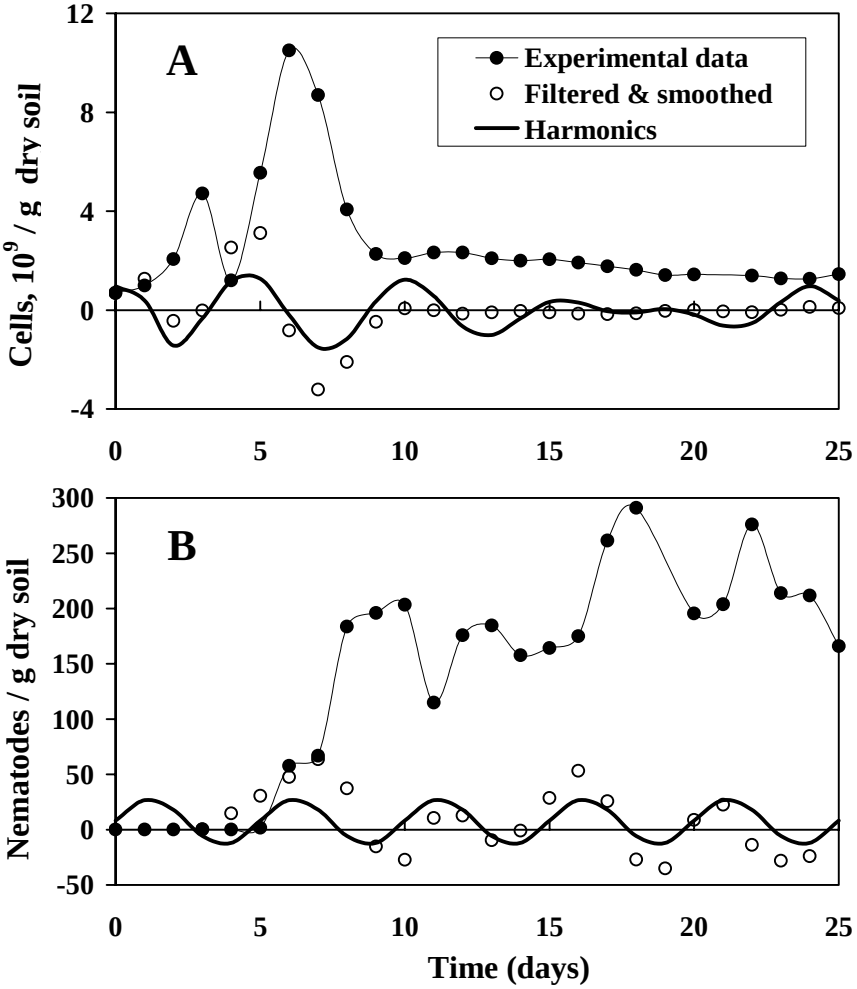

Figure 3. Dynamics of total numbers of bacterial cells (A) and bacterial-feeding nematodes (B) during microcosm experiment 1, each accompanied by the sum of significant harmonics (P<0.1) of filtered (first difference filter) and smoothed series.

Harmonics analysis of filtered and smoothed bacterial data in CG amended soil resulted in two significant (P<0.10) harmonics in the first experiment, namely the fifth and sixth harmonics (Table 2; Fig. 3A). These harmonics contributed 55.8% to the observed variation in bacterial counts. In the second experiment, the fifth and seventh harmonic were significant, accounting for 50.7% of the variation (Table 2). The frequencies of the oscillations in bacterial populations were very similar in both experiments. There were also significant wave-like fluctuations for daily population changes (i.e. first difference filters) of BFN (Table 2; Fig. 3B), but not for those of total nematode communities. The frequencies of these fluctuations were similar to those of bacterial populations in experiment 1, while the phases were shifted (Table 2; Fig. 3B). The frequencies of daily population changes in BFN were lower than those of bacterial populations in the second experiment (Table 2). However, the composition of the bacterial-feeding nematode community was more diverse in this experiment than in the first with dried soil.

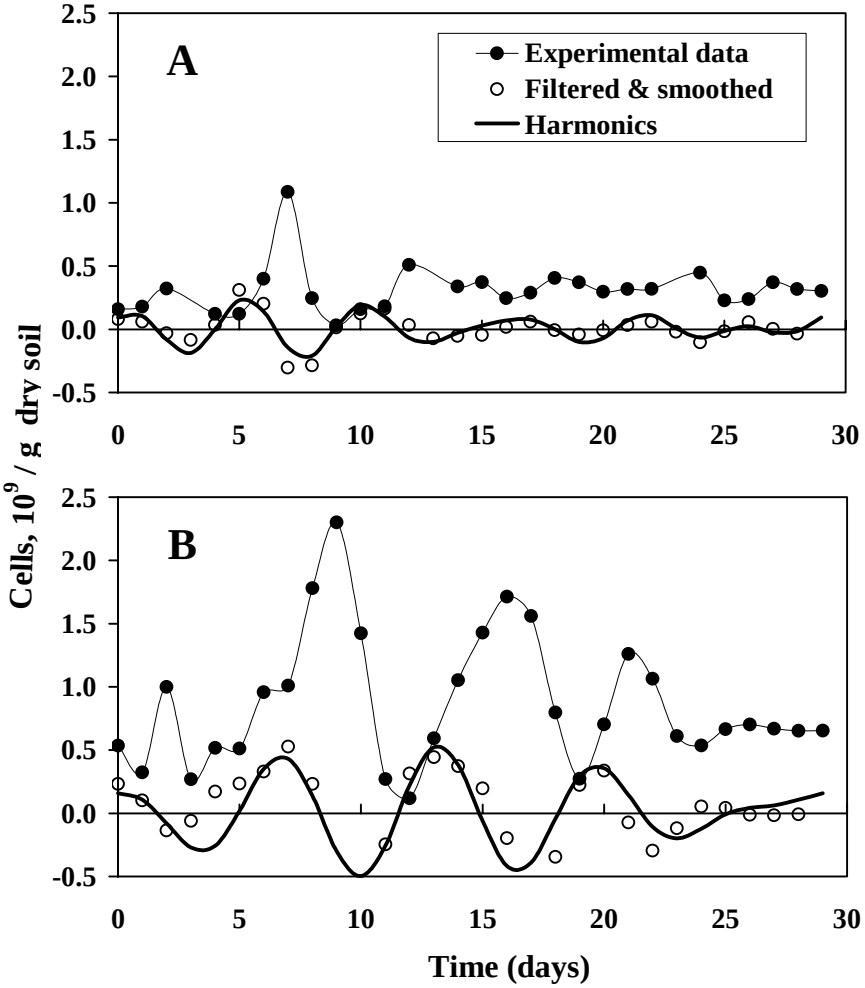

Figure 4. Dynamics of total numbers of bacteria in microcosm experiment 3 for non-irradiated (A) and irradiated (B) soil, each accompanied by the sum of significant harmonics (P<0.1) of filtered (first difference filter) and smoothed series.

Experiment with irradiated and non-irradiated soil

Similar to the previous experiments, bacterial populations showed a small and a large peak within a week after CG incorporation in non-irradiated soil. These two peaks were followed by weak fluctuations (Fig. 4A). Fourier analysis revealed three significant harmonics, the fifth harmonic with a period of almost 6 days being most influential (Table 2). Gamma-irradiation of soil significantly increased the periods and amplitudes of bacterial oscillations (Fig. 4B). For this soil, the fourth harmonics was most influential, with a period of 7 days (Table 2). The number of nematodes detected in irradiated soil decreased from 3.2 individuals per g dry soil on day 0 through 0.8 on day 11 down to 0 on day 28. The initially detected nematodes were probably already dead at the time of sampling, but not yet decomposed, since formaldehyde was added at each sampling period. In the irradiated soil, pronounced fluctuations in bacterial populations continued until day 25, while in natural field soil, fluctuations in bacterial populations were minimized in about 15 days after addition of fresh plant material to soil. At the end of the experiment, some protozoa were detected in the gamma-irradiated

soil. The number of nematodes in the non-irradiated soil increased from 8.5 individuals per g dry soil on day 0 to 255 on day 11 and then dropped to 188 individuals per g dry soil on day 29. The dominating family was, as in the other two experiments, Rhabditidae (47 and 54% of the total nematode population on days 11 and 29, respectively) together with Diplogasteridae (31-21%) as in the second experiment. Dauerlarvae comprised 21-18% of the total number of nematodes.

Discussion

Recently, we demonstrated significant regular wave-like patterns in bacterial population dynamics after a strong perturbation, namely addition of fresh organic matter to soil (Zelenev et al., 2003). In several microcosm experiments (Zelenev et al., 2003), the bacterial community responded with a small and a large peak in growth within one week after the perturbation, followed by more or less regular oscillations in bacterial numbers (or CFUs). Similar small and large peaks within one week were found in terms of total phospholipid fatty acids (PFLA) in tilled soil (Caldéron et al., 2001). In the research reported here, the same pattern was observed again, and the question if BFN could be partially responsible for the intermittent decreases in bacterial populations was addressed.

For the first time, we demonstrated that BFN did not increase simply in a sigmoid fashion after addition of fresh plant material to soil, but with significant fluctuations in growth ‘rates’ (more precisely daily changes in populations). These fluctuations were not random but regular. Such fluctuations had not been reported previously, partially because observations were not made frequently enough (for example: Fu et al., 2000).

The periods of the waves in growth ‘rates’ of BFN were similar to the periods of wave-like fluctuations in bacterial growth and death ‘rates’ in one of two experiments, when the majority of BFN were Rhabditidae. In the other experiment, wave-like fluctuations in growth ‘rates’ of BFN had larger periods than the oscillations in bacterial growth and death ‘rates’. In that experiment, Diplogasteridae also became abundant, next to Rhabditidae. Members of the Rhabditidae have short generation periods of about 4 to 5 days (Ferris et al., 1996) while Diplogasteridae have sometimes longer generation periods. Indeed, at high prevalence of Rhabditidae in the CG-treatment of experiment 1 a significant harmonic ($P<0.05$) with a period of 5 days was detected for BFN dynamics, whereas in experiment 2 with lower Rhabditidae abundance, the population dynamics of BFN was characterized by harmonics with other periods reflecting the greater contribution of the other families (Table 2).

Interestingly, dauerlarvae, the surviving but still inactive juveniles of BFN after drying and rewetting of the soil, did not disappear. Instead, their proportion declined in the first four days, then increased in a stepwise fashion with periods of about 4 days in the first 2 weeks after CG incorporation in soil (Fig.2B). The eighth harmonics with a period of almost 4 days was significant for the changes in populations of dauerlarvae (Table 2). This indicates that BFN (primarily Rhabditidae) grew very fast, ran out of prey and produced new dauerlarvae every 4 days. Older dauerlarvae were converted to active BFN, so that active BFN and dauerlarvae fluctuated in opposite direction. This observation led us to hypothesise that an alternation in active and inactive stages in a synchronized predator

community after a disturbance could allow periods of bacterial growth alternated with periods of death. Could such predator-prey relationships be a sufficient explanation for the initiation of bacterial fluctuations?

Regular oscillations in bacterial populations have for long been ascribed to predator-prey relationships in microcosm studies, but numbers of prey consumed by the predators do not always account for the fall in prey numbers (Clarholm, 1981; Danso and Alexander, 1975; Habte and Alexander, 1977). In our experiments, the drop in bacterial numbers after the first and second peaks amounted to 0.1 – 5 billion cells per g dry soil in one day, when the bacterial-feeding nematode populations ranged from 1-60 nematodes per g dry soil. Assuming that members of the Rhabditidae can consume about 0.6-1.5 million bacterial cells per day (Ferris et al., 1996), the number of Rhabditidae was too low to completely account for the decline after the first two peaks in bacterial cells. Thus, despite the coincidence of wave-like fluctuations in changes in BFN and bacteria, nematodes were likely not the sole responsible factor for the bacterial fluctuations in the first week after CG incorporation. Nevertheless, BFN probably contributed significantly to the decrease in bacteria after the second peak (Fig. 3A and B).

Protozoa can respond quickly to increases in bacteria and reach a peak a few days after the first peak in fast-growing culturable bacteria (Thirup et al., 2000). However, maxima in protozoan populations do not always coincide with minima in bacterial populations (Clarholm, 1981, Gorbenko and Panikov, 1989; Thirup et al., 2000), or protozoa may account for only part of the decline in microbial biomass (Clarholm, 1981). In a separate experiment on effects of CG addition to soil, protozoa were enumerated (Zelenev, unpublished). The numbers of flagellates and amoebae were about 2000 per g dry soil at the beginning of the experiment, and 140,000 after one week. Assuming a generation time of half a day and consumption of 5000 cells per individual (Ekelund et al., 2002), protozoa were likely at least partly responsible for the decline of 0.1 to 3 billion bacteria on day three or four (after the first bacterial peak), and likely contributed significantly to the decline after the second peak on day 7 or 8. However, additional factors were probably responsible for the first fluctuation in bacterial populations, since bacterial fluctuations also occur in sterilized soil (Pussard et al., 1994; Semenov, unpublished). Thus, it is possible that initial fluctuations in bacteria lead to subsequent fluctuations in their predators rather than the other way around.

The most likely factors responsible for the first decline in bacterial populations are competition for easily available substrate or temporary oxygen shortages due to the fast increase in microbial populations immediately after addition of fresh organic matter to soil. The anoxia hypothesis is supported by the fact that short-term peaks in N_2O emission after freezing-thawing and drying-rewetting were at least partially due to denitrification within 1-2 days after the thawing or rewetting event (Priemé and Christensen, 2001). From the graphs presented by Priemé and Christensen (2001) it appears that N_2O peaks occurred at the same time when CO_2 dropped. Rønn et al. (1996) also noted increased N_2O production after amendment of soil with barley roots, and ascribed this to the intense local decomposition activity. Similarly, Jackson (2000) attributed short-term losses of nitrogen from a ^{15}N-labeled cover crop to denitrification as a result of high microbial activity. This conclusion was

supported by Calderón et al. (2001) who showed that a peak in denitrification four days after tillage was associated with an increase in a PFLA marker for anaerobic eubacteria.

Thus our research on bacterial fluctuations has broader implications than unraveling predator-prey relationships. The amplitude of the fluctuations could also be associated with losses of nitrogen via denitrification, including N_2O emissions. Large losses of nutrients from a system are indicative of an unhealthy ecosystem (van Bruggen and Semenov, 2000). Ecosystem health could be measured by resistance and resilience of the system after a disturbance, expressed by the amplitudes and dampening period of fluctuations in biological populations (Griffiths et al., 2000 and 2001). In these experiments, we had three levels of soil stress: recently collected natural field soil (experiment 2), the same soil but stored air-dry for several months (experiment 1), and gamma-irradiated soil compared to its non-irradiated counterpart (air-dried for one month). Gamma-irradiated soil had the lowest resistance and resilience as evidenced from the wide and extensive fluctuations in bacterial populations compared to its non-irradiated counterpart. The fluctuations in bacterial populations were also much greater in the soil that had been air-dry for several months than the same soil when it was recently collected (amplitudes of detected harmonics were around $6 \cdot 10^8$ cells/g dry soil for dried soil vs $6.7 \cdot 10^7$ for newly collected soil, Table 2), although the periods in the fluctuations were similar. Similarly, the amplitudes in nematode growth rates were greater in the air-dried than in the fresh soil (20 vs 5 nematodes per g dry soil per day), although the periods were smaller in the air-dried soil.

These observations are in agreement with the general idea that natural or slightly stressed ecosystems reveal greater resistance to a perturbation and are more resilient, being able to return to a steady state faster than severely stressed ecosystems can do. Irradiated soil can be considered as a highly stressed ecosystem with much less biodiversity and reduced competition, allowing fast-growing bacteria to reach a higher level of biomass. In experiments on the effects of reductions in biodiversity by soil fumigation on biological functioning, soil respiration and initial decomposition of added organic material were higher for fumigated than for non-fumigated samples (Griffiths et al., 2000). Substrate-induced respiration was initially reduced but within a day enhanced in the fumigated samples; thus, greater CO_2 emissions could be expected after addition of organic matter to stressed or highly disturbed soils. In other experiments, soil respiration rates were lower in petrol-polluted than in unpolluted soil amended with grass, glucose or sawdust (Griffiths et al., 2001), but oscillations were much more pronounced. Relative oscillations (% of the baseline) in CO_2 flux were also greater in soil from ploughed fields than from grasslands (Priemé and Christensen, 2001). Thus, research on microbial fluctuations can gain important insights into conditions that stimulate losses of nutrients and greenhouse gases.

We conclude that fluctuations in bacterial populations were not directly related to similar fluctuations in populations of BFN, as expected from classical Lotka-Volterra equations for predator-prey relationships, but were related to changes in growth rates of BFN. An alternation in active and inactive stages in a synchronized predator community after a disturbance could allow periods of bacterial growth alternated with periods of death. In largely defaunated soil, fluctuations in bacterial populations were dampened much later than in natural field soil, indicating that the soil fauna did play

a role in regulation of bacterial populations. However, it is unlikely that the death phase after the very first peak in bacteria was initiated by the soil fauna. Additional research is needed to clarify the initiation of bacterial fluctuations and the conditions that lead to intensive and relatively long fluctuations.

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Chapter 7

"BACWAVE-WEB", a simulation model for oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil

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Abstract

Recently regular oscillations in bacterial populations and bacterial feeding nematodes (BFN) were shown to occur after addition of fresh organic matter to soil. This paper presents a model developed to investigate potential mechanisms of those oscillations, and whether they were initiated by bacteria-substrate interactions or predatory regulation of bacteria. The model was also used to investigate mineral nitrogen release during short-term organic matter decomposition. Experimental data originated from several microcosm experiments with a sandy soil amended with clover-grass mixture. Numbers of bacteria and BFN, nitrate and ammonium content in soil were measured daily during about a month, while protozoa were counted occasionally. A substrate-based food web model was constructed with 3 plant residue and 5 soil organic matter compartments, 3 trophic groups of bacteria (copiotrophic, oligotrophic and hydrolytic), and two predatory groups (BFN and protozoa). Both carbon and nitrogen flows were modelled. Fluctuations in microbial populations in soil after plant residue incorporation could be reproduced with and without participation of predators. The first two peaks in bacterial numbers were mainly related to bacteria-substrate interactions, while predators (particularly protozoa) influenced bacterial dynamics during later stages of bacterial community development. Oligotrophic bacteria had a stabilizing effect on fluctuations of other trophic groups, and were the main source of nutrients for predators. A peak in soil ammonium occurred within one week after residue incorporation. Nitrate increased sigmoidally after a short lag phase. The final nitrate concentration was primarily determined by bacterial dynamics, and to a lesser extent by protozoa and nematodes. This model emphasized the importance of substrate-consumer relations for regulation of populations at different trophic levels and nutrient release from fresh organic matter added to soil.

Keywords: copiotrophic, oligotrophic, hydrolytic, nematodes, protozoa, perturbation, fluctuations, waves, plant residues, carbon flows, N mineralization.

Introduction

Temporal dynamics of bacteria, fungi, microscopic animals, and their interrelationships have been important subjects for research by soil microbiologists and microbial ecologists. Although population dynamics of soil microorganisms and their predators were studied in various soils and under different environmental conditions, most studies were concentrated on long-term dynamics, for example with weekly or less frequent observations for several months (Bloem et al., 1994). However, many important processes, including nutrient release and loss, take place during the first few days after a disturbance, such as tillage or incorporation of fresh plant residues in soil (Calderón et al., 2001; Griffiths et al., 2001; Priemé and Christensen, 2001).

Nevertheless, most investigations concerning plant residue and soil organic matter decomposition have focused on long-term dynamics (Bouwman et al., 1994). In a few short-term experiments, fluctuations in measured microbial biomass N and C and mineral N could be observed, but were not reproduced in accompanying model results (Grant et al., 1993a and b; Kätterer and Andrén, 2001). During several years we investigated the initial stages of fresh plant residue decomposition in soil and observed

significant oscillations of two trophic groups of bacteria (copiotrophs[1] and oligotrophs) immediately after residue incorporation in soil (Hu et al., 1999; van Bruggen and Semenov, 2000; Zelenev et al., 2003b). The dynamics of nematodes and inorganic nitrogen availability was also monitored intensively (Hu et al., 1997; Semenov et al., 2002; Zelenev et al., 2003a). However, potential relationships between the wave-like fluctuations of bacterial populations and ammonium and nitrate contents in soil were not apparent, since concentrations of these nutrients did not oscillate over time (Zelenev et al., 2003b). Similarly, no clear relationship was observed between microbial fluctuations and changes in redox potential. Instead, bacterial oscillations seemed to be associated with concentrations of easily consumable substrate (Zelenev et al., 2000) or possibly with fluctuations in predators (Zelenev et al., 2003a).

The role of predators for regulation of bacterial numbers and N release in soil has been emphasized in many publications (Darbyshire, 1994; Ferris et al., 1998; Kuikman et al., 1991; Mamilov et al., 2001; Verhoeff and Brussaard, 1990). Microbial response can be very rapid and lead to considerable mineralization of nitrogen during the first week following incorporation of low C/N plant material in soil (Jackson, 2000). When daily or even hourly observations were made during the first week after an impulse of nutrients in soil, regular oscillations were detected in various microbial measures and release of N_2O from soil (Calderón et al., 2001; Griffiths et al., 2001; Jackson, 2000; Priemé and Christensen, 2001; Zelenev et al., 2003b). Although fluctuations in bacterial and predator populations were often apparent in experimental data, the observations were frequently not detailed enough to prove that there were significant oscillations in predator populations following similar oscillations in prey populations (Griffiths et al., 1995; Mamilov et al., 2001). Peaks in protozoa and BFN may follow peaks in bacterial populations within a few days, respectively weeks (Ekelund et al., 2002; Zelenev et al., 2003a). However, these predators may be only partially responsible for declines in bacterial populations (Clarholm, 1981; Ekelund et al., 2002; Ferris et al., 1998; van Bruggen and Semenov, 2000; Zelenev et al., 2003a). Thus, various mechanisms may be involved in regulation of bacterial populations, and the role of predators may have been over-estimated (Ferris et al., 1998; Mamilov et al., 2001).

A simulation model could provide insight in potential relationships between the various players in organic mater decomposition and nitrogen release without the need to carry out detailed, difficult experiments. Many simulation models have been constructed for residue decomposition and microbial biomass dynamics, but 'wave-like' fluctuations in bacterial populations and mineral nitrogen concentrations were not modeled (de Ruiter et al., 1993; Grant et al., 1993a and b; Kätterer and Andrén, 2001; Neutel et al., 2002; Nicolardot and Molina, 1994; van Veen et al., 1984). Soil biology models could be divided in two main types as suggested by Brussaard (1998), namely food web models and SOM decomposition models. In soil food web models usually only one single group of bacteria and one group of decomposer fungi are taken into account. Distinction of different groups of primary decomposers may better clarify events involved in organic matter decomposition (Kersebaum

[1] Copiotrophic bacteria grow relatively fast on C rich media (1000 mg C/L), while oligotrophic bacteria grow more slowly, but better on C poor (10 mg C/L) than on C rich media. The term "oligotrophic bacteria" is used here to include so-called "wide range" bacteria (Semenov, 1991) that can grow on both dilute and more concentrated media.

and Richter, 1994). SOM models are more detailed at the basis of the food web, but frequently ignore effects of predators. A combination model that includes food web components, several trophic groups of primary decomposers and compartments of fresh and older organic matter could be a powerful instrument for understanding and forecasting various events in the soil.

Current models for decomposition of organic matter do not simulate fluctuations in microbial populations and their predators adequately. The main reason for the lack of fluctuations is likely the independence of microbial relative death rates on readily consumable substrate concentrations (Henriksen and Breland, 1999; Nicolardot et al., 2001; van Veen et al., 1984), or absence of a cross-over point between relative growth and death rate curves in relation to substrate concentration (Blagodatsky and Richter, 1998; Blagodatsky et al., 1998). We constructed the model 'BACWAVE' to simulate microbial oscillations in time and space, based on the realization that microbial relative growth and death rates are dependent on easily utilizable substrate content. This model resulted in wave-like dynamics and distribution of copiotrophic bacteria in the rhizosphere in response to nutrient input from a passing root tip (Zelenev et al., 2000). For the current paper, 'BACWAVE' was extended to simulate the oscillatory dynamics of 3 trophic groups of bacteria (copiotrophic, oligotrophic and hydrolytic bacteria) and 2 groups of predators (protozoa and BFN) in relation to substrate concentration, and mineral nitrogen release at early decomposition stages of fresh plant material incorporated in soil. Elements of earlier models (e.g. de Ruiter et al., 1993; Rutherford and Juma, 1992; van Veen et al., 1984) were included in this model.

Specific objectives of this research were:

1. to simulate early peaks in total bacterial numbers after incorporation of fresh plant material in soil and investigate if these peaks consist of different trophic groups of bacteria in succession or of a mixture of groups that each fluctuate simultaneously;
2. to simulate relationships of different trophic groups of bacteria with two groups of predators, and investigate their role in regulation of bacterial dynamics and release of inorganic nitrogen from organic matter in soil;
3. to simulate decomposition of fresh plant material incorporated in soil and flows of carbon and nitrogen released from different fractions of SOM, plant residues, and biotic components.

Materials and methods

Experimental data

Experimental data sets used in this research consisted of direct microscopic counts of bacteria, and soil nitrate and ammonium contents determined daily during continuous incubation of sandy soil amended with a fresh clover-grass mixture in pot experiments in Spring 2000 and Fall 2001 (Zelenev et al., 2003b). Numbers of bacterial feeding nematodes (BFN) were counted daily in the course of two similar experiments in the fall of 2000 and 2001 (Zelenev et al., 2003a). The experiments lasted 30 to 34 days. Numbers of protozoa (flagellates and amoebae) were determined 2 or 5 times during the course of two similar experiments in 2002. To determine the numbers of bacteria per g dry soil, a diluted soil suspension was stained with Fluorescein Isothiocyanate (FITC), and filtered through a nylon membrane

with 0.2 μm pores. Stained bacterial cells were counted under a fluorescent microscope as described earlier (Zelenev et al., 2003b). Soil NO_3^- and NH_4^+ contents were determined after extraction of both ions from soil with 1 M KCl (20 g of dry soil in 50 ml of solution) (Zelenev et al., 2003b). Nematodes were counted in soil subsamples preserved with 4% formaldehyde. After decanting from soil the nematodes were extracted with the centrifugation method and enumerated (Zelenev et al., 2003a). Numbers of BFN were calculated from the proportion of BFN in 100 nematodes identified to the family level (Zelenev et al., 2003a). Numbers of protozoa were estimated in soil samples of 20 g with the MPN technique (Bamforth, 1995).

Model assumptions

Three groups of bacteria were considered in the model: copiotrophic, oligotrophic and hydrolytic bacteria (Semenov, 1991). Oligotrophic bacteria are characterized by much lower kinetic characteristics (maximal relative growth rate and substrate half saturation constant) than copiotrophs. At the same time, they have a higher tolerance of low substrate concentrations, but a higher sensitivity to high substrate content resulting in their death at concentrations higher than 1200 μg of substrate carbon per ml (Klimova and Nikitin, 1994). Hydrolytic bacteria were assumed to have kinetic characteristics close to copiotrophic bacteria, but were considered as a separate trophic group being able to excrete extra-cellular enzymes. All bacteria were assumed to consume only substrate dissolved in soil solution, which was considered as the sum of low-molecular sugars, aminosugars, organic acids, and aminoacids, but distinct from water-soluble total organic carbon extracted from soil. A soil enzyme pool was introduced into the model to effectuate decomposition of complex polymers into water-soluble low-molecular weight substances. Fungi were not included in the model since fungal biomass in Dutch arable soil at pH 6.5-7 was about 100-fold lower than the bacterial biomass (Bloem et al., 1994) and our own attempts to find fungal hyphae under the microscope in soil suspensions prepared for direct observation failed.

Model structure

The model consists of two linked structures for carbon and nitrogen components in soil. State and rate variables are expressed in units of [μg] or [μg/h] of C or N per cm^3 of homogeneous soil. Soil organic matter consisted of the following state variables (Fig. 1, Table 1): soluble organic carbon {SOLOM}, soluble extra-cellular enzyme pool {E}, soluble substrate directly consumable for bacteria {S}, structural soil organic matter {STROM} and humic organic matter {HUMOM}. Plant residues are divided into water-soluble plant residues {SORES}, non-lignin structural plant residues {STRES} and a lignin fraction of plant residues {LIRES}. {SOLOM} is calculated as a fixed fraction (0.025) of total soil organic carbon (Alvarez and Alvarez, 2000). Extra-cellular enzymes {E} are secreted by hydrolytic bacteria during their growth, and are released after their death. {STRES} consists of cellulose and hemicellulose carbon, while {STROM} consists of carbon of residual cellulose and hemicellulose plus cell wall materials of dead soil biota. {SORES} is calculated as the difference between carbon of dry weight of added plant material and the sum of carbon of analytically determined cellulose, hemicellulose, and lignin (Hu et al., 1997). The following organisms (living components) are considered: bacteria (copiotrophic {COP}, oligotrophic {OLIG} and hydrolytic {HB}), protozoa {PRTZ}, and bacterial feeding nematodes {BFN}. Carbon losses from the system due to respiration accumulate in a common sink, the {A_CO_2}-compartment. No distinction was made between CO_2 in the water and gas

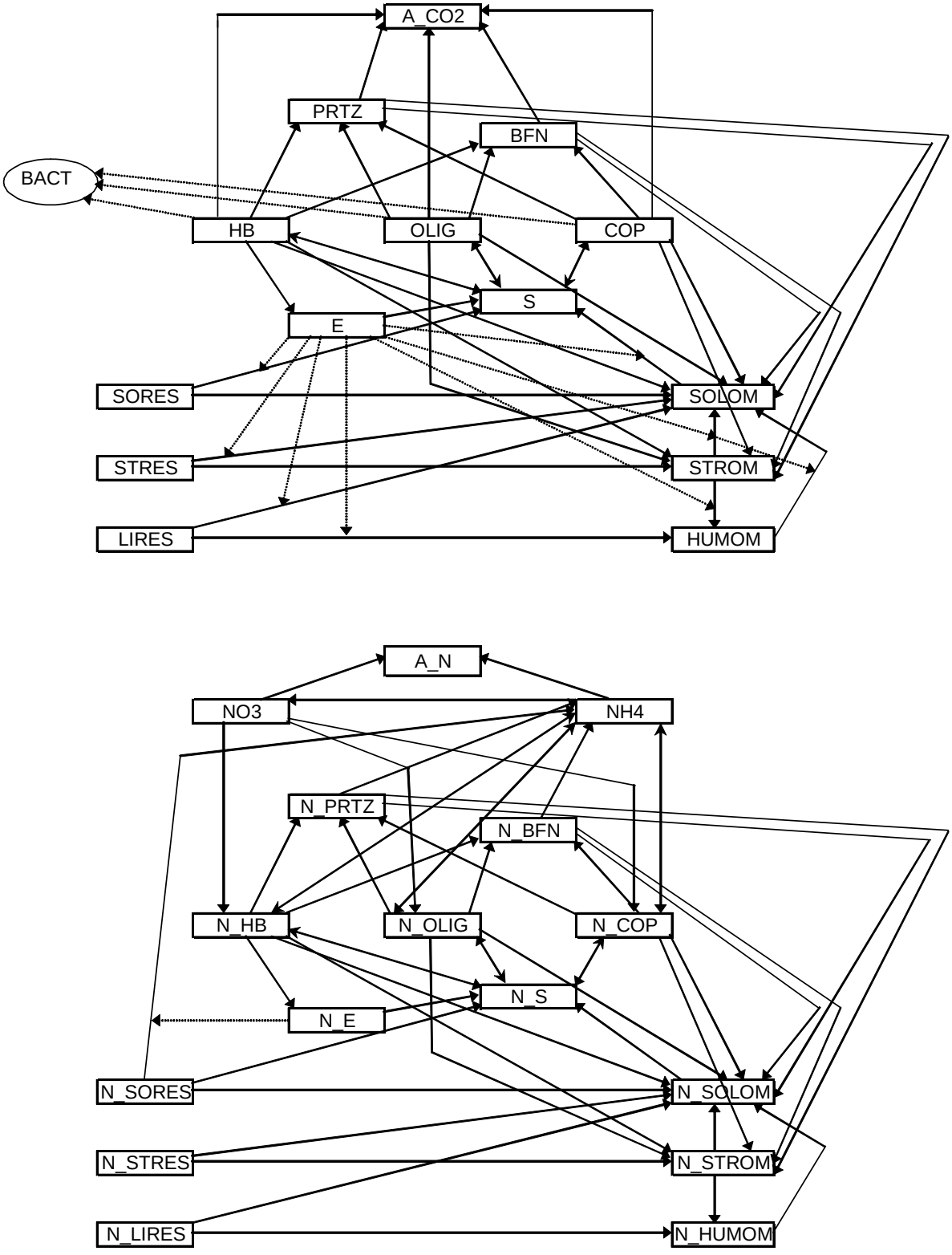

Figure 1. Relational diagram of the model, with two linked flow systems. Rectangles represent state variables, ovals auxiliary variables, full lines flows of matter (C or N), and dashed lines flows of information. Flows of information ending in flows of matter indicate that the matter flow is influenced by the respective information flow. For description of symbols see Table 1.

Table 1. List of model components and their initial values for data sets of experiments 1 and 2.

Compartment	Symbol	Unit	Experiment		Source*
			1	2	
Cumulative CO_2 sink to atmosphere	A_CO2	[µg C / cm^3]	0	0	-
Cumulative Nitrogen sink to atmosphere	A_N	[µg N / cm^3]	0	0	-
Total number of bacteria	BACT	[#/g dry soil]	$3.05 \cdot 10^9$	$1.5 \cdot 10^9$	m
Bacterial Feeding Nematodes	BFN	[µg C / cm^3]	0.1	0.1	o
COPiotrophic Bacteria	COP	[µg C / cm^3]	59	25	o
Enzymes	E	[µg C / cm^3]	0.4	0.4	o
Hydrolytic Bacteria	HB	[µg C / cm^3]	3	2	l
HUMus Organic Matter of soil	HUMOM	[µg C / cm^3]	27844	27844	m
LIgnin fraction of plant RESidues	LIRES	[µg C / cm^3]	410	410	m
Nitrogen of Bacterial Feeding Nematodes	N_BFN	[µg N / cm^3]	0.02	0.02	m
Nitrogen of COPiotrophic Bacteria	N_COP	[µg N / cm^3]	13	6	o+l
Nitrogen of Enzymes	N_E	[µg N / cm^3]	0.13	0.13	l
Nitrogen of Hydrolytic Bacteria	N_HB	[µg N / cm^3]	0.7	0.4	l
Nitrogen of HUMus Organic Matter of soil	N_HUMOM	[µg N / cm^3]	2784	2784	m+l
Nitrogen of LIgnin fraction of plant RESidues	N_LIRES	[µg N / cm^3]	10	10	m+l
Nitrogen of OLIGotrophic Bacteria	N_OLIG	[µg N / cm^3]	4	3	o+l
Nitrogen of protozoa	N_PRTZ	[µg N / cm^3]	0.2	2.4	o+l
Nitrogen of Soluble Substrate	N_S	[µg N / cm^3]	20	20	o+l
Nitrogen of SOLuble Organic Matter of soil	N_SOLOM	[µg N / cm^3]	83	83	l
Nitrogen of SOluble fraction of plant RESidues	N_SORES	[µg N / cm^3]	415	370	m
Nitrogen of STable fraction of plant RESidues	N_STRES	[µg N / cm^3]	11	11	m
Nitrogen of STRuctural Organic Matter of soil	N_STROM	[µg N / cm^3]	206	206	m+l
Ammonium	NH4	[µg N / cm^3]	7.0	10	m
Nitrate	NO3	[µg N / cm^3]	0.1	2	m
OLIGotrophic Bacteria	OLIG	[µg C / cm^3]	20	15	o
Protozoa	PRTZ	[µg C / cm^3]	1	12	o
Soluble Substrate	S	[µg C / cm^3]	200	200	o
SOLuble Organic Matter of soil	SOLOM	[µg C / cm^3]	825	825	m+l
SOluble plant RESidues	SORES	[µg C / cm^3]	3250	3729	m
STable fraction of plant RESidues	STRES	[µg C / cm^3]	1767	1766	m
STRuctural Organic Matter of soil	STROM	[µg C / cm^3]	4125	4125	m+l

* m = measured, o = optimised, l = literature (see Materials and Methods section)

phase in soil. The state variables for nitrogen pools in the model are similar to the carbon state variables. Mineral forms of nitrogen, ammonium {NH4} and nitrate {NO3}, are also included (Fig. 1). An atmospheric N compartment {A_N} functions as a common sink of nitrogen from the soil due to denitrification and ammonia volatilization.

Model input

Total numbers of bacterial cells per g dry soil were entered into the model as obtained from two experiments described earlier (Zelenev et al., 2003b). These numbers were divided over copiotrophic and oligotrophic bacteria based on proportions of 'large' and 'small' cells, respectively, as obtained in an experiment on microbial dynamics after incorporation of a vetch-oats cover crop into soil (van Bruggen and Semenov, 2001). The volume of 'small' cells was calculated from the length and width according to Baath (1994) as 0.132 μm^3. That of 'large' cells was estimated as 0.35 μm^3. The number of 'large' cells was subdivided into numbers of hydrolytic and non-hydrolytic bacteria according to the proportion of copiotrophic CFU's (initial value: 0.04) that revealed hydrolytic activity (Alekhina et al., 1997). The non-hydrolytic copiotrophic bacteria were called {COP} and the hydrolytic copiotrophic bacteria {HB} in the model. Nitrifying bacteria were not modelled separately, but were considered as a small proportion (0.001) of oligotrophic bacteria, since their relative growth rate is about 0.04 (Berg, 1986), which is slightly higher than that of oligotrophs. The biomass carbon content of each group of bacteria per cm^3 of soil was calculated from their numbers and cell volumes assuming a cell wet density of 1.2 g/cm^3, cell dry weight of 25% of the wet weight, and cell carbon content of 49% of the dry weight (Jørgensen et al., 1991). Initital nitrogen content of each bacterial group was calculated based on a mean C/N ratio of 4.5 (Rutherford and Juma, 1992b).

BFN numbers were transformed into wet biomass by multiplication with 0.11 µg of fresh weight per individual nematode as determined in soil under barley (Sohlenius and Sandor, 1987). Carbon content of BFN was assumed to be 10% of fresh nematode biomass (Jensen, 1984). Initial nitrogen content was calculated based on a mean C/N ratio of 5.89 for 8 nematode species (Ferris et al., 1997). Numbers of protozoa were transformed into biomass carbon per cm^3 according to their biovolume (50 and 1600 μm^3 for flagellates and amoebae, respectively) (Bloem et al., 1992) and $1 \cdot 10^{-7}$ µg of carbon per μm^3 of protozoa biovolume (Brussaard et al., 1990). Initial nitrogen content of protozoan biomass was calculated from its biomass carbon and C/N ratio of 5 (Darbyshire, 1994; Rutherford and Juma, 1992b).

Carbon and nitrogen contents of coarse soil organic matter (extracted by water flotation) and plant material added to soil were determined by chemical analyses as described previously (Zelenev et al., 2003b). Water soluble material, cellulose, hemicellulose and lignin fractions of added plant material were determined by fiber analysis as described in Hu et al. (1997). Carbon contents of structural {STRES} and lignin-like {LIRES} fractions in the model were considered equivalent to those of cellulose (plus hemicellulose) and lignin, and were calculated based on the molecular composition of these compounds. Initial nitrogen content of cellulose was assumed the same as that in cotton fiber, namely 0.16% (Schwarzinger et al., 2002), and that of lignin similar to the humus fraction in soil, namely 2% (Tuev, 1989). Carbon and nitrogen contents of the soluble fraction {SORES} were calculated so that the total C and N content of all fractions equalled those of measured C and N contents

in plant material. Soil nitrate and ammonium contents were measured as µg N per g soil (Zelenev, 2003b) and transformed to µg N per cm^3 soil taking soil bulk density (1.1 g/cm^3) into account. The calculated values for C and N contents of bacterial, protozoan and nematode biomass, various soil and plant fractions, and mineral nitrogen compounds were entered as initial values at time 0 in the model. The initial values were later optimised to fit measured values during a month of observations (Table 1).

Growth and death of bacteria

Bacteria are assumed to take up carbon only in the form of soluble directly consumable substrate {S}. Bacterial relative growth rate is dependent on substrate concentration according to the Monod equation (see also Appendix A, equations 1, 2, 3):

$$G_b = B \cdot \mu_{max} \cdot (S/W)/(K_S+(S/W)) , \quad (1)$$

where

G_b = growth rate of a trophic group of bacteria [µg C h^{-1} cm^{-3} soil];

B = biomass of a trophic group of bacteria [µg C cm^{-3} soil];

μ_{max}= maximal relative growth rate of a trophic group of bacteria [h^{-1}];

S = consumable substrate content [µg C cm^{-3} soil];

W = soil moisture content [ml cm^{-3} soil];

K_S = half saturation constant for substrate [µg C ml^{-1} soil solution],

with different parameter values (Mu and Ks) for each of the trophic groups (Appendix B). Consumption of substrate by bacteria is calculated by dividing bacterial growth rate by the yield coefficient characteristic for each trophic group. Maintenance respiration is taken into account implicitly as part of growth respiration. Bacterial relative death rates decline with increasing substrate concentration according to a hyperbolic function:

$$Db = B \cdot Dm \cdot Kd/(Kd+(S/W)) , \quad (2)$$

where

Db = death rate of a trophic group of bacteria [µg C $\cdot$ h^{-1} $\cdot$ cm^{-3} soil],

Dm = maximal relative death rate of oligotrophic bacteria [h^{-1}],

Kd = death constant for substrate [µg C $\cdot$ ml^{-1} soil solution].

Each trophic group of bacteria has different death parameters Dm and Kd (Appendix B). The relative death rate of oligotrophic bacteria first decreases then increases with substrate concentration, as they are sensitive to substrate concentrations above 1200 µg C/ml (Klimova and Nikitin, 1994):

$$D_O = B_O \cdot (Dm \cdot Kd/(Kd+(S/W))+L \cdot (S/W)) , \quad (3)$$

where

D_O = death rate oligotrophic bacteria [µg C $\cdot$ h^{-1} $\cdot$ cm^{-3} soil],

B_O = biomass of oligotrophic bacteria [μg C · cm^{-3} soil],
L = factor for linear increase of relative death rate of oligotrophic bacteria with substrate [ml · $μg^{-1}$ C · h^{-1}].

Dead bacterial biomass is divided over {S}, {SOLOM} and {STROM} according to fixed proportions, estimated from the cytoplasm fractions KrC, KrO, and KrH going to {S} + {SOLOM}, and the cell wall fraction (1-KrC etc.) going to {STROM} (Juma and McGill, 1986). The cytoplasm of dead copiotrophic {COP} and oligotrophic bacteria {OLIG} is further divided into {S} and {SOLOM} according to the fractions KpC and KpO (Appendix B). The cytoplasm of dead hydrolytic bacteria {HB} is divided over the enzyme pool {E}, {S} and {SOLOM} according to fixed proportions, KpeH, KpH, and (1-KpeH-KpH), namely 0.15, 0.5, and 0.35 (Appendix B).

Growth and death of predators

Consumption of bacteria by protozoa and BFN is described by Monod equations (with different kinetic parameters for each group of organisms) in relation to the amount of bacterial biomass available above a certain threshold, TN for nematodes and TP for protozoa (see also Appendix A, equations 4, 5, and Appendix B):

$$C_P = Mc_P \cdot P \cdot (B-T_P)/(K_B+B_{tot}) \quad \text{if } B > T_P,$$
$$C_P = 0 \quad \text{if } B \leq T_P, \qquad (4)$$

where
C_P = consumption rate by a predator group [μg C · h^{-1} · cm^{-3} soil],
Mc_P = maximal relative consumption rate by a predator group [h^{-1}],
P = biomass of a predator group [μg C · cm^{-3} soil],
T_P = threshold for predation of a trophic group of bacteria [μg C · cm^{-3} soil],
K_B = half saturation constant for bacterial consumption by predator group [μg C · cm^{-3} soil],
B_{tot} = biomass of all trophic groups of bacteria [μg C · cm^{-3} soil].

Protozoa and BFN obtain carbon and nitrogen only from bacterial biomass. The relative death rate of protozoa and nematodes is assumed to be constant (Appendix B). Bacterial biomass consumed by protozoa and nematodes is used for their growth, growth respiration, and excretion. The growth rate of protozoa and nematodes is calculated as net rate of carbon assimilation (excretion and maintenance excluded) multiplied by production efficiency coefficient (see also Appendix A):

$$G_P = C_P \cdot (1-Ex_P) \cdot Y_P , \qquad (5)$$

where
G_P = growth rate by a predator group [μg C · h^{-1} · cm^{-3} soil],
Ex_P = portion of excrements from consumed biomass of bacteria by a predator group [-],
Y_P = production efficiency of a predator group [μg C · $μg^{-1}$ C].

The excretion rates of protozoa and nematodes are a fixed fraction (0.3 and 0.4, respectively) of the

consumed biomass C of microorganisms. Both excrements and necromass of protozoa and nematodes are distributed between {SOLOM} and {STROM} in certain constant proportions. Twenty percent of the excrements of protozoa and nematodes go into {SOLOM}. Of the necromass of protozoa, 90%, and of the nematode necromass 85% go into {SOLOM}.

Organic matter dynamics

Decomposition of any soil organic matter component is described in the model according to first order kinetics corrected by a factor (E/W)/Em:

$$D_Q = R_m \cdot (E/W)/E_m \cdot Q \,, \qquad (6)$$

where

D_Q = decomposition rate of an organic matter component [μg C · h^{-1} · cm^{-3} soil],

R_m = standard relative decomposition rate of an organic matter fraction [h^{-1}],

E = extracellular enzymes content in soil [μg C · cm^{-3} soil],

E/W = extracellular enzymes concentration in soil solution [μg C · ml^{-1}],

E_m = standard extracellular enzymes concentration in soil solution [μg C · ml^{-1}],

Q = content of an organic matter component in soil [μg C · cm^{-3}].

The value of Em was estimated as 1 μg C/ml (Appendix B) on the basis of the relationship between cellulase activity and enzyme protein concentration in water solution (Semenov et al., 1996) and soluble protein content in soil (Naseby et al., 1999). The relative decomposition rates (R) were dependent on the organic matter fractions to be decomposed and were taken from the literature (Appendix B).

Transitions of carbon from one organic matter component to other components is regulated by extracellular enzymes, excreted by hydrolytic bacteria. Enzyme excretion takes place only if the concentration of consumable substrate {S} in soil solution drops below a certain threshold (40 μg C/ml), and then depends on the substrate concentration hyperbolically: the lower the substrate concentration, the higher is the relative enzyme excretion rate (Ljungdahl and Eriksson, 1985) (see also Appendix A, equation 3):

$$E_{ENZ} = \mu_E \cdot B_H \cdot K_E/(K_E + S/W) \qquad \text{if } S/W < T_{AS} \,,$$
$$E_{ENZ} = 0 \qquad \text{if } S/W \geq T_{AS} \,, \qquad (7)$$

where

E_{ENZ} = rate of enzyme excretion [μg C · h^{-1} · cm^{-3} soil],

μ_E = maximal relative rate of enzyme excretion by hydrolytic bacteria [h^{-1}],

B_H = biomass of hydrolytic bacteria [μg C · cm^{-3} soil],

K_E = substrate constant for enzyme excretion [μg C · ml^{-1} soil solution],

T_{AS} = substrate threshold for enzyme excretion [μg C · ml^{-1} soil solution].

When the concentration of consumable substrate drops below the threshold level, the yield coefficient (Yhyd) and substrate half saturation constant (KsHB) of {HB} decrease from 0.5 to 0.09 μg C/μg C and

from 2 to 0.1 μg C/ml, respectively. This switch is included to take into account the extra energy that is probably needed to produce and excrete extra-cellular enzymes and the competitive ability of {HB} to take up catabolites released by their own extra-cellular enzymes, simulated as a higher affinity to the substrate released. Extra-cellular enzymes are subject to degradation into consumable substrate following first order kinetics with the correction factor (E/W)/Em as described above.

Release of substrate {S} from {SORES} is a fixed proportion (0.05) of the decomposing {SORES}; the rest (0.95) of the decomposing {SORES} flows into {SOLOM}. The carbon compounds resulting from decomposition of structural plant residues {STRES} are divided over soluble soil organic matter {SOLOM} and soil structural organic matter {STROM} at a ratio of 7:3 (Appendix B). Similarly, decomposing {STROM} is split between {SOLOM} and {HUMOM} at a ratio of 99:1, and decomposing {LIRES} into {SOLOM} and {HUMOM} at a ratio of 3:7. Soil humus {HUMOM} is subjected to very slow decomposition into soluble soil organic matter {SOLOM}.

Release of {S} and {SOLOM} from fresh plant residues {SORES} is initially restricted by the decomposition of the structural residue fraction {STRES} until 5% of this fraction is decomposed. After this threshold is reached {SORES} decomposition is accelerated by multiplication of the relative decomposition rate (Rsos) with an acceleration factor (Rsa) (see also Appendix A, equation 11):

$$\begin{aligned} Dsos &= Rsos \cdot (E/W)/Em \cdot SORES && \text{if } STRES/STRES_O > Psts\ , \\ Dsos &= Rsa \cdot Rsos \cdot (E/W)/Em \cdot SORES && \text{if } STRES/STRES_O \leq Psts\ , \end{aligned} \qquad (8)$$

where
Dsos = decomposition rate of a soluble fraction of plant residues [μg C · h^{-1} · cm^{-3} soil],
Rsos = initial relative decomposition rate of a soluble fraction of plant residues [h^{-1}],
Rsa = acceleration constant of initial relative decomposition rate of {SORES}
after a proportion Psts of {STRES} remained [-].

This construction was added to simulate restricted access of plant cell protoplasm to bacterial decomposition as long as plant cell walls are intact. But once a cell wall is damaged, the content of the cell is easily available for decomposition.

Nitrogen dynamics
The N-flow between two compartments is linked to the corresponding C-flow by dividing the C-flow by the current C/N ratio of the source compartment. Each group of bacteria takes up an amount of nitrogen from the substrate nitrogen {N_S} pool proportionally to the rate of carbon consumption from the {S} pool according to the C/N ratio of {S}. If the current C/N ratio of a bacterial group is higher than the standard C/N ratio for that group, consumption of nitrate and ammonium is switched on. The relative uptake rates are dependent on the concentration of each form of mineral nitrogen according to the Monod expression with their own specific parameter values:

$$U_N = U_m \cdot B \cdot (N/W)/(K_N + (N/W)) \qquad \text{if } CN_B > CN_B^S\ ,$$

$$U_N = 0 \qquad \text{if } CN_B \leq CN_B^S , \tag{9}$$

where

U_N = rate of ammonium or nitrate consumption by a trophic group of bacteria [µg N · h^{-1} · cm^{-3} soil],

U_m = maximal relative rate of ammonium or nitrate consumption by a trophic group of bacteria [µg N · μg^{-1} C · h^{-1}],

N = ammonium or nitrate content in soil [µg N · cm^{-3}],

CN_B = current C/N ratio of a trophic group of bacteria [-],

CN_B^S = standard C/N ratio of a trophic group of bacteria [-], −

with a higher maximal relative uptake rate and higher affinity for NH_4^+ than for NO_3^-. Predators receive nitrogen only through consumption of bacteria according to the C/N ratio of the specific bacterial group, defecate at predator own C/N ratio, and secrete excessive N as ammonia (see next paragraph). Nitrogen from dead organisms returns to {SOLOM}, {S} and {STROM} according to their current C/N ratio, disregarding differences in C/N ratio between cytoplasm and cell wall.

Release of nitrogen in the form of ammonium takes place from six different sources: {N_SORES}, live and dead protozoa and their excrements {N_PRTZ}, live and dead nematodes and their excrements {N_BFN}, and dead copiotrophic, oligotrophic and hydrolytic bacteria {N_COP},{N_OLIG}, and {N_HB}. Nitrogen mineralisation to ammonium is modelled by enzymatic decomposition of {N_SORES} into {N_S}, {N_SOLOM} and {NH4} according to a fixed division over these pools, namely 5% to {N-S}, 38% to {N_SOLOM} and 57% to {NH4}. In addition, ammonium is released directly from the cytoplasm of dead organisms and excrements rather than from {SOLOM}, because {SOLOM} includes humic acids and other break-down products from {STROM} and (HUMOM}. The fraction of the N flow from dead cytoplasm and excrements to {SOLOM} that is released as NH_4^+ is taken as 0.2 (Appendix B). Ammonium is also released from live protozoa and BFN, because the C/N ratio of bacteria is lower than that of their predators (Appendix B). The amount of NH_4^+ released is calculated as [assimilation]/[C/Nbact] – [growth]/[C/Npred]:

$$V_{NH4} = C_P \cdot (1 - Ex_P)/CN_B - G_P/CN_P^S \qquad \text{if } CN_P \leq CN_P^S ,$$
$$V_{NH4} = 0 \qquad \text{if } CN_P > CN_P^S , \tag{10}$$

where

V_{NH4} = rate of NH_4^+ release from a live predator group [µg N · h^{-1} · cm^{-3} soil],

CN_P = current C/N ratio of a predator group [-],

CN_P^S = standard C/N ratio of a predator group [-].

The rate of ammonium nitrification is modelled according to a Monod equation as dependent on ammonium content per cm^3 of soil. Nitrifying bacteria are considered a small proportion (0.001) of oligotrophic bacteria (see also Appendix A, equations 28, 29):

$$V_{NO3} = Vnit \cdot 0.001 \cdot B_O \cdot NH4/(Knit+NH4), \quad (11)$$

where
V_{NO3} = rate of nitrification [μg N · h^{-1} · cm^{-3} soil],
Vnit = maximal relative rate of nitrification by nitrifying bacteria [μg N · μg^{-1} C · h^{-1}],
NH4 = ammonium content in soil [μg N · cm^{-3}],
Knit = half saturation constant of ammonium content for nitrification [μg N · cm^{-3} soil].

As small pockets of anaerobic conditions can exist under otherwise aerobic conditions (especially after addition of easily decomposable organic substrates to soil), denitrification is also included in the model. However, oxygen concentrations are not described in this model, and nitrification and denitrification are assumed to occur side by side. The rate of denitrification is described by a double term Monod equation depending on concentration of nitrates {NO3} and consumable substrate {S}, and on the sum of {COP} and {HB} (see also Appendix A, equations 29, 30):

$$V_{DN} = Vdnit \cdot (B_C+B_H) \cdot \{(NO3/W)/(Kdnit+(NO3/W))\} \cdot \{(S/W)/(Ksdnit+(S/W))\}, \quad (12)$$

where
V_{DN} = rate of denitrification [μg N · h^{-1} · cm^{-3} soil],
Vdnit = maximal relative rate of denitrification by denitrifying bacteria [μg N · μg^{-1} C · h^{-1}],
$B_C + B_H$ = estimate of biomass of denitrifying bacteria, presumed to be the sum of copiotrophic and hydrolytic bacteria [μg C · cm^{-3} soil],
NO3 = nitrate content in soil [μg N · cm^{-3}],
Kdnit = half saturation constant of nitrate concentration for denitrification [μg N · ml^{-1} soil solution],
Ksdnit = half saturation constant of substrate concentration for denitrification [μg C · ml^{-1} soil solution].

To determine the rate of ammonia volatilisation (Appendix A, equation 30), first the ammonia concentration in soil solution was calculated from extractable ammonium content in soil according to Barnabe (1990). Next, the concentration of ammonia in soil air was calculated from the concentration of ammonia in soil solution using the distribution coefficient (solution:air) for ammonia K_{distr} = 1829 at 25 ^oC (Hales and Drewes, 1979). Finally, the rate of ammonia volatilisation from soil air to the atmosphere was calculated by muliplication of the difference between the ammonia concentration in soil air and in the atmosphere with the inverse of the residence time (English et al., 1980).

Model runs
The model was run in the program 'Model Maker' (Cherwell Scientific Publ. Ltd., Oxford, UK), with initial values calculated from experimental results (Zelenev et al., 2003a and b) or optimized to fit experimental results (Table 1). Optimization was done manually. Parameter values were taken as much as possible from the literature (Appendix B). The Runge-Kutta method of integration was selected with a variable time step starting from 0.01 hour. The model was run for 800 hours with input

data from two similar experiments as described above. To test the internal workings of the model, simulated C/N ratios of all components were checked for consistency. Different trophic groups of bacteria and predators were omitted from the model to assess their role in the modeled food web, and their effects on model performance and on nitrogen release from decomposing residues.

Model output

Modeled biomass carbon of each bacterial group was converted to number of bacteria per g dry soil taking into account cell volumes and other cell characteristics given above. The sum of the calculated numbers in each bacterial group, {BACT}, was compared with experimentally measured total microscopic counts of bacteria stained with FITC (Zelenev et al., 2003b). Experimentally determined nitrate and ammonium content, and numbers of protozoa and BFN (Zelenev et al., 2003a) were converted into μg of nitrogen or carbon per cm^3 soil, and were compared with modeled values in the same units. To assess the agreement of the model output with the experimental data, the coefficient of determination r^2 was estimated by the "Goodness of fit" procedure in Model Maker.

Results

Model performance

The results of the simulation model were in good agreement with experimental data with respect to bacteria, protozoa, nematode, and mineral nitrogen dynamics. These results were obtained using mostly the same parameter values for both data sets (Appendix B), except for 3 parameters, which were related to the thresholds of bacterial biomass above which predation by protozoa would take place (Table 2). As the dynamics of microorganisms and mineral nitrogen were very similar for both model runs, only the data of the first experiment are presented, except for the modeled and experimental bacterial numbers.

Table 2. List of 'BACWAVE-WEB' parameter values that varied between experiment 1 and 2.

Parameter	Symbol	Unit	Experiment	
			1	2
Minimal biomass of Hydrolytic Bacteria {HB}, which is available for Protozoa consumption (Threshold)	TPH	[μg C / cm^3]	2.2	10.0
Minimal biomass of COPiotrophic Bacteria {COP}, which is available for Protozoa consumption (Threshold)	TPC	[μg C / cm^3]	3.0	10.0
Minimal biomass of OLIGotrophic Bacteria {OLIG}, which is available for Protozoa consumption (Threshold)	TPO	[μg C / cm^3]	13.2	25.0
Soil moisture	W	[ml / cm^3]	0.17	0.18

Simulated bacterial numbers fluctuated over time in a similar manner as observed bacterial populations in both experiments (Fig. 2a and b). The model reproduced a small initial peak in total bacterial numbers followed by a large peak and then a series of lesser dampening peaks reaching an equilibrium after 400-600 hours since CG incorporation. The r^2 of the total fit was 0.80 for the first experiment and 0.47 for the second experiment.

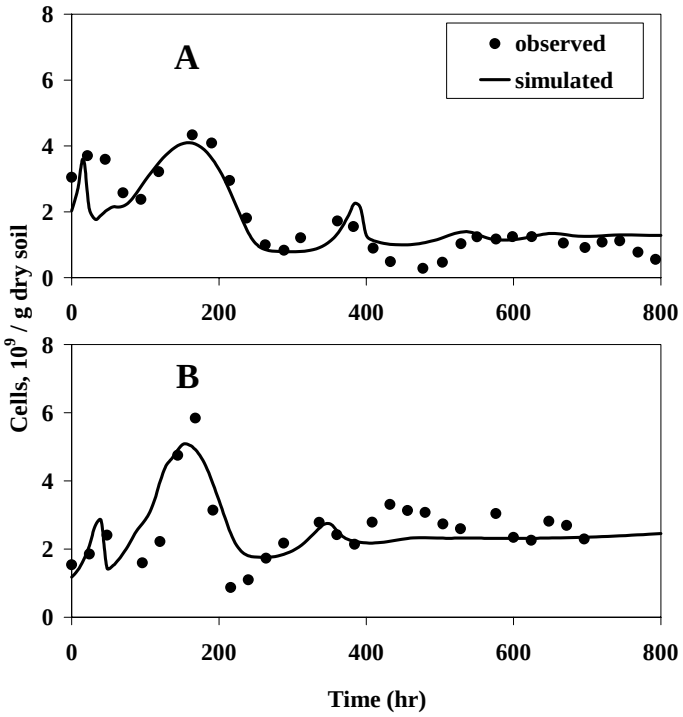

Figure 2. Dynamics of FITC-stained bacterial counts (circles) and simulated total bacterial numbers (lines) after incorporation of clover-grass material in soil for microcosm experiment 1 (A), and microcosm experiment 2 (B).

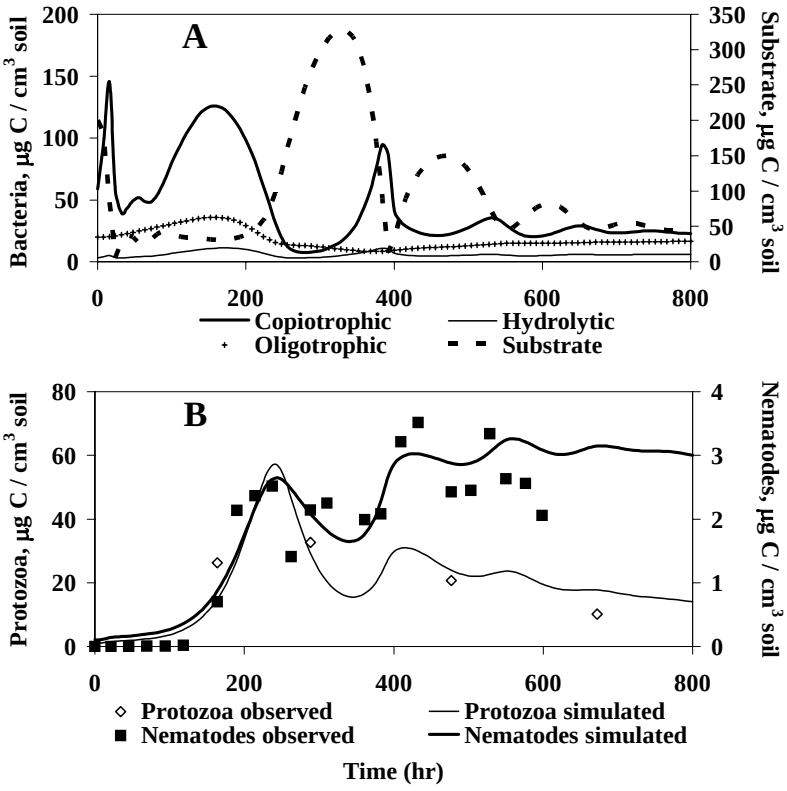

Figure 3. Simulated dynamics of consumable substrate and biomass of different trophic groups of bacteria (A), and observed and simulated biomass of bacterial predators (B) for microcosm experiment 1.

Modeled copiotrophic biomass was the main component of bacterial biomass responsible for the oscillating behavior of the total number of bacteria. The main peaks in biomass of copiotrophic bacteria took place at 16, 160 and 384 hours after CG incorporation in the first experiment (Fig. 3a), and at 40, 160 and 352 hours in the second experiment (data not shown). Hydrolytic bacteria oscillated at a similar frequency as copiotrophs but with a reduced amplitude (Fig. 3a). Oligotrophs revealed a different pattern of fluctuations characterized by small amplitudes and large periods tending to stabilization after 400 hours (Fig. 3a).

Simulated dynamics of consumable substrate content was almost in opposite phase with modeled copiotrophic and hydrolytic biomass dynamics. Oscillations in substrate fell in the range of 6-328 μg C/cm^3 soil and were also dampening to the end of the period of simulation (Fig. 3a). Modeled changes in oligotrophic biomass were only slightly related to substrate dynamics.

Modeled protozoan biomass had a lag phase of about 100 hours, and then increased in response to the second peak in bacterial biomass, and fluctuated thereafter (Fig. 3b). The peaks in protozoa followed peaks in bacterial biomass, except for the initial peak in bacterial biomass. Amplitudes of peaks in protozoan biomass decreased with dampening of bacterial biomass, declining monotonously when bacteria approached a steady state. Modeled BFN oscillated with the same frequency as protozoa, but did not decline after 400 (376 for second experiment) hours as protozoa did, so that they reached a higher biomass than protozoa at the end of the model run (Fig. 3b).

The most labile components of the organic materials apart from consumable substrate {S} were soluble soil organic matter {SOLOM} and soluble plant residues {SORES}. The {SORES} content decreased monotonically and practically disappeared within 200 hours after addition of plant material (Fig. 4a). The fraction{SOLOM} had one peak of about 3000 μg C/cm^3 soil at about 136 hours for both experiments and then gradually decreased to the end of the run. The plant structural fraction {STRES} slowly decreased from the beginning to the end of the model run by 24-37% from the initial content. The soil structural fraction {STROM} did not change very much in the course of 800 hours, first slightly increasing and then slowly decreasing in both experiments (Fig. 4a). The lignin fraction {LIRES} decreased very slowly and the modelled loss during 800 hours was about 0.5-0.9% from its initial content. The humus content {HUMOM} changed extremely slowly, and 800 hours after incorporation of plant material it had increased by about 0.04-0.07%.

The modelled dynamics of nitrate and ammonium were close to the measured values (Fig. 4b). The ammonium content peaked at about 128 hours in both experiments, just before the second bacterial peak and considerably (90-100 hours) before the first peak in predators, and then dropped down to almost zero after 376 hours. The observed nitrate content increased in a sigmoid manner after a lag period of about 50 hours, in which the first peak in bacteria occurred. The simulated nitrate content increased slightly faster between 50 and 200 hours and then stabilised to a slightly lower level than the measured nitrate content (Fig. 4b).

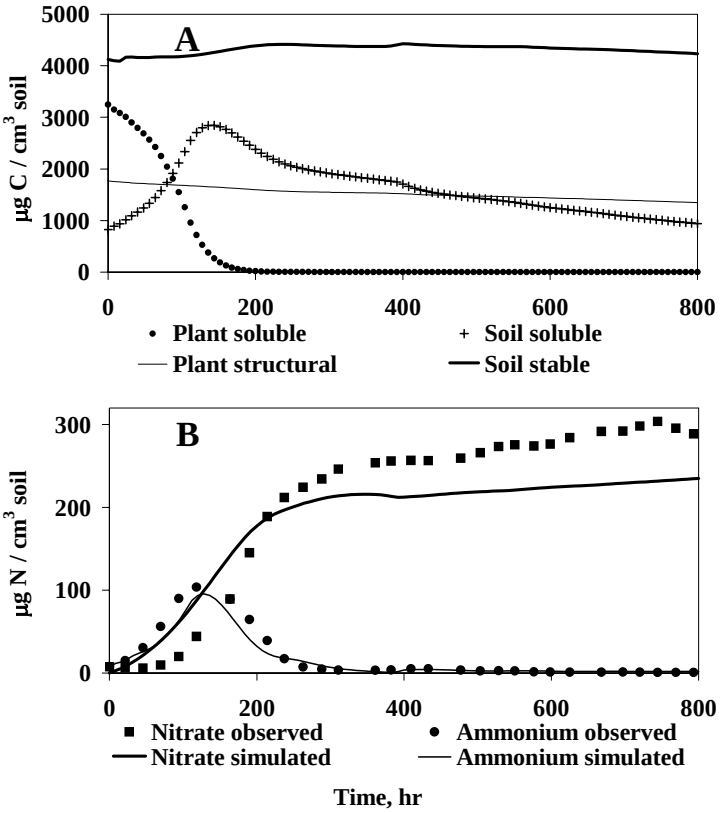

Figure 4. Simulated dynamics of soil and plant organic matter fractions (A), and observed and simulated soil nitrate and ammonium content (B) for microcosm experiment 1. Curves for humus {HUMOM} and plant lignin fractions {LIRES} are not shown in figure 4A, since values of those fractions practically did not change for 800 hours and were about 27843 and 409 µg / cm^3 soil respectively.

Parameter estimation

The majority of the parameters were taken from the literature and were not changed during optimisation of the model. Parameters regulating growth and death of bacterial groups were slightly modified, but are not very different from the values estimated previously (Appendix B). Values of maximal relative rates of nitrate and ammonium consumption by bacteria used in the model were considerably lower than reported in the literature. As a rule the C/N ratio of each bacterial group was slightly below their standard values, consumption of mineral forms of nitrogen by bacteria did not usually take place. Parameters responsible for consumption, growth and death of predators were kept close to the average estimates reported in the literature. Relative death rates for protozoa as well as for BFN had to be increased about 10-fold in order to fit experimentally determined levels of their biomass, probably because higher level predators were not included in the model. Maximal relative consumption rate of BFN was optimised to a lower value relative to that in the literature (Appendix B). Other parameter values were close to those in the literature.

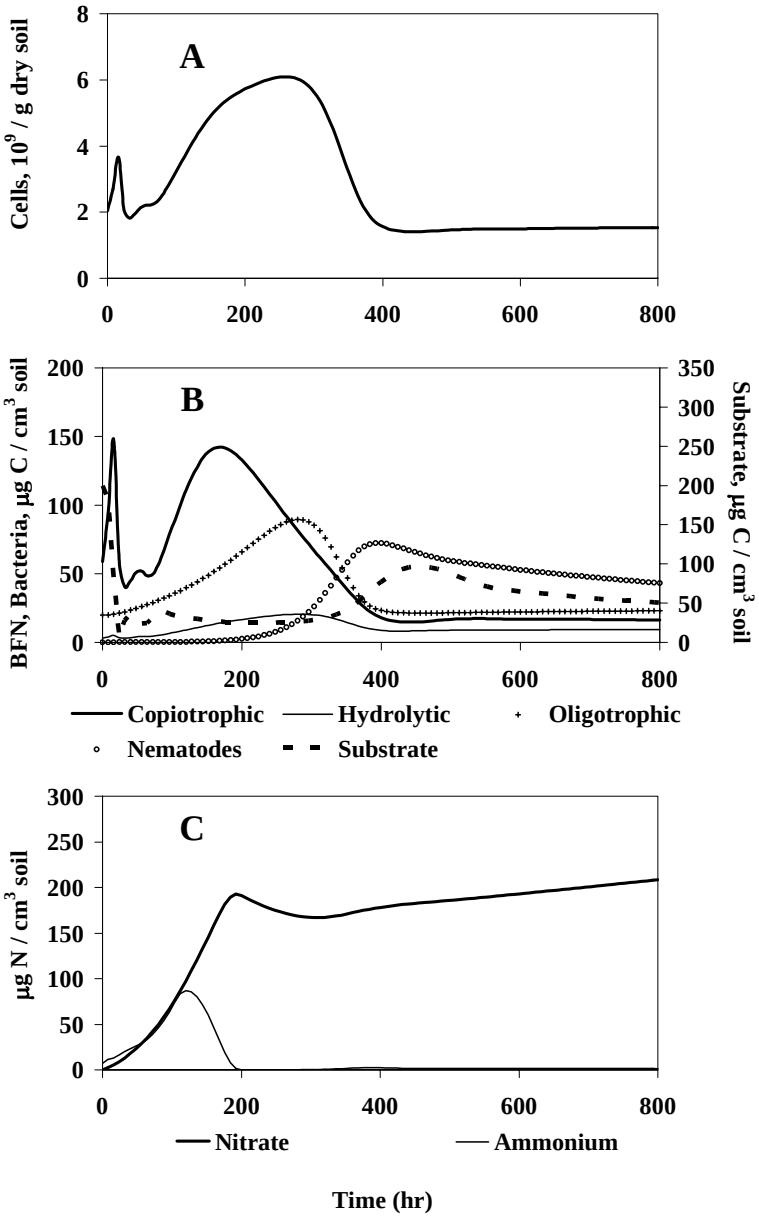

Figure 5. Simulated dynamics of total bacterial numbers (A), consumable substrate and biomass of different trophic groups of bacteria (B), and soil nitrate and ammonium content (C) for microcosm experiment 1 in the absence of protozoa.

Model investigations

Elimination of BFN from the food web caused no visible changes in bacterial as well as nitrate and ammonium dynamics (data not shown) as compared to the results of the full version of the model (Fig. 2a and b, Fig. 3a and b). However, elimination of protozoa from the food web considerably changed bacterial dynamics (Fig. 5a and b) and noticeably modified mineral nitrogen (Fig. 5c). Absence of protozoa led to a 2.5 (3.5) –fold increase in oligotrophic biomass at 280 (384) hours in the first and second model runs (second between brackets), respectively. Peaks in copiotrophic and hydrolytic bacteria became wider (Fig. 5b), so that the second peak of total numbers of bacteria was shifted to 264 (376) hours and reached values of 6.1 (6.6) billion cells per g dry soil (Fig. 5a). Then total

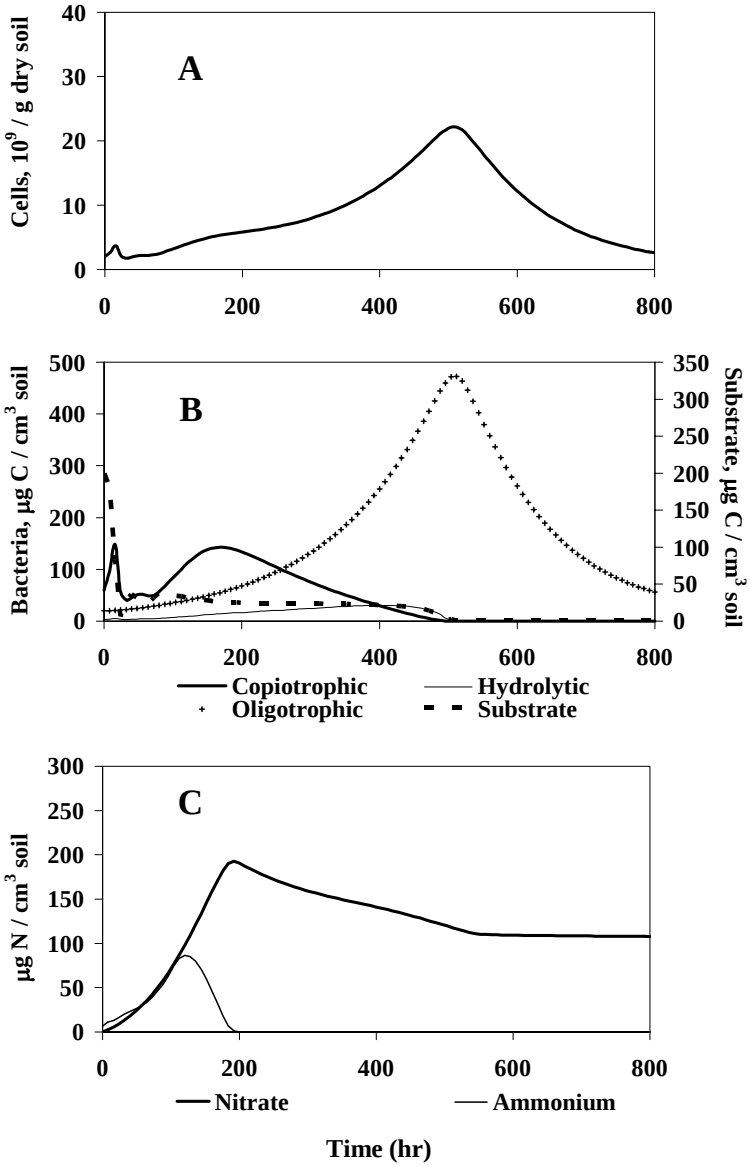

Figure 6. Simulated dynamics of total bacterial numbers (A), consumable substrate and biomass of different trophic groups of bacteria (B), and soil nitrate and ammonium content (C) for microcosm experiment 1 in the absence of both predators.

numbers of bacteria decreased about 4-fold and did not show any oscillations after 416 (568) hours of model run associated with a considerable growth of BFN from 200 hours onwards (Fig. 5b). BFN reached a peak of 72 (48) µg biomass C per cm^3 soil at 392 (504) hours. Later BFN biomass gradually decreased down to values of 43 (16) µg C/cm^3 soil (Fig. 5b). The dynamics of ammonium without protozoa was the same as with protozoa up to about 100 hours of the model run (Fig. 5c). After a peak the content of ammonium dropped slightly faster in the absence of protozoa. The nitrate content in this case was similar until about 200 hours, and then after a small peak, remained 30 (75) µg N/cm^3 soil lower than the final nitrate content in the presence of protozoa.

Removal of both BFN and protozoa from the food web completely altered the dynamics of total

bacterial numbers in soil (Fig. 6a), which was caused by enormous growth of oligotrophic bacterial biomass (Fig. 6b) because oligotrophs were not eliminated by predators. One peak was observed in total numbers of bacteria, reaching about 22 billion cells/g dry soil after 510 (570) hours of the model run. The subsequent decrease in total numbers of bacteria was attributed to the death of oligotrophic bacteria due to exhaustion of substrate. The dynamics of copiotrophic bacteria was similar to the situation with predators showing two peaks within 200 hours. Without predators, hydrolytic bacteria did not follow the same dynamics as copiotrophic bacteria, but {HB} biomass continued to increase to 30 (40) μg C/cm^3 soil at 400 (456) hours of the model run. Lack of substrate led to extinction of copiotrophic and hydrolytic bacteria when oligotrophic bacteria peaked (512 (560) hours) (Fig. 6b). However, in the first 100 hours the total numbers as well as biomass of individual groups of bacteria largely coincided with the dynamics in the presence of predators indicating limited influence of predators on bacterial population during early stages of residue decomposition (Fig. 3a, Fig. 6b). The dynamics of ammonium in the absence of predators was practically identical to the run in the absence of protozoa, whereas the simulated nitrate content was the same only within 232 (320) hours from the start of those runs and then, in the case without predators, continued to decrease before it took almost a constant value of 110 (44) μg N/cm^3 soil after 552 (592) hours (Fig. 5c, Fig. 6c).

Elimination of copiotrophic bacteria led to formation of an enormous peak in hydrolytic bacteria of about 1490 (1550) μg biomass C per cm^3 soil after 176 (200) hours of the model run. This peak comprised 26.6 (27.6) billion cells per g dry soil (Fig. 7a). After this peak bacterial numbers decreased down to values of 0.3 billion cells per g dry soil and then formed one or two small peaks at about 550 (500 and 750) hours with 2.8 billion cells per g dry soil (Fig. 7a). These peaks were formed mainly by hydrolytic bacteria since the biomass of oligotrophic bacteria was very small due to a high consumable substrate content, which prevented growth of oligotrophs (data not shown). Protozoa had the first big peak at 264 (248) hours when their biomass reached 129 (253) μg C/cm^3 soil and then one or two small peaks of 40 (50) μg C/cm^3 soil at 600 (544 and 792) hours. BFN reached maxima simultaneously with the protozoa and had a biomass of 21 and 45 μg C/cm^3 soil at the first and the second peak, respectively (Fig 7b). Peaks in predator biomass followed those in bacterial biomass after 40-90 hours and played a considerable role in the decrease of bacterial biomass and numbers. Elimination of copiotrophs led to a complete change in mineral nitrogen dynamics: instead of one peak in ammonium there were three peaks within 800 hours, and the final concentration was much higher than in the real situation (Fig. 7c). In its turn nitrate did not go up in a sigmoid manner, but had a peak of 33.1 (29.8) μg N/cm^3 after 136 hours of the model run, went down to almost zero at 200 hours and then increased again ending at about 79 (26) μg N/cm^3 soil after 800 hours. This abnormal behavior in nitrate dynamics was probably related to the limited biomass of oligotrophs, which included nitrifying bacteria in this model.

Elimination of hydrolytic bacteria had as implication that the enzyme pool was not replenished by extracellular enzyme production, although residual soil enzymes continued to affect decomposition of various organic matter fractions. Consequently the level of consumable substrate was relatively low, and copiotrophic bacteria practically did not contribute to the total bacterial numbers after 250 hours

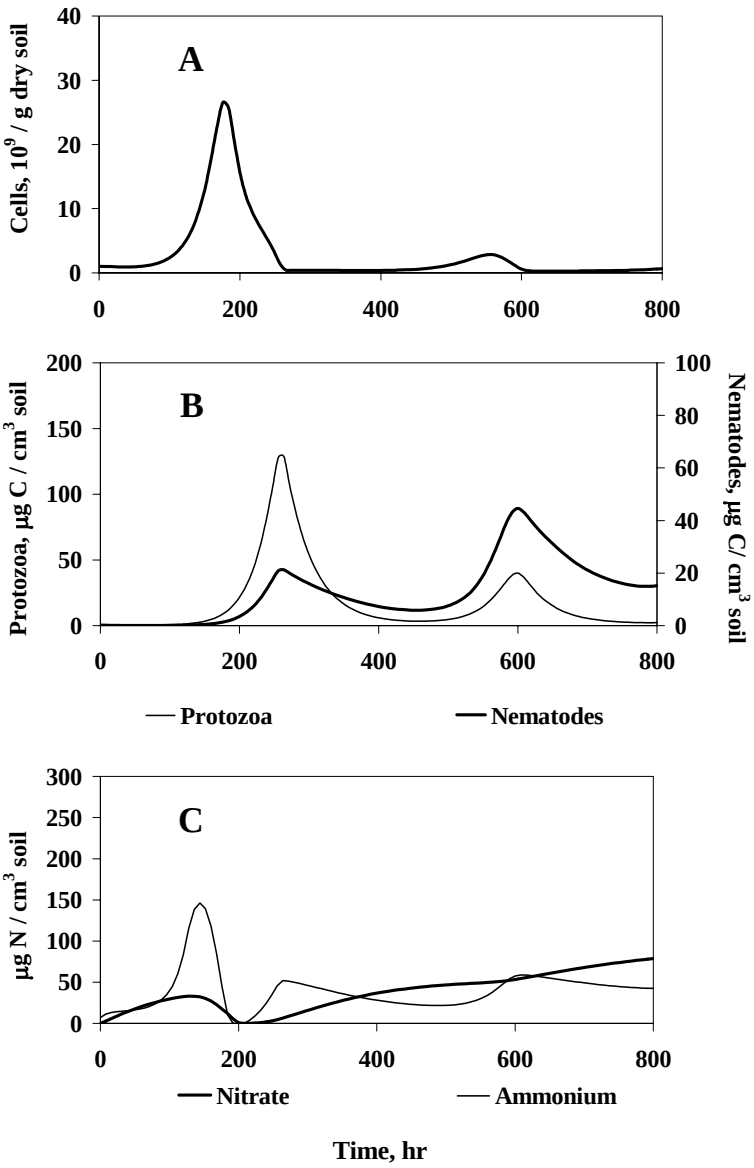

Figure 7. Simulated dynamics of total bacterial numbers (A), biomass of bacterial predators (B), and soil nitrate and ammonium content (C) for microcosm experiment 1 in the absence of copiotrophic bacteria.

(data not shown). Copiotrophic bacteria formed only the regular early peak of 3.6 (2.8) billion cells per g dry soil at 16 (40) hours. After this peak copiotrophic bacteria numbers decreased to 0.1 (0.2) billion cells per g dry soil and had 2 low peaks before they almost disappeared. In this situation oligotrophic bacteria played a dominant role and formed one main peak of 3.5 (4.0) billion cells per g dry soil at 300 (350) hours (Fig. 8a). Predators had a relatively low biomass compared to the original situation. Protzoan and BFN biomass did not exceed 8.8 and 0.5 µg C/cm^3, respectively, at around 410 hours in the first model run (Fig. 8a). The behavior of protozoa and nematodes was completely different in the second model run, with one large peak in protozoa and a small peak in nematodes after about 40 hours (data not shown). The dynamics of nitrate and ammonium were similar to the original situation in the first experiment, except for an earlier and lower peak in ammonium content (Fig. 4b,

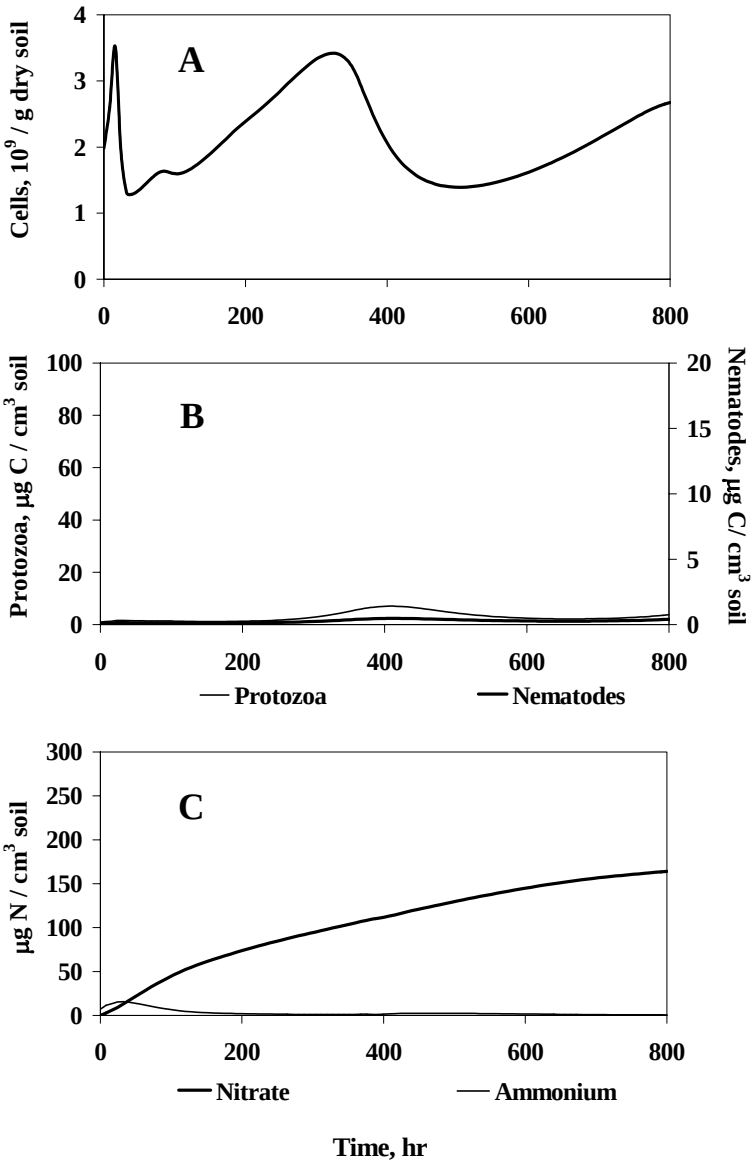

Figure 8. Simulated dynamics of total bacterial numbers (A), biomass of bacterial predators (B), and soil nitrate and ammonium content (C) for microcosm experiment 1 in the absence of hydrolytic bacteria.

Fig. 8c). In the second model run, the dynamics of ammonium and nitrate were similar to that of the original experimental situation, except that the nitrate content suddenly collapsed to zero after 395 hours of model run (data not shown).

Elimination of oligotrophic bacteria led to lower total numbers of bacteria on average than the complete version of the model, and resulted in appearance of more pronounced peaks of total bacterial numbers, which fluctuated in a dampening mode within the range of 0.1-2.7 (0.5-3.1) billion cells per g dry soil (Fig. 9a). These peaks were formed by copiotrophic and hydrolytic bacteria fluctuating simultaneously. Initial peaks in copiotrophic bacterial biomass reached 144 (150) µg C/cm^3 soil while peaks in hydrolytic bacterial biomass were 10- (3-) fold lower. More pronounced peaks in bacterial

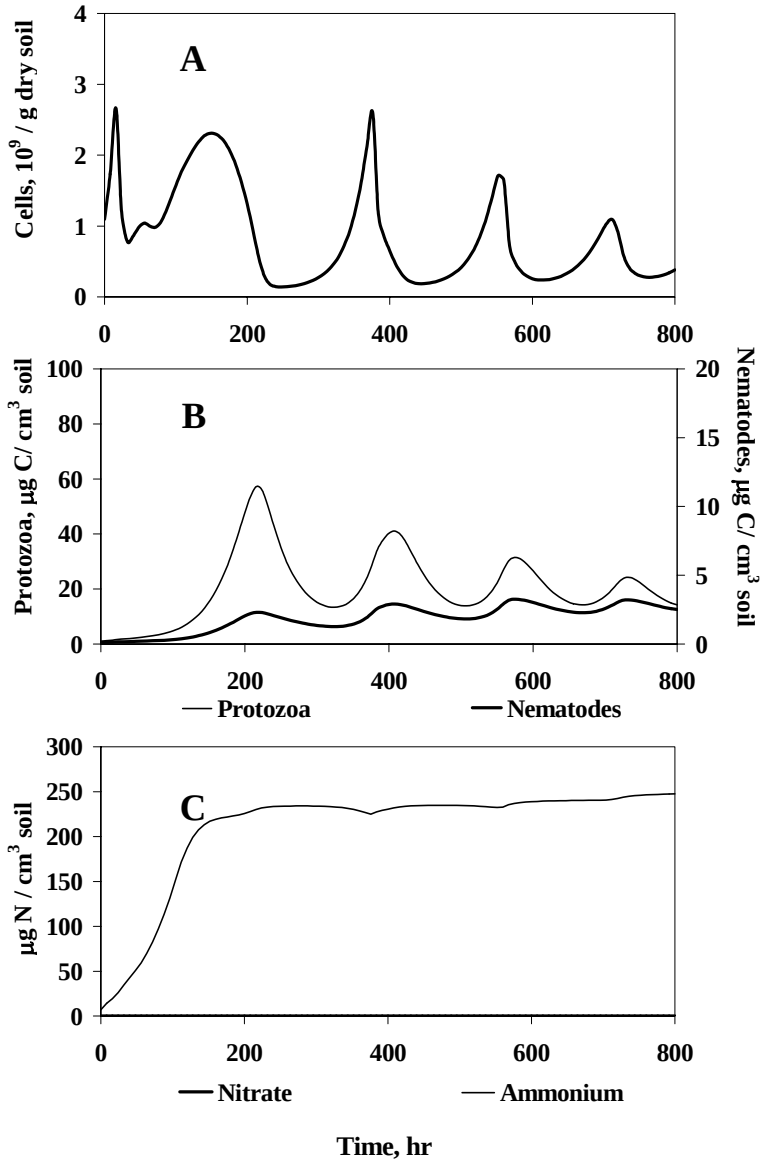

Figure 9. Simulated dynamics of total bacterial numbers (A), biomass of bacterial predators (B), and soil nitrate and ammonium content (C) for microcosm experiment 1 in the absence of oligotrophic bacteria.

biomass resulted in more distinct peaks of both predator groups (Fig. 9b), although the mean biomass of each predator group remained close to the values obtained in the complete version of the model (Fig. 3b), where fluctuations in predators dampened more quickly (Fig 2a). Dynamics of mineral nitrogen in the absence of oligotrophic (and nitrifying) bacteria were completely altered with a sigmoid increase in ammonium up to 240 (210) µg N/cm^3 without conversion to nitrate (Fig. 9c).

Discussion

Short-term dynamics of bacteria, protozoa, BFN and mineral nitrogen were modelled accurately after incorporation of fresh plant material into soil. The model satisfactorily reproduced the release of ammonium and conversion to nitrate at early stages of plant material decomposition. For the first time, dynamics of three main soil bacterial trophic groups, namely copiotrophs, oligotrophs and hydrolytics were modelled separately, contrary to the usual modelling of one generalised total bacterial population (Brussaard, 1998). These groups have long ago been recognised as the most important components of the soil heterotrophic bacterial community (Semenov, 1991). Distinct peaks in total bacterial populations coincided with peaks in copiotrophic and hydrolytic bacteria, demonstrating that the peaks did not consist of different trophic groups in succession, but that these two trophic groups fluctuated simultaneously (objective 1). Oligotrophic bacteria had only one main maximum, coinciding with the second peak in copiotrophic and hydrolytic bacteria.

Copiotrophic bacteria are fast-growing and -dying bacteria that respond quickly to substrate changes and are mainly responsible for the fluctuations in bacterial populations in soil. When copiotrophic nonhydrolytic bacteria were eliminated from the model, there was a considerable increase in average substrate content in soil compared with the run when copiotrophic bacteria were present, indicating that copiotrophic bacteria quite rapidly bring substrate content to a low level. So, copiotrophic bacteria create favourable conditions for growth of oligotrophic bacteria, since they are suppressed at high substrate content (Semenov, 1991). When oligotrophic bacteria were omitted from the model, fluctuations in total bacterial numbers and substrate concentrations were enhanced, indicating that one of the functions of oligotrophs is their stabilizing capacity (van Bruggen and Semenov, 2000). When hydrolytic bacteria were removed from the model, only the first peak in copiotrophic bacteria remained, and oligotrophic bacteria had a chance to grow, so that the second peak in total bacteria consisted primarily of oligotrophs. The behavior of protozoa and nematodes changed in response to changes in dynamics of the various trophic groups of bacteria, which were primarily determined by interactions with substrate.

During the first 600 hours (400 hours in the second experiment) of residue decomposition, the model reflected the observed fluctuations in microbial populations better than during the last part of the model runs, when simulated bacterial populations ceased to fluctuate while observed populations continued to oscillate. This discrepancy could be due to many reasons, for example the inability to properly quantify (and model) substrate concentrations for bacteria and the response of predators to bacterial fluctuations. Especially in the second experiment, nematode populations continued to increase from 400 hours onwards, and were overestimated after 600 hours of model run. Thus, there was no proper regulation of BFN populations, possibly due to the absence of higher-order predators (de Ruiter et al., 1993). However, proper regulation was achieved in the first part of the model run, when regulation of bacterial populations was accomplished by fluctuations in substrate concentration while predators hardly played a role in regulation. Thus the lack of fluctuations in bacterial populations in the last part of the model run, when the bacterial community consisted mainly of oligotrophs, may also be due to absence of regulation by substrate concentrations at low average

substrate concentrations. This may be due to an unrealistically low cross-over point of the relative death and growth rate curves for oligotrophs in relation to substrate concentration (see chapter 4). As a result, oligotrophs continued to grow at a very slow rate, and BFN continued to grow in tandem. Nevertheless, fluctuations in microbial biomass were simulated with our model, while other models failed to do this, despite obvious fluctuations in observed data (Blagodatsky et al., 1998; Kätterer and Andrén, 2001).

Two groups of bacterial predators, namely nematodes and protozoa were included in the model. Nematode populations were simulated quite accurately. Simulated protozoa populations were slightly lower than observed populations, however protozoa were not observed frequently enough to see fluctuations in their numbers. Thus, modelled populations are more likely correct. Neither of the predator groups seemed to contribute significantly to the decline in copiotrophic bacteria after the first peak. Yet, calculations of bacterial consumption based on feeding rates of protozoa indicated a potential small role of protozoa in the first decline in bacterial numbers (Zelenev et al., 2003a). Both predators likely contributed to the decline in bacteria after the second peak, when their numbers started to increase, but substrate was the main regulating factor. Protozoa had a biomass 10-20 times that of BFN, and were possibly more responsible for bacterial regulation after the second peak in bacterial numbers than BFN were. Indeed, removal of nematodes from the model had no effect on population dynamics of bacteria and patterns of ammonium release and transformation to nitrate. Removal of protozoa or both predators resulted in a slight increase in copiotrophic bacteria and a major increase in oligotrophic bacteria, with a major effect on total bacterial numbers, namely delaying the second peak, and eliminating subsequent fluctuations. The dynamics of ammonium release and transformation to nitrate were not significantly altered, although the final nitrate concentration was slightly lower in the absence of predators, as had been found experimentally too (Ferris et al., 1998).

Flows of carbon and nitrogen are simulated in detail in 'BACWAVE-WEB'. Similar to many other models, a distinction is made between labile, structural and stable fractions (Brussaard, 1998), named soluble, structural and lignin or humus containing fractions in this model. These fractions were distinguished for both fresh plant material and soil organic matter. The main difference with other decomposition models is that a separate state variable is introduced for directly consumable substrate, which allows proper simulation of fluctuations in bacterial populations. This state variable was introduced earlier in the model 'BACWAVE' (Zelenev et al., 2000). In 'BACWAVE' predators were not included; yet, wave-like oscillations were accurately modelled along wheat roots thanks to the interaction of bacterial populations with substrate concentrations. In addition to the organic matter fractions and substrate, an extra state variable for enzymes was introduced into 'BACWAVE-WEB' to control rates of decomposition and population dynamics of different functional groups of bacteria. Soil enzymes were also introduced in the model of Henriksen and Breland (1999), and significantly improved the performance of their model.

'BACWAVE-WEB' simulated nitrogen flows satisfactorily. The C:N ratio's of the living components of the model fluctuated only very slightly, at most about 10% (data not shown). The C:N ratio of microbial biomass could vary considerably (Hassink et al., 1994; van Veen et al., 1984), but these

changes may be attributed to variations in microbial composition. We assumed that the C:N ratio for specific trophic groups is rather constant, especially in the same soil under controlled conditions.

As long as all microbial functional groups were included in the model, release of ammonium and conversion to nitrate were modelled accurately. Initial release of ammonium was partially accomplished by direct ammonification from {SORES}, dead cytoplasm of bacteria and predators, and soluble excrements from predators, as affected by hydrolytic enzyme concentration. The reason for this construction was the capacity of soil enzymes to degrade proteins even after soil fumigation (Ladd and Jackson, 1982). Yet, live bacteria were also (indirectly) important for ammonium release and transformation, as removal of copiotrophic and hydrolytic bacteria from the model significantly changed ammonium and nitrate concentrations over time. Removal of predators had little effect on ammonium and nitrate dynamics, but did reduce final nitrate concentrations somewhat. Hassink et al. (1994) also concluded from their experimental and modelled results on C and N mineralization in various soils that differences in N mineralization rates had to be attributed to the microbial biomass and its C:N ratio rather than the microfauna. Other authors found significant effects of predators on ammonium release, but not on nitrate concentrations (Rutherford and Juma, 1992c).

The selected parameter values resulted in acceptable fits of the modelled curves to the experimental data. By far the majority of the parameter values were the same for both model runs with experimental data from Spring 2000 and Fall 2001. Only very few parameters had to be changed (Table 2) in order to get the best fit for each of the experiments. The differences in thresholds (TPC, TPO and TPH) for consumption of different groups of bacteria by protozoa could be attributed to higher bacterial numbers and hence biomass of bacteria in the second experiment, so that protozoa had better nutritive conditions and thresholds for consumption of bacteria could be higher, although they were still within the values for protected biomass given by Hunt et al. (1984). Alternatively, slight differences in soil moisture and inhabitable pore space could explain the differences in predation thresholds between experiments (Hassink et al., 1993; Rutherford and Juma, 1992a). Thus, the values selected from the literature or estimated by fitting the model to experimental observations were quite realistic. Some of these parameters are very difficult to measure experimentally. For example, quantification of the flows of consumable substrate from various organic matter sources is extremely difficult. These flows could now be estimated and specific techniques could be devised to try to quantify them.

A shortcoming of 'BACWAVE-WEB' may be the large number of parameters included in the model. Yet, the model is already quite limited in some respects. For example the main group of hydrolytic microorganisms, fungi, were not included. However, hydrolytic bacteria could take over this function, especially in agricultural soils, since in various agricultural systems bacteria comprised 75-94% of total below-ground biomass (Bloem et al., 1994; Zwart et al., 1994). De Ruiter et al. (1994) also concluded that foodweb analysis could possibly be restricted to microbes and protozoans for agricultural soils. Another shortcoming may be that death of bacteria is simulated primarily by a shortage of substrate and secondarily by predators. In reality various other factors may induce bacterial death. For example, parasites (phages or Bdellovibrio) can have a strong influence on bacterial survival (Ashelford et al., 2003). Also fluctuations in temperature, moisture and oxygen

content could affect growth and death of bacteria. However, it is interesting that variations in environmental conditions are not a prerequisite for oscillations in bacterial populations (Zvyagintsev and Golimbet, 1983; Zelenev et al. 2000). Yet, for long-term predictions of mineralization inclusion of temperature and moisture fluctuations are essential. Under fluctuating environmental conditions it also becomes essential to include dormant stages of bacteria and their predators into the model (Blagodatsky and Richter, 1998; Ekelund et al., 2002; Kuikman et al., 1991). Finally, the model assumes homogeneity in soil and does not take into account spatial variability, which can lead to varying thresholds of predation and varying oxygen concentrations among different pockets in soil. Diffusion of CO_2 from and O_2 into soil was not taken into account. Local and temporal anoxia could be one of the factors contributing to cell death besides shortage of substrate and predation (van Bruggen et al, 2000; Zelenev, 2003a and b). Moreover, denitrification could be modelled more accurately if the gas-composition in soil pores would be included in the model.

Besides these shortcomings of the model 'BACWAVE-WEB', there are many advantages of this model. It allowed us to simulate oscillations in bacterial populations as observed after a disturbance of soil under experimental conditions. Although such oscillations are apparent from numerous papers, they were generally not modelled before (Blagodatsky et al., 1998; Kätterer and Andrén, 2001). Modelling of fluctuations after a disturbance will give us an opportunity to evaluate factors that influence the amplitudes of the oscillations, and thus the stability of a soil ecosystem (van Bruggen and Semenov, 2000). Further, the model gave us a better insight in the interrelated dynamics of three trophic groups of bacteria and two predator groups in soil, as well as the role of each of the groups in carbon decomposition and nitrogen release from fresh plant material added to soil. The practical significance of the research reported here is a better understanding of nutrient supply and availability from decomposing organic matter, the main source of nutrients in low-input and organic farming systems.

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Appendix A. Set of model equations. Each equation is represented as a sum of flows (column "Equations"). For each flow there are indicated source and sink compartments (column "Direction") and formula (column "Flows description").

Initial Value	Equations	Direction	Flows description	Equation number
COP0	dCOP / dt =			(1)
	+Fsc	from S to COP	+(MuCOP*S/(KsCOP*W+S)*COP/Ycop)	
	-Fcopstm	from COP to STROM	-((1-krC)*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	-Fcopslm	from COP to SOLOM	-((1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	-Fcprtz	from COP to PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((COP-TPC),0))	
	-Fcbfn	from COP to BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((COP-TNC),0))	
	-Fcs	from COP to S	-(KpC*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	-Fce	from COP to E	-(krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	-Fcco2	from COP to A_CO2	-(MuCOP*S/(KsCOP*W+S)*COP*(1-Ycop)/Ycop)	
OLIG0	dOLIG / dt =			(2)
	+Fso	from S to OLIG	+(MuOLI*S/(KsOLIG*W+S)*OLIG/Yoli)	
	-Folistm	from OLIG to STROM	-((1-krO)*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
	-Folislm	from OLIG to SOLOM	-((1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
	-Foprtz	from OLIG to PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((OLIG-TPO),0))	
	-Fobfn	from OLIG to BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((OLIG-TNO),0))	
	-Fos	from OLIG to S	-(KpO*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
	-Foe	from OLIG to E	-(krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
	-Foco2	from OLIG to A_CO2	-(MuOLI*S/(KsOLIG*W+S)*OLIG*(1-Yoli)/Yoli)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
HB0	dHB / dt =			(3)
	-FhE	from HB to E	-(KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB) if S>=Tas*W; -(MuSR*HB*Ksr/(Ksr+S/W)+KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB) if S<Tas*W	
	+Fsh	from S to HB	+(MuHB*HB*S/(KsHB*W+S)/Yhyd) if S>=Tas*W; +(MuHBf*HB*S/(KsHBf*W+S)/Yhydf) if S<Tas*W	
	-Fhstm	from HB to STROM	-((1-krH)*DmHB*KdHB/(KdHB+S/W)*HB)	
	-Fhslm	from HB to SOLOM	-((1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB)	
	-Fhprtz	from HB to PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((HB-TPH),0))	
	-Fhbfn	from HB to BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((HB-TNH),0))	
	-Fhs	from HB to S	-(KpH*krH*DmHB*KdHB/(KdHB+S/W)*HB)	
	-Fhco2	from HB to A_CO2	-(MuHB*HB*S/(KsHB*W+S)*(1-Yhyd)/Yhyd) if S>=Tas*W; -(MuHBf*HB*S/(KsHBf*W+S)*(1-Yhydf)/Yhydf) if S<Tas*W	
BFN0	dBFN / dt =			(4)
	+Fcbfn	from COP to BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((COP-TNC),0))	
	+Fobfn	from OLIG to BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((OLIG-TNO),0))	
	+Fhbfn	from HB to BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((HB-TNH),0))	
	-Fbfnstm	from BFN to STROM	-(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*(1-Kfaeslm)+BFN*Dbfn*(1-Kbfnslm))	
	-Fbfnslm	from BFN to SOLOM	-(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*Kfaeslm+BFN*Dbfn*Kbfnslm)	
	-Fbnco2	from BFN to A_CO2	-(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*(1-ExBN)*(1-Ybfn))	
PRTZ0	dPRTZ / dt =			(5)
	+Fcprtz	from COP to PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((COP-TPC),0))	
	+Foprtz	from OLIG to PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((OLIG-TPO),0))	
	+Fhprtz	from HB to PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((HB-TPH),0))	
	-Fprslm	from PRTZ to SOLOM	-(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*Kfaeslm+PRTZ*Dprtz*Kprslm)	
	-Fprstm	from PRTZ to STROM	-(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*(1-Kfaeslm)+PRTZ*Dprtz*(1-Kprslm))	
	-Fprco2	from PRTZ to A_CO2	-(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*(1-ExPr)*(1-Yprtz))	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
S0	dS / dt =			(6)
	-Fsc	from S to COP	-(MuCOP*S/(KsCOP*W+S)*COP/Ycop)	
	+FslmS	from SOLOM to S	+(RslmS*E/W/Em*SOLOM)	
	+FsosS	from SORES to S	+(KsosS*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1)*Rsa) *E/W/Em*Rsos*SORES)	
	-Fso	from S to OLIG	-(MuOLI*S/(KsOLIG*W+S)*OLIG/Yoli)	
	-Fsh	from S to HB	-(MuHB*HB*S/(KsHB*W+S)/Yhyd) if S>=Tas*W; -(MuHBf*S/(KsHBf*W+S)*HB/Yhydf) if S<Tas*W	
	+Fes	from E to S	+(RenzS*E/W/Em*E)	
	+Fcs	from COP to S	+(KpC*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	+Fos	from OLIG to S	+(KpO*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
	+Fhs	from HB to S	+(KpH*krH*DmHB*KdHB/(KdHB+S/W)*HB)	
SOLOM0	dSOLOM / dt =			(7)
	-FslmS	from SOLOM to S	-(RslmS*E/W/Em*SOLOM)	
	+Fhumslm	from HUMOM to SOLOM	+(Rh*E/W/Em*HUMOM)	
	+Fstmslm	from STROM to SOLOM	+(Rstm*E/W/Em*STROM)	
	+Fsosslm	from SORES to SOLOM	+((1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1)*Rsa) *E/W/Em*Rsos*SORES)	
	+Fstsslm	from STRES to SOLOM	+(Kstsslm*Rsts*E/W/Em*STRES)	
	+Flisslm	from LIRES to SOLOM	+(Klisslm*Rlis*E/W/Em*LIRES)	
	+Fcopslm	from COP to SOLOM	+((1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	+Folislm	from OLIG to SOLOM	+((1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W) +Lolig*S/W)*OLIG)	
	+Fhslm	from HB to SOLOM	+((1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB)	
	+Fprslm	from PRTZ to SOLOM	+(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*Kfaeslm +PRTZ*Dprtz*Kprslm)	
	+Fbfnslm	from BFN to SOLOM	+(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*Kfaeslm+BFN*Dbfn*Kbfnslm)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
E0	dE / dt =			(8)
	+FhE	from HB to E	+(KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB) if S>=Tas*W; +(MuSR*HB*Ksr/(Ksr+S/W)+KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB) if S<Tas*W	
	-Fes	from E to S	-(RenzS*E/W/Em*E)	
	+Fce	from COP to E	+(krC*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	+Foe	from OLIG to E	+(krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG)	
STROM0	dSTROM / dt =			(9)
	-Fstmslm	from STROM to SOLOM	-((1-KstmH)*Rstm*E/W/Em*STROM)	
	+Fstsstm	from STRES to STROM	+(Rsts*E/W/Em*STRES*(1-Kstsslm))	
	-Fstmhum	from STROM to HUMOM	-(KstmH*Rstm*E/W/Em*STROM)	
	+Fcopstm	from COP to STROM	+((1-krC)*DmCOP*Kdcop/(Kdcop+S/W)*COP)	
	+Folistm	from OLIG to STROM	+((1-krO)*(DmOLIG*KdOLIG /(KdOLIG+S/W)+Lolig*S/W) *OLIG)	
	+Fhstm	from HB to STROM	+((1-krH)*DmHB*KdHB/(KdHB+S/W)*HB)	
	+Fprstm	from PRTZ to STROM	+(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*(1-Kfaeslm) +PRTZ*Dprtz*(1-Kprslm))	
	+Fbfnstm	from BFN to STROM	+(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*(1-Kfaeslm)+BFN*Dbfn*(1-Kbfnslm))	
HUMOM0	dHUMOM / dt =			(10)
	-Fhumslm	from HUMOM to SOLOM	-(Rh*E/W/Em*HUMOM)	
	+Flishum	from LIRES to HUMOM	+((1-Klisslm)*Rlis*E/W/EM*LIRES)	
	+Fstmhum	from STROM to HUMOM	+(KstmH*Rstm*E/W/Em*STROM)	
SORES0	dSORES / dt =			(11)
	-FsosS	from SORES to S	-(KsosS*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1)*Rsa)*E/W/Em *Rsos*SORES)	
	-Fsosslm	from SORES to SOLOM	-((1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1)*Rsa)*E/W/Em *Rsos*SORES)	
STRES0	dSTRES / dt =			(12)
	-Fstsslm	from STRES to SOLOM	-(Kstsslm*Rsts*E/W/Em*STRES)	
	-Fstsstm	from STRES to STROM	-((1-Kstsslm)*Rsts*E/W/Em*STRES)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
LIRES0	dLIRES / dt =			(13)
	-Flishum	from LIRES to HUMOM	-((1-Klisslm)*Rlis*E/W/Em*LIRES)	
	-Flisslm	from LIRES to SOLOM	-(Klisslm*Rlis*E/W/EM*LIRES)	
A_CO2_0	dA_CO2 / dt =			(14)
	+Fprco2	from PRTZ to A_CO2	+(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*(1-ExPr)*(1-Yprtz))	
	+Fbnco2	from BFN to A-CO2	+(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*(1-ExBN)*(1-Ybfn))	
	+Fcco2	from COP to A_CO2	+(MuCOP*S/(KsCOP*W+S)*COP*(1-Ycop)/Ycop)	
	+Foco2	from OLIG to A_CO2	+(MuOLI*S/(KsOLIG*W+S)*OLIG*(1-Yoli)/Yoli)	
	+Fhco2	from HB to A_CO2	+(MuHB*HB*S/(KsHB*W+S)*(1-Yhyd)/Yhyd) if S>=Tas*W; +(MuHBf*S/(KsHBf*W+S)*HB*(1-Yhydf)/Yhydf) if S<Tas*W	
N_COP0	dN_COP / dt =			(15)
	-Ncopstm	from N_COP to N_STROM	-((1-krC)*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	+NScop	from N_S to N_COP	+(MuCOP*S/(KsCOP*W+S)*COP/Ycop/CN_S)	
	-Ncopslm	from N_COP to N_SOLOM	-((1-Kcnh4)*(1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	-Ncopnh4	from N_COP to NH4	-(Kcnh4*(1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	+Nno3cop	from NO3 to N_COP	+(COP*Uno3_COP*NO3/(Kno3_COP*W+NO3)) if CN_COP > CN_COPo; 0 if CN_COP <= CN_COPo	
	+Nnh4cop	from NH4 to N_COP	+(COP*Unh4_COP*NH4/(Knh4_COP*W+NH4)) if CN_COP > CN_COPo; 0 if CN_COP <= CN_COPo	
	-Ncbn	from N_COP to N_BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((COP-TNC),0)/CN_COP)	
	-Ncpr	from N_COP to N_PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((COP-TPC),0)/CN_COP)	
	-NcopS	from N_COP to N_S	-(KpC*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	-Nce	from N_COP to N_E	-(krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
N_OLIG0	dN_OLIG / dt =			(16)
	+NSoli	from N_S to N_OLIG	+(MuOLI*S/(KsOLIG*W+S)*OLIG/Yoli/CN_S)	
	-Nolinh4	from N_OLIG to NH4	-(Konh4*(1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
	-Nolislm	from N_OLIG to N_SOLOM	-((1-Konh4)*(1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W) *OLIG/CN_OLIG)	
	-Nolistm	from N_OLIG to N_STROM	-((1-krO)*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
	+Nno3oli	from NO3 to N_OLIG	+(OLIG*Uno3_OLIG*NO3/(Kno3_OLIG*W+NO3)) if CN_OLIG>CN_OLIGo; 0 if CN_OLIG<=CN_OLIGo	
	+Nnh4oli	from NH4 to N_OLIG	+(OLIG*Unh4_OLIG*NH4/(Knh4_OLIG*W+NH4)) if CN_OLIG>CN_OLIGo; 0 if CN_OLIG<=CN_OLIGo	
	-Nobn	from N_OLIG to N_BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((OLIG-TNO),0)/CN_OLIG)	
	-Nopr	from N_OLIG to N_PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((OLIG-TPO),0)/CN_OLIG)	
	-NoligS	from N_OLIG to N_S	-(KpO*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
N_HB0	dN_HB / dt =			(17)
	+Nnh4hyd	from NH4 to N_HB	+(HB*Unh4_HB*NH4/(Knh4_HB*W+NH4)) if CN_HB>CN_HBo; 0 if CN_HB<=CN_HBo	
	-Nhydnh4	from N_HB to NH4	-(Khnh4*(1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	+Nno3hyd	from NO3 to N_HB	+(HB*Uno3_HB*NO3/(Kno3_HB*W+NO3)) if CN_HB>CN_HBo; 0 if CN_HB<=CN_Hbo	
	-NhE	from N_HB to N_E	-(KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB) if S>=Tas*W; -(MuSR*HB*Ksr/(Ksr+S/W)/CN_Eo+krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB) if S<Tas*W)	
	-Nhstm	from N_HB to N_STROM	-((1-krH)*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	-Nactslm	from N_HB to N_SOLOM	-((1-Khnh4)*(1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	+NShyd	from N_S to N_HB	+(MuHB*HB*S/(KsHB*W+S)/Yhyd/CN_S) if S>=Tas*W; +(MuHBf*S/(KsHBf*W+S)*HB/Yhydf/CN_S) if S<Tas*W	
	-Nhbn	from N_HB to N_BFN	-(McN*BFN/(KbN+COP+OLIG+HB)*max((HB-TNH),0)/CN_HB)	
	-Nhpr	from N_HB to N_PRTZ	-(McP*PRTZ/(KbP+COP+OLIG+HB)*max((HB-TPH),0)/CN_HB)	
	-NhydS	from N_HB to N_S	-(KpH*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
N_BFN0	dN_BFN / dt =			(18)[a]
	+Nhbn	from N_HB to N_BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((HB-TNH),0)/CN_HB)	
	+Nobn	from N_OLIG to N_BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((OLIG-TNO),0)/CN_OLIG)	
	+Ncbn	from N_COP to N_BFN	+(McN*BFN/(KbN+COP+OLIG+HB)*max((COP-TNC),0)/CN_COP)	
	-Nbfnstm	from N_BFN to N_STROM	-(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*(1-Kfaeslm)+BFN*Dbfn*(1-Kbfnslm)/CN_BFN)	
	-Nbnslm	from N_BFN to N_SOLOM	-((1-Kbfnnh4)*(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*Kfaeslm+BFN*Dbfn*Kbfnslm)/CN_BFN)	
	-Nbnnh4	from N_BFN to NH4	(1-NCBFN)*McB*BFN/(KbB+COP+OLIG+HB)*(max(COP-TNC,0)+max(OLIG-TNO,0) +max(HB-TNH,0))*(1-ExBn)*(1/CN_HB-Ybfn/CN_BFNo) +Kbfnnh4*((McB*BFN/(KbB+COP+OLIG+HB)*(max(COP-TNC,0)+max(OLIG-TNO,0) +max(HB-TNH,0))*ExBN*Kfaesml+BFN*Dbfn*Kbfnslm)/CN_BFN)	
N_PRTZ0	dN_PRTZ / dt =			(19)[a]
	+Nhpr	from N_HB to N_PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((HB-TPH),0)/CN_HB)	
	+Nopr	from N_OLIG to N_PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((OLIG-TPO),0)/CN_OLIG)	
	+Ncpr	from N_COP to N_PRTZ	+(McP*PRTZ/(KbP+COP+OLIG+HB)*max((COP-TPC),0)/CN_COP)	
	-Nprstm	from N_PRTZ to N_STROM	-(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*(1-Kfaeslm)+PRTZ*Dprtz*(1-Kprslm)/CN_PRTZ)	
	-Nprslm	from N_PRTZ to N_SOLOM	-((1-Kprnh4)*(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*Kfaeslm+PRTZ*Dprtz*Kprslm)/CN_PRTZ)	
	-Nprnh4	from N_PRTZ to NH4	-(1-NCPRTZ)*McP*PRTZ/(KbB+COP+OLIG+HB)*(max(COP-TPC,0)+max(OLIG-TPO,0) +max(HB-TPH,0))*(1-ExPR)*(1/CN_HB-Yprtz/CN_PRTZo) +Kprnh4*((McP*PRTZ/(KbB+COP+OLIG+HB)*(max(COP-TPC,0)+max(OLIG-TPO,0) +max(HB-TPH,0))*ExPR*Kfaesml+PRTZ*Dprtz*Kprsml)/CN_PRTZ)	
N_E0	dN_E / dt =			(20)
	+NhE	from N_HB to N_E	+(KpeH*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB) if S>=Tas*W; +(MuSR*HB*Ksr/(Ksr+S/W)/CN_Eo+krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB) if S<Tas*W	
	-NES	from N_E to N_S	-(RenzS*E/W/Em*E/CN_E)	
	+Nce	from N_COP to N_E	+(krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	

[a] - in this equation NCBFN (or NCPRTZ) = 1 if current C/N ratio of BFN (or PRTZ) is greater than its standard C/N ratio, otherwise NCBFN (or NCPRTZ) = 0.

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
N_S0	dN_S / dt =			(21)
	-NScop	from N_S to N_COP	-(MuCOP*S/(KsCOP*W+S)*COP/Ycop/CN_S)	
	+NslmS	from N_SOLOM to N_S	+(RslmS*E/W/Em*SOLOM/CN_SOLOM)	
	-NSoli	from N_S to N_OLIG	-(MuOLI*S/(KsOLIG*W+S)*OLIG/Yoli/CN_S)	
	+NES	from N_E to N_S	+(RenzS*E/W/Em*E/CN_E)	
	-NShyd	from N_S to N_HB	-(MuHB*HB*S/(KsHB*W+S)/Yhyd/CN_S) if S>=Tas*W; -(MuHBf*S/(KsHBf*W+S)*HB/Yhydf/CN_S) if S<Tas*W	
	+NsosS	from N_SORES to N_S	+(KsosS*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1)*Rsa)*E/W/Em *Rsos*SORES/CN_SORES)	
	+NcopS	from N_COP to N_S	+(KpC*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	+NoligS	from N_OLIG to N_S	+(KpO*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
	+NhydS	from N_HB to N_S	+(KpH*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
N_SOLOM0	dN_SOLOM / dt =			(22)
	+Ncopslm	from N_COP to N_SOLOM	+((1-Kcnh4)*(1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	-NslmS	from N_SOLOM to N_S	-(RslmS*E/W/Em*SOLOM/CN_SOLOM)	
	+Nolislm	from N_OLIG to N_SOLOM	+((1-Konh4)*(1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W) *OLIG/CN_OLIG)	
	+Nstsslm	from N_STRES to N_SOLOM	+(Kstsslm*Rsts*E/W/Em*STRES/CN_STRES)	
	+Nstmslm	from N_STROM to N_SOLOM	+(Rstm*E/W/Em*STROM/CN_STROM)	
	+Nlisslm	from N_LIRES to N_SOLOM	+(Klisslm*Rlis*E/W/Em*LIRES/CN_LIRES)	
	+Nhumslm	from N_HUMOM to N_SOLOM	+(Rh*E/W/Em*HUMOM/CN_HUMOM)	
	+Nsosslm	from N_SORES to N_SOLOM	+((1-Ksosnh4)*(1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1) *Rsa)*E/W/Em*Rsos*SORES/CN_SORES)	
	+Nactslm	from N_HB to N_SOLOM	+((1-Khnh4)*(1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	+Nprslm	from N_PRTZ to N_SOLOM	((1-Kprnh4)*(McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*Kfaeslm+PRTZ*Dprtz*Kprslm)/CN_PRTZ)	
	+Nbnslm	from N_BFN to N_SOLOM	+((1-Kbfnnh4)*(McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*Kfaeslm+BFN*Dbfn*Kbfnslm)/CN_BFN)	

Appendix A (continued).

Initial Value	Equations	Direction	Flows description	Equation number
N_STROM0	dN_STROM / dt =			(23)
	+Ncopstm	from N_COP to N_STROM	+((1-krC)*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	-Nstmslm	from N_STROM to N_SOLOM	-((1-KstmH)*Rstm*E/W/Em*STROM/CN_STROM)	
	+Nolistm	from N_OLIG to N_STROM	+((1-krO)*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
	-Nstmhum	from N_STROM to N_HUMOM	-(KstmH*Rstm*E/W/Em*STROM/CN_STROM)	
	+Nstsstm	from N_STRES to N_STROM	+(Rsts*E/W/Em*STRES*(1-Kstsslm)/CN_STRES)	
	+Nhstm	from N_HB to N_STROM	+((1-krH)*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	+Nprstm	from N_PRTZ to N_STROM	+((McP*PRTZ*(max((COP-TPC),0)+max((OLIG-TPO),0)+max((HB-TPH),0)) /(KbP+COP+OLIG+HB)*ExPr*(1-Kfaeslm)+PRTZ*Dprtz*(1-Kprslm))/CN_PRTZ)	
	+Nbfnstm	from N_BFN to N_STROM	+((McN*BFN*(max((COP-TNC),0)+max((OLIG-TNO),0)+max((HB-TNH),0)) /(KbN+COP+OLIG+HB)*ExBN*(1-Kfaeslm)+BFN*Dbfn*(1-Kbfnslm))/CN_BFN)	
N_HUMOM0	dN_HUMOM / dt =			(24)
	+Nlishum	from N_LIRES to N_HUMOM	+((1-Klisslm)*Rlis*E/W/Em*LIRES/CN_LIRES)	
	-Nhumslm	from N_HUMOM to N_SOLOM	-(Rh*E/W/Em*HUMOM/CN_HUMOM)	
	+Nstmhum	from N_STROM to N_HUMOM	+(KstmH*Rstm*E/W/Em*STROM/CN_STROM)	
N_SORES0	dN_SORES / dt =			(25)
	-Nsosslm	from N_SORES to N_SOLOM	-((1-Ksosnh4)*(1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1) *Rsa)*E/W/Em*Rsos*SORES/CN_SORES)	
	-NsosS	from N_SORES to N_S	-(KsosS*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1) *Rsa)*E/W/Em*Rsos*SORES/CN_SORES)	
	-Nsosnh4	from N_SORES to NH4	-(Ksosnh4*(1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1) *Rsa)*E/W/Em*Rsos*SORES/CN_SORES)	
N_STRES0	dN_STRES / dt =			(26)
	-Nstsslm	from N_STRES to N_SOLOM	-(Kstsslm*Rsts*E/W/Em*STRES/CN_STRES)	
	-Nstsstm	from N_STRES to N_STROM	-(Rsts*E/W/Em*STRES*(1-Kstsslm)/CN_STRES)	

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
N_LIRES0	dN_LIRES / dt =			(27)
	-Nlishum	from N_LIRES to N_HUMOM	-((1-Klisslm)*Rlis*E/W/Em*LIRES/CN_LIRES)	
	-Nlisslm	from N_LIRES to N_SOLOM	-(Klisslm*Rlis*E/W/Em*LIRES/CN_LIRES)	
NH4_0	dNH4 / dt =			(28[a])
	+Ncopnh4	from N_COP to NH4	+(Kcnh4*(1-KpC)*krC*DmCOP*Kdcop/(Kdcop+S/W)*COP/CN_COP)	
	+Nolinh4	from N_OLIG to NH4	+(Konh4*(1-KpO)*krO*(DmOLIG*KdOLIG/(KdOLIG+S/W)+Lolig*S/W)*OLIG/CN_OLIG)	
	-Nnit	from NH4 to NO3	-(Vnit*0.001*OLIG*NH4/(Knit+NH4))	
	-Nnh4oli	from NH4 to N_OLIG	-(OLIG*Unh4_OLIG*NH4/(Knh4_OLIG*W+NH4)) if CN_OLIG>CN_OLIGo; 0 if CN_OLIG<=CN_OLIGo	
	-Nnh4cop	from NH4 to N_COP	-(COP*Unh4_COP*NH4/(Knh4_COP*W+NH4)) if CN_COP > CN_COPo; 0 if CN_COP <= CN_COPo	
	-Nvol	from NH4 to A_N	-(Rnh3v*(NH4/(PS-W+exp(-1.694+1477.7/Tk)*W*(1+exp(23.1824-0.076*(Tk-273.15)-2.3026*pH)))-Anh3))	
	-Nnh4hyd	from NH4 to N_HB	-(HB*Unh4_HB*NH4/(Knh4_HB*W+NH4)) if CN_HB>CN_HBo; 0 if CN_HB<=CN_HBo	
	+Nhydnh4	from N_HB to NH4	+(Khnh4*(1-KpeH-KpH)*krH*DmHB*KdHB/(KdHB+S/W)*HB/CN_HB)	
	+Nbnnh4	from N_BFN to NH4	(1-NCBFN)*(McB*BFN/(KbB+COP+OLIG+HB)*(max(COP-TNC,0)+max(OLIG-TNO,0) +max(HB-TNH,0))*(1/CN_HB-(1-ExBn)*Ybfn/CN_BFNo) +Kbfnnh4*((McB*BFN/(KbB+COP+OLIG+HB)*(max(COP-TNC,0)+max(OLIG-TNO,0) +max(HB-TNH,0))*ExBn*Kfaesml+BFN*Dbfn*Kbnsml)/CN_BFN)	
	+Nprnh4	from N_PRTZ to NH4	(1-NCPRTZ)*(McP*PRTZ/(KbB+COP+OLIG+HB)*(max(COP-TPC,0)+max(OLIG-TPO,0) +max(HB-TPH,0))*(1-ExPR)*(1/CN_HB-Yprtz/CN_PRTZo) +Kprnh4*((McP*PRTZ/(KbB+COP+OLIG+HB)*(max(COP-TPC,0)+max(OLIG-TPO,0) +max(HB-TPH,0))*ExPR*Kfaesml+PRTZ*Dprtz*Kprsml)/CN_PRTZ)	
	+Nsosnh4	from N_SORES to NH4	+(Ksosnh4*(1-KsosS)*(1+min((EXP(Kb*(1-STRES/STRES0))-1)/(EXP(Kb*(1-Psts))-1),1) *Rsa)*E/W/Em*Rsos*SORES/CN_SORES)	

[a] - NCBFN and NCPRTZ have the same meaning as explained in footnote for equations (18) and (19)

Appendix A (continued).

Initial value	Equations	Direction	Flows description	Equation number
NO3_0	dNO3 / dt =			(29)
	-Nno3oli	from NO3 to N_OLIG	-(OLIG*Uno3_OLIG*NO3/(Kno3_OLIG*W+NO3)) if CN_OLIG>CN_OLIGo; 0 if CN_OLIG<=CN_OLIGo	
	-Nno3cop	from NO3 to N_COP	-(COP*Uno3_COP*NO3/(Kno3_COP*W+NO3)) if CN_COP > CN_COPo; 0 if CN_COP <= CN_COPo	
	+Nnit	from NH4 to NO3	+(Vnit*0.001*OLIG*NH4/(Knit+NH4))	
	-Ndnit	from NO3 to A_N	-(Vdnit*(COP+HB)*NO3/(Kdnit*W+NO3)*S/(Ksdnit*W+S))	
	-Nno3hyd	from NO3 to N_HB	-(HB*Uno3_HB*NO3/(Kno3_HB*W+NO3)) if CN_HB>CN_HBo; 0 if CN_HB<=CN_HBo	
A_N0	dA_N / dt =			(30)
	+Ndnit	from NO3 to A_N	+(Vdnit*(COP+HB)*NO3/(Kdnit*W+NO3)*S/(Ksdnit*W+S))	
	+Nvol	from NH4 to A_N	+(Rnh3v*(1+exp(23.1824-0.076*(Tk-273.15)-2.3026*pH))-Anh3))	

Appendix B. List of 'BACWAVE-WEB' model parameters and their values (same for both experiments).

Parameter	Symbol	Value	Unit	Min	Max	Reference
Air NH3 concentration	Anh3	$1.8 \cdot 10^{-5}$	[µg N/cm^3 air]	$1.7 \cdot 10^{-6}$	$1.8 \cdot 10^{-5}$	Beauchamp et al.(1978)
Standard C/N ratio for Bacteria feeding nematodes {BFN}	CN_BFNo	6	[-]*	5.16	6.83	Ferris et al. (1997)
Standard C/N ratio for COPiotrophic bacteria {COP}	CN_COPo	4.5	[-]	3.65	4.92	Ferris et al. (1997)
C/N ratio for Enzymes {E}	CN_Eo	3	[-]			Generalized formula of a protein
Standard C/N ratio for Hydrolytic Bacteria {HB}	CN_HBo	4.5	[-]	3.65	4.92	Ferris et al. (1997)
Standard C/N ratio for OLIGotrophic bacteria {OLIG}	CN_OLIGo	4.5	[-]	3.65	4.92	Ferris et al. (1997)
Standard C/N ratio for Protozoa {PRTZ}	CN_PRTZo	5	[-]	5	10	De Ruiter et al. (1993); Clarholm (1985)
Relative death rate of BFN	Dbfn	0.01	[h^{-1}]	0.0004	0.011	Hunt et al. (1987); Moorhead et al. (1987)
Death constant for COPiotrophic bacteria {COP}	DmCOP	0.82	[h^{-1}]	0.26		Zelenev et al. (2000)
Death constant for Hydrolytic Bacteria {HB}	DmHB	0.39	[h^{-1}]	0.26		Zelenev et al. (2000)
Death constant for OLIGotrophic bacteria {OLIG}	DmOLIG	0.01	[h^{-1}]	0.005		Zelenev et al. (2003c)
Relative death rate of Protozoa	Dprtz	0.025	[h^{-1}]	0.0007		Hunt et al. (1987)
Standard enzymes concentration in soil solution	Em	1	[µg C / ml]	0.5	86	Semenov et al. (1996); Naseby et al. (1999)
Fraction of excrements from consumption for BFN	ExBN	0.4	[-]		0.4	Hassink et al. (1994)
Fraction of excrements from consumption for Protozoa	ExPr	0.3	[-]	0.1	0.4	Brussaard et al. (1990); Rutherford & Juma (1992b)
Constant regulating an acceleration of {SORES} decomposition at certain (Psts) proportion of {STRES} remained	Kb	100	[-]			Optimized
Portion of Nitrogen releasing as {NH4} from decomposition of soluble fractions of excrements and dead BFN	Kbfnnh4	0.2	[-]			Optimized
Soluble fraction of dead {BFN}	Kbfnslm	0.85	[-]		0.85	Hunt et al. (1987)
Half saturation constant of bacteria consumption by {BFN}	KbN	90	[µg C / cm^3]	100	180	Hunt et al. (1987); Schiemer et al. (1980)
Half saturation constant of bacteria consumption by Protozoa {PRTZ}	KbP	30	[µg C / cm^3]	50	175	Hunt, 1984; Rutherford & Juma (1992b)

* - unitless

Appendix B (continued).

Parameter	Symbol	Value	Unit	Min	Max	Reference
Portion of dead COP Nitrogen {N_COP} going to {N_SOLOM} released as {NH4}	Kcnh4	0.2	[-]			Optimized
Substrate constant for death of Copiotrophic Bacteria {COP}	KdCOP	14.5	[µg C / ml]		14.5	Zelenev et al. (2000)
Substrate constant for death of Hydrolytic Bacteria {HB}	KdHB	14.5	[µg C / ml]		14.5	Zelenev et al. (2000)
Half Saturation constant of Nitrates {NO3} for Denitrification	Kdnit	5	[µg N / ml]			Optimized
Substrate constant for death of Oligotrophic Bacteria {OLIG}	KdOLIG	39.4	[µg C / ml]	10		Zelenev et al. (2000c)
Fraction of faeces going to SOLuble Organic Matter {SOLOM}	Kfaeslm	0.2	[-]			Hunt et al. (1987)
Portion of dead {HB} Nitrogen {N_HB} going to {N_SOLOM} released as {NH4}	Khnh4	0.2	[-]			Optimized
Fraction of decomposing LIgnin fraction of plant RESidues {LIRES} going to {SOLOM}	Klisslm	0.3	[-]			Optimized
Half Saturation constant of {NH4} for uptake by {COP}	Knh4_COP	5	[µg N / ml]		100	Rutherford & Juma (1992b)
Half Saturation constant of {NH4} for uptake by {HB}	Knh4_HB	1	[µg N / ml]			Optimized
Half Saturation constant of {NH4} for uptake by {OLIG}	Knh4_OLIG	1	[µg N / ml]			Optimized
Half Saturation constant of {NH4} for nitrification	Knit	15	[µg N / cm^3]	0.32	56	Jørgensen et al. (1991)
Half Saturation constant of {NO3} uptake by {COP}	Kno3_COP	50	[µg N / ml]		50	Smith (1979)
Half Saturation constant of {NO3} uptake by {HB}	Kno3_HB	25	[µg N / ml]			Optimized
Half Saturation constant of {NO3} uptake by {OLIG}	Kno3_OLIG	1	[µg N / ml]			Optimized
Portion of dead {OLIG} Nitrogen {N_OLIG} going to {N_SOLOM} released as {NH4}	Konh4	0.2	[-]			Optimized
Portion of soluble fraction of dead {COP} going to Substrate {S}	KpC	0.5	[-]			Optimized
Portion of soluble fraction for dead Hydrolytic Bacteria {HB} going to Enzymes {E}	KpeH	0.15	[-]			Optimized
Portion of soluble fraction of dead Hydrolytic Bacteria {HB} going to Substrate {S}	KpH	0.5	[-]			Optimized

Appendix B (continued).

Parameter	Symbol	Value	Unit	Min	Max	Reference
Portion of soluble fraction of dead OLIGotrophic Bacteria {OLIG} going to Substrate {S}	KpO	0.2	[-]			Optimized
Portion of Nitrogen releasing as {NH4} from decomposition of soluble fractions of excrements and dead protozoa	Kprnh4	0.2	[-]			Optimized
Fraction of dead Protozoa going to {SOLOM}	Kprslm	0.9	[-]		0.9	Hunt et al. (1987)
Soluble fraction of dead Copiotrophic Bacteria {COP}	KrC	0.4	[-]			Educated Guess
Soluble fraction of dead Hydrolytic Bacteria {HB}	KrH	0.2	[-]			Educated Guess
Soluble fraction of dead Oligotrophic Bacteria {OLIG}	KrO	0.1	[-]			Educated Guess
Half Saturation constant of Substrate {S} for uptake by Copiotrophic Bacteria {COP}	KsCOP	3	[µg C / ml]	1	10	Newman (1978), Toal et al. (2000)
Half Saturation constant of Substrate {S} for Denitrification	Ksdnit	3	[µg C / ml]			Educated Guess
Half Saturation constant of Substrate {S} for uptake by {HB}	KsHB	2	[µg C / ml]	1	10	Newman (1978), Toal et al. (2000)
Half Saturation constant of Substrate {S} for uptake by {HB} at Substrate content below threshold	KsHBf	0.1	[µg C / ml]	1	10	Newman (1978), Toal et al. (2000)
Half Saturation constant of Substrate {S} for uptake by Oligotrophic Bacteria {OLIG}	KsOLIG	0.01	[µg C / ml]		0.01	Zelenev et al. (2000c)
Portion of decomposed Nitrogen of {N_SORES} released as Ammonium {NH4}	Ksosnh4	0.6	[-]			Optimized
Fraction of {SORES} going straight to {S}	KsosS	0.05	[-]			Optimized
Half Saturation constant for substrate repression of enzyme production. Relates to substrate content {S}	Ksr	0.3	[µg C / ml]			Optimized
Fraction of decomposing {STROM} going to {HUMOM} (humification)	KstmH	0.01	[-]			Optimized
Fraction of decomposing {STRES} going to {SOLOM}	Kstsslm	0.7	[-]			Optimized
Constant for OLIGotrophs relative death rate increase with substrate	Lolig	$8.31 \cdot 10^{-6}$	[ml/h·µg C]		$8.31 \cdot 10^{-6}$	Zelenev et al. (2000c)
Maximum relative consumption rate of bacteria by {BFN}	McN	0.24	[h^{-1}]		0.42	Brussaard et al. (1990)
Maximum relative consumption rate of bacteria by {PRTZ}	McP	0.16	[h^{-1}]	0.15		Rutherford & Juma (1992b)
Maximal relative growth rate for Copiotrophic Bacteria {COP}	MuCOP	0.07	[h^{-1}]	0.012	0.5	Jørgensen et al. (1991); Toal et al. 2000.

Appendix B (continued).

Parameter	Symbol	Value	Unit	Min	Max	Reference
Maximal relative growth rate for Hydrolytic Bacteria {HB}	MuHB	0.04	[h^{-1}]	0.012	0.5	Jørgensen et al. (1991); Toal et al. 2000.
Maximal relative growth rate for Hydrolytic Bacteria {HB}at Substrate content {S} below threshold	MuHBf	0.04	[h^{-1}]	0.012	0.5	Jørgensen et al. (1991); Toal et al. 2000.
Maximal relative growth rate for {OLIG}	MuOLI	0.01	[h^{-1}]	0.0004	0.14	Semenov (1991)
Maximal relative rate of Enzymes {E} excretion by Hydrolytic Bacteria {HB}	MuSR	0.2	[h^{-1}]			Optimized
pH of soil	pH	6.8	[-]			Experimental
Soil porosity	PS	0.59	[cm^3 / cm^3]			Experimental
Fraction of {STRES} remained when acceleration of {SORES} decomposition occurs	Psts	0.95	[-]			Optimized
Relative rate of enzymes {E} degrading to {S}	RenzS	0.04	[h^{-1}]	0.005	0.019	Albrecht & Rasmussen (1995); Paul & Clark (1989)
Relative decomposition rate of {HUMOM}	Rh	$2.4 \cdot 10^{-7}$	[h^{-1}]	$3.3 \cdot 10^{-8}$	$2.3 \cdot 10^{-6}$	Voroney et al. (1981); Berkenkamp et al. (2002)
Relative decomposition rate of {LIRES}	Rlis	0.00001	[h^{-1}]	$5.3 \cdot 10^{-7}$	$4.2 \cdot 10^{-4}$	Paul, Clark (1989); Voroney et al. (1981)
Inverse of ammonia (NH3) Residence time in soil	Rnh3v	$7.0 \cdot 10^{-2}$	[h^{-1}]			Optimized
Soil bulk density	RoE	1.09	[g / cm^3]			Experimental
Constant for acceleration of {SORES} relative decomposition rate after a proportion Psts of {STRES} remained	Rsa	20	[-]			Optimized
Relative rate for transformation of {SOLOM} into {S}	RslmS	0.006	[h^{-1}]	0.0008	0.008	Molina & Smith (1998)
Relative decomposition rate of {SORES}	Rsos	0.002	[h^{-1}]	0.002	0.008	Molina & Smith (1998)
Relative decomposition rate of {STROM}	Rstm	0.0006	[h^{-1}]	0.00002	0.0008	Molina & Smith (1998)
Relative decomposition rate of {STRES}	Rsts	0.0005	[h^{-1}]	0.0002	0.0006	Molina & Smith (1998)
Threshold for Hydrolytic Bacteria {HB} to increase affinity to substrate and to start enzyme excretion	Tas	40	[µg C / ml]	20		Ljungdahl & Eriksson (1985)
Temperature of amended soil incubation (25°C)	Tk	298.15	[K]			Experimental
Minimal biomass of {COP} which is available for {BFN} consumption (Threshold)	TNC	5	[µg C / cm^3]	5	60	Hunt (1984), Vranken (1986)

Appendix B (continued).

Parameter	Symbol	Value	Unit	Min	Max	Reference
Minimal biomass of {HB} which is available for {BFN} consumption (Threshold)	TNH	5	[µg C / cm^3]	5	60	Hunt (1984), Vranken (1986)
Minimal biomass of {OLIG}, which is available for {BFN} consumption (Threshold)	TNO	20	[µg C / cm^3]	5	60	Hunt (1984), Vranken (1986)
Maximal relative rate of ammonium {NH4} uptake by COPiotrophic Bacteria {COP}	Unh4_COP	0.1	[µg N/h µg C]	0.15	0.28	Rutherford & Juma (1992b)
Maximal relative rate of ammonium {NH4} uptake by Hydrolytic Bacteria {HB}	Unh4_HB	0.1	[µg N/h µg C]	0.15	0.28	Rutherford & Juma (1992b)
Maximal relative rate of ammonium {NH4} uptake by OLIGotrophic Bacteria {OLIG}	Unh4_OLIG	0.05	[µg N/h µg C]			Educated Guess
Maximal relative rate of nitrates {NO3} uptake by Copiotrophic Bacteria {COP}	Uno3_COP	0.03	[µg N/h µg C]	0.28	0.35	Rutherford & Juma (1992b)
Maximal relative rate of nitrates {NO3} uptake by Hydrolytic Bacteria {HB}	Uno3_HB	0.01	[µg N/h µg C]	0.28	0.35	Rutherford & Juma (1992b)
Maximal relative rate of nitrates {NO3} uptake by Oligotrophic Bacteria {OLIG}	Uno3_OLIG	0.005	[µg N/h µg C]			Educated Guess
Maximal relative rate of Denitrification per unit of bacterial biomass	Vdnit	0.0003	[µg N/h µg C]			Optimized
Mean volume of large cells	Vl	0.35	[µm3]	0.001	15.8	Bölter et al. (2002)
Maximal relative rate of Nitrification per unit of bacterial biomass	Vnit	45	[µg N/h µg C]	4	1170	Berg (1986); Verhagen (1992)
Mean volume of small cells	Vs	0.132	[µm3]	0.001	15.8	Bölter et al. (2002)
A coefficient for recalculation of total number of [cells/g ds] to biomass [ugC/ cm^3 soil]	Wnb	$1.62 \cdot 10^{-7}$	[µg C g ds /$µm^3$ cm^3]			See Results section
Production efficiency for bacterial feeding nematodes {BFN}	Ybfn	0.37	[µg C / µg C]	0.37		Hassink et al. (1994); Brussaard et al. (1990)
Yield coefficient for Copiotrophic Bacteria {COP}	Ycop	0.45	[µg C / µg C]	0.4	0.6	Darrah (1991)
Yield coefficient for Hydrolytic Bacteria {HB}	Yhyd	0.5	[µg C / µg C]	0.4	0.6	Darrah (1991)
Yield coefficient for Hydrolytic Bacteria {HB} at Substrate content below threshold	Yhydf	0.09	[µg C / µg C]	0.1	0.8	Toal et al.(2000)
Yield coefficient for Oligotrophic Bacteria {OLIG}	Yoli	0.6	[µg C / µg C]	0.4	0.6	Darrah (1991)
Production efficiency for Protozoa {PRTZ}	Yprtz	0.57	[µg C / µg C]		0.6	Rutherford & Juma (1992)

Chapter 8

General Discussion

The phenomenon of fluctuations in soil microbial populations was observed many times in detail both in laboratory experiments and in the field with native bacterial communities (Aristovskaya, 1980; Zvyagintsev and Golimbet, 1983). Under natural conditions, microbial fluctuations in soil generally did not closely correlate with variations in external environmental characteristics, such as soil temperature and moisture. There was some regularity in the observed fluctuations, but regular oscillations were not demonstrated using appropriate statistical techniques such as time series analysis.

Irregular fluctuations can turn into regular oscillations with distinct waves after a disturbance such as addition of fresh organic matter to soil (Clarholm, 1981; Doebeli and Ruxton, 1997 and 1998). Soil is generally low in easily available nutrients, especially fallow arable soil. Any disturbance providing a nutrient impulse under these conditions, such as incorporation of fresh organic matter, rewetting after drying, or mechanical disturbance, likely initiates a wave-like response of the microbial community. Hints of wave-like fluctuations were obtained in a field experiment after incorporation of cover crop debris into soil (van Bruggen and Semenov, 2000), but the observations were too sparse for time series analysis.

Growth of plant roots can be considered as the most basic source of nutrient influx and disturbance for the soil microbial community that leads to wave-like development of this community in space and in time (Kozhevin, 1989; Semenov et al., 1999). In the greenhouse experiments reported in this thesis, wave-like distributions of microbial populations along roots of wheat were demonstrated for the first time using harmonics analysis (Chapter 2). There were shifting oscillations over time, justifiably called ‘moving waves’ (Semenov et al., 1999). The moving wave hypothesis was confirmed in experiments with a moving artificial nutrient source in soil (van Bruggen et al., 2000). Although not demonstrated, it is likely that wave-like distribution patterns of the microbial community are generated along any roots, including lateral roots, initiated from the growing root tips. Exudation from root tips leads to creation of waves both in vertical direction (macro-waves along the root) and in horizontal direction (micro-waves perpendicular to the root).

Temporal oscillations were observed in microcosm experiments with soil amended with fresh plant material incubated at constant temperature and moisture (Chapter 5). In all these experiments, the soil bacterial community experienced considerable disturbance in the form of substrate introduction. Bacterial populations fluctuated with different periods and amplitudes, depending on specific conditions of each experiment, but immediately after the disturbance they revealed remarkable oscillations with increased frequencies and amplitudes. Yet, the patterns of the oscillations were quite predictable, for example always with a small and large peak within one week after incorporation of a clover+grass mixture in soil. In a simulation model (Chapter 7), this pattern could only be reproduced when a two-step release of readily utilizable substrate from plant material was assumed (immediately after incorporation in soil, and when the cell walls started to decompose).

No oscillations were detected in other measured variables in the microcosm experiments, such as mineral nitrogen, pH, or redox potential (ROP). The only peak in ammonium concentration occurred when bacterial numbers formed the second large peak, confirming the essential role of bacteria in organic nitrogen mineralization (Ladd and Jackson, 1982). In other words, after incorporation of fresh plant material bacteria influenced the dynamics of mineral nitrogen, but not the other way around. Changes in soil pH and ROP were clearly associated with changes in NH_4^+ concentrations. Thus, the most important and often checked factors as pH or redox potential did not seem to play an important

role in the cycle of growth and death of soil bacteria. Nevertheless, at microscales temporary oxygen shortages could have contributed to the death phase after the first peak in bacteria (Chapter 5). Temporary oxygen shortages could lead to denitrification during the first days after plant residue incorporation. Short-term peaks in N_2O emissions were observed after freezing-thawing, drying-rewetting and simulated tillage (Priemé and Christensen, 2001; Caldéron et al., 2001). In the microcosm experiment with clover-grass, NO_3^- accumulation did not occur until 4-5 days after residue incorporation (Chapter 5). Nevertheless, 60 to 80% of easily mineralisable organic nitrogen was released within 7-10 days after incorporation of the residues.

Plant residue decomposition and nitrogen release have been included in many simulation models, but these models did not describe wave-like oscillations of bacteria and their predators in soil (Nicolardot et al., 2001). Similarly, oscillations of bacterial populations along roots were not simulated in rhizosphere models (Scott et al., 1995). In this thesis, two models were developed for simulation of bacterial oscillations in space and time. First, a simple model ("BACWAVE") was developed for growth and death of copiotrophic and 'broad range' bacteria interacting with substrate primarily coming from a moving root tip (Zelenev et al., 2000). Only a small part of total soluble organic matter was needed for the observed bacterial growth; the rest could apparently not be used and may have consisted of various polymers and humic acids (Naseby et al., 1999). The model accurately described oscillating distributions of bacterial populations isolated on C rich and C poor media along roots (Chapters 3 and 4). True oligotrophic bacteria that could not grow on C rich media constituted only a small part of colonies on C poor media (Maloney et al., 1997). These did not oscillate in the model, but all bacteria growing on C poor media ('broad range' bacteria) did oscillate, as observed in experiments. The model accurately reproduced the observed differences in behaviour of each trophic group.

The model "BACWAVE" was extended to a substrate-based simplified food web model ("BACWAVE-WEB") to describe temporal dynamics of 3 trophic groups of bacteria (copiotrophic, oligotrophic and hydrolytic), and two predatory groups (bacteria-feeding nematodes [BFN] and protozoa) after incorporation of fresh plant material in soil (Chapter 7). Short-term dynamics of both carbon and nitrogen flows were modelled accurately.

The most important innovations in the models "BACWAVE" and "BACWAVE-WEB" were introduction of a state variable for readily utilizable substrate and dependence of bacterial relative death rates on substrate concentration. This allowed to model fluctuations not only in copiotrophic and oligotrophic bacterial biomass, but also in predatory biomass. The initial biomass oscillations appeared to be due to growth and death of copiotrophic (and hydrolytic) bacteria which respond rapidly to available substrate. Oligotrophic bacteria are better able to grow at low substrate levels and are responsible for a later peak in total bacterial numbers. In the rhizosphere, oligotrophic bacteria dominate at the root base instead of the root tip (Maloney et al., 1997). Results from "BACWAVE-WEB" indicate that oligotrophic bacteria play a dual role in soil: 1. bringing substrate levels below the threshold for catabolite repression (Semenov, 1991) allowing hydrolytic micro-organisms to produce hydrolytic enzymes, 2. being a stabilizing factor by accumulation of C in their biomass at low concentrations of readily utilizable substrate in soil, thereby limiting copiotrophic bacteria in their growth. Indeed, elimination of oligotrophs from the simulation model resulted in wild fluctuations of copiotrophic bacteria.

Although many authors have looked for external reasons to explain observed fluctuations in microbial populations, there is little support for any hypothesis relating microbial population densities directly to heterogeneity in the environment. Indeed, there were no correlations between numbers of lateral roots or soil moisture and population densities of copiotrophic and oligotrophc bacteria in the rhizosphere of wheat (Chapter 2). Similarly, there were no correlations between soil pH, moisture, temperature or ROP and oscillations in bacterial populations after clover+grass incorporation (Chapter 5). A disturbance like a rain event may initiate a surge in growth, but may not be directly responsible for the ensuing fluctuations (Clarholm, 1981). Oscillations in microbial populations are likely due to growth and death phases in response to a release of easily available nutrients after a rain event or any other disturbance such as a passing root tip or incorporated organic substances (Semenov et al., 1999).

Death of microbes is probably primarily a consequence of depletion of substrate, as assumed in the models "BACWAVE" and "BACWAVE-WEB". One obvious justification for this assumption is that predators generally evolved much later than their prey (bacteria), and it is unlikely that there would not be any density-dependent regulation before predators evolved. It is also unlikely that substrate – consumer regulation would be completely replaced by predator-prey regulation in later evolutionary periods. In this thesis research, there was only a weak correlation between dynamics of numbers of BFN and bacteria, although changes in growth rates of BFN were related to bacterial numbers (Chapter 6). Estimation of the number of bacterial cells that could be consumed by experimentally determined numbers of BFN and protozoa, showed that these predators could not be responsible for the decrease in bacterial numbers after the first peak and only partially responsible for the decline after the second peak in bacteria. Similarly, it would be unlikely that parasites could multiply so fast that they could be responsible for the first oscillations in bacterial populations (Ashelford et al., 2000). On the other hand, it is likely that predators were involved in bacterial regulation at stages of substrate decomposition. Based on virtual experiments with "BACWAVE-WEB", protozoa played a much more important role in bacterial regulation than BFN. This is understandable given their much faster growth rates than those of BFN (Darbyshire, 1994).

Regrowth of bacteria after the death phase takes place when substrate levels have increased, possibly due to autolysis of dead bacterial cells. Since there are continuous losses of CO_2 due to growth and maintenance respiration, the fluctuations would dampen very quickly if there were no replenishment of carbon sources, for example by substances released from decomposing organic matter or sloughed off cortex cells. Soil enzymes play an important role in the supply of readily utilizable substrate after the death phase in the bacterial oscillations (Chapter 7). Soil enzymes are also important for speedy ammonification of soluble substrate, especially immediately after incorporation of plant residues.

A major contribution of this thesis is that it can provide a better understanding of the concept of soil health. A healthy soil is defined as a stable system with resilience to stress, high biological diversity, and high levels of internal nutrient cycling (van Bruggen and Semenov, 1999). The state of health could possibly be tested by characterizing microbial fluctuations after a disturbance such as addition of organic material to soil (van Bruggen and Semenov, 2000). The strongest wave-like response of microbial communities occurs in soils low in organic matter (Chapter 2). In high-organic matter soils, wave-like responses are also observed but the amplitudes and periods of these waves may be less noticeable. The amplitude of the waves in microbial populations and the period to return to initial conditions before organic amendment may be used as indicators for soil health. The amplitudes are likely lower and resilience greater in a soil with a high microbial biomass and diversity than in a

microbiologically poor soil (van Bruggen and Semenov, 2000). This notion is supported by the reduced resilience of gamma-irradiated soil compared to the corresponding natural field soil (Chapter 6). Moreover, removal of typical K strategists (oligotrophic bacteria) from the model "BACWAVE-WEB" resulted in much larger fluctuations in copiotrophic bacteria compared to results from the complete model. Yet, the hypothesis about soil health still needs to be tested in much more detail. A comparison of the response of microbial populations to added fresh organic material in conventionally and organically managed soils would contribute to elucidating this matter. The period required for dampening of microbial oscillations after a disturbance could then be used as an indicator of soil health (van Bruggen, Semenov, 2000).

The practical significance of the research reported here is an improved predictability of nutrient supply and availability from decomposing organic matter, the main source of nutrients in low-input and organic farming systems. Moreover, the model "BACWAVE-WEB" would be useful for qualitative investigation and quantitative evaluation of nitrogen flows and retention in different agricultural soils.

Although the experimental work and models developed in this thesis contribute significantly to our understanding of the observed phenomenon of wave-like fluctuations in microbial populations after soil disturbance, there are still some questions to be answered. It is not yet completely clear what the reasons are for declines in microbial populations after the first peaks. Could a local lack of oxygen result in anaerobic fermentation and the production of toxic organic acids and subsequent death of bacteria? Or is the death phase simply a matter of substrate depletion? Detailed measurements of oxygen concentrations coupled to measurements of oxygen stress in bacteria could partially solve this question. Also, better estimates of readily utilizable substrate would be needed instead of soluble total organic carbon.

Additional research into the role of protozoa in the successive declines in bacterial populations would also be warranted. In this thesis protozoa were enumerated about once a week; this was not frequently enough to estimate fluctuations in their numbers. In the literature no daily observations on protozoa populations were found either (Darbyshire, 1994). The model "BACWAVE-WEB" simulated oscillations in protozoa numbers. Removal of protozoa from the model significantly changed microbial dynamics, but only after the first two peaks in bacterial populations, indicating that oscillations were initiated by bacteria – substrate interactions, and that oscillations in predators followed those in their prey.

Another question that needs to be answered is whether consecutive peaks in bacterial populations consist of similar microbial communities or different communities in succession. This question has been addressed by analysing bacterial communities along roots and over time after incorporation of plant debris in soil using denaturing gradient gel electrophoresis on bacterial 16S DNA (de Vos and van Bruggen, 2001; van Diepeningen, personal communication). The communities appeared similar from peak to peak, but there was a succession within each peak.

Several improvements could be made to the model "BACWAVE-WEB". In the current version there are 3 trophic groups of bacteria. Nitrifying bacteria were considered a subgroup of oligotrophic bacteria because they have similar growth rates. However, nitrifying bacteria are chemo-litho-autotrophic bacteria and deserve to be included as a separate state variable. Similarly, denitrifying bacteria are considered part of the copiotrophic bacteria in the current model; this is acceptable, but

denitrification takes place only under anaerobic conditions. To model this properly, an oxygen compartment would be needed, including oxygen diffusion and consumption during respiration. Finally, dormancy in bacteria is not included in this model. Yet, especially oligotrophic bacteria can accumulate storage substances such as polyhydroxybutiric acid, so that they can survive harsh environmental conditions for months (Semenov et al., 1989).

Despite these potential improvements to the models, the following new insights were obtained in this thesis:

1. Heterotrophic bacterial populations develop in a wave-like fashion in time and space after an impulse of readily utilizable substrate followed by growth and death cycles of bacterial communities;
2. Spatial wave-like distributions can be derived from temporal oscillations initiated by a moving nutrient source (root tip);
3. The concept of growth and death cycles was formalized in two simulation models, one for the rhizosphere, and one for bulk soil. Wave-like development of soil bacterial communities could only be simulated when a pulse of substrate was provided; the presence of predators did not guarantee wave-like oscillations in bacterial populations;
4. Waxing and waning of population growth rates of predators (BFN) in soil can be associated with oscillations in bacterial populations, even if no oscillations are observed in predator populations themselves;
5. Although predators, in particular protozoa, seem to play a role in regulation of bacterial populations, this role may only be secondary compared to the interaction of primary consumers with their substrate;
6. Bacteria play a more important role in release of ammonium (and secondarily nitrate) from decomposing plant material than predators;
7. As oscillatory development of bacterial populations may be a universal phenomenon after a disturbance, this phenomenon could possibly be used to compare soils in terms of stability and resilience, and consequently soil health.

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Summary

Fluctuations of bacterial populations can be observed when frequent and sufficiently long series of samples are obtained for direct microscopic or plate counts of bacteria. Such fluctuations in time and space have been observed for both bacteria and other soil inhabitants. These fluctuations of bacterial numbers are especially noticeable after some disturbance of soil such as tillage, drying and rewetting, and substrate addition, for example in the form of fresh plant material. However, very seldom were bacterial fluctuations subjected to proper statistical analysis to detect significant periodical components in the analyzed data (Chapter 1). The phenomenon of wave-like bacterial oscillations was investigated in short-term (1 month) controlled experiments for rhizosphere and bulk soil after substrate input from plant roots and fresh plant debris, respectively. Short-term oscillating dynamics of bacterial populations were simulated in a mechanistic model, which may contribute significantly to our understanding of the reasons and consequences of bacterial oscillations after addition of substrate to soil.

To determine the spatial variation in density of different trophic bacterial groups (copiotrophic and oligotrophic) and carbon sources in the rhizosphere, colony-forming units (CFUs) and soluble total organic carbon (TOC) were quantified along the root from rhizosphere and corresponding bulk soil samples at 2 cm intervals along wheat roots two, three, and four weeks after planting (Chapter 2). There was a moderate rhizosphere effect in one experiment with soil rich in fresh plant debris (1% C in soil), and a very pronounced rhizosphere effect in the second experiment with soil low in organic matter (0.7% C). Wave-like patterns of both trophic groups of bacteria as well as TOC could be discerned along the whole root length (60 or 90 cm). Harmonical analysis revealed significant oscillations in bacterial populations and TOC. TOC concentrations were maximal at the root tip and base and minimal in the middle part of the roots. Populations of copiotrophic and oligotrophic bacteria had two maxima close to the root tip and at the root base, or three maxima close to the tip, in the middle section, and at the root base. Phases and periods of the two trophic groups differed slightly. The location and pattern of the waves in bacterial populations changed progressively from week to week, and was not consistently correlated with TOC concentrations or the location of lateral root formation. Thus, the traditional view that patterns in bacterial numbers along the root directly reflect patterns in exudation and rhizodeposition from several fixed sources along the root may not be true. We attributed the observed wave-like patterns in bacterial populations to bacterial growth and death cycles (due to autolysis or grazing by predators). Considering the root tip as a moving nutrient source, temporal oscillations in bacterial populations at any location where the root tip passed would result in moving waves along the root. This change in concept about bacterial populations in the rhizosphere could have significant implications for plant growth promotion and soil health.

To check the hypothesis that the principal mechanism underlying the wave-like distribution of bacteria along the root is a cycle of growth, death, autolysis, and regrowth of copiotrophic bacteria in response to a moving substrate source (root tip) a simulation model was created (Chapter 3). 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 relative 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. The original source of substrate was the root tip, supplemented with a background flux (BGF) of substrate from soil organic matter and dead root cells. Dead necromass from bacteria was partially recycled into substrate. This model was named “BACWAVE”, standing for ‘bacterial waves’. The model generated cyclic dynamics of bacteria, which 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. The model was validated with an independent data set of bacteria along wheat roots in relatively C-rich soil. Calculated microbial biomass values produced spatial fluctuations similar to those obtained for experimental biomass data derived from colony forming units. Concentrations of readily utilizable substrate (RUS) calculated from biomass dynamics did not mimic measured concentrations of TOC, which consists not only of substrate but also various polymers and humic acids. Thus, a moving impulse of nutrients into soil resulting in cycles of growth and death of bacteria can explain the observed phenomenon of moving bacterial waves along roots. This was the first report of wave-like dynamics of micro-organisms in soil along a root resulting from the interaction of a single organism group with its substrate.

The model "BACWAVE" for wave-like dynamics of copiotrophic bacteria (CB) was extended to include dynamics of oligotrophic bacteria (OB) (Chapter 4). CFUs of OB and CB along wheat roots (24 samples) in a low C soil were transformed to temporal biomass taking root growth rate and cell sizes into account. Growth rates of both groups of bacteria increased with readily utilizable substrate (RUS) according to Monod equations, but each with their own characteristic parameter values. The death rate of CB decreased monotonically with substrate concentration, while the death rate of OB first decreased and then increased with substrate concentration. Model parameters were estimated from literature and with an iterative optimization method. Initial biomass and kinetic parameters were lower for OB than for CB, and fell in the range of values in the literature. The model was validated with an independent data set of bacteria along wheat roots in relatively C-rich soil, so that BGF and initial microbial populations were higher, but other model parameters were the same for both data sets. A satisfactory fit was obtained between experimental and modeled data. This is the first rhizosphere model in which oligotrophic bacteria are taken into account.

Several microcosm experiments were carried out to investigate the hypothesis that an impulse of fresh substrate into soil would invoke oscillations in bacterial populations (Chapter 5). Soil bacterial populations, mineral nitrogen content, pH, and redox potential (ROP) were monitored daily for one month after incorporation of clover-grass (CG) plant material in soil. Colony-forming units (CFUs) and direct microscopic counts of FDA-stained and FITC-stained bacteria increased immediately after incorporation of the plant material, dropped within 2 days, and fluctuated thereafter. Harmonics analysis demonstrated that there were significant wave-like fluctuations with three or four significant peaks within one month after incorporation of clover-grass material. Ammonium (NH_4^+)

concentrations increased from the start of the experiments until nitrification commenced. Nitrate (NO_3^-) concentrations dropped immediately after plant incorporation, and then rose monotonically until the end of the experiments. There were no wave-like fluctuations in NH_4^+ and NO_3^- concentrations, so that bacterial fluctuations could not be attributed to alternating mineral N shortage and sufficiency. pH levels rose and declined with NH_4^+ levels. ROP dropped shortly before NH_4^+ concentrations rose, and increased before NH_4^+ concentrations decreased; there were no regular fluctuations in ROP, so that temporary oxygen shortages may not have been responsible for the observed fluctuations in bacterial populations. Thus, for the first time, regular wave-like dynamics were demonstrated for bacterial populations after perturbation by addition of fresh organic matter to soil, and several potential reasons for the death phase of the fluctuations could be excluded from further consideration.

To elucidate possible reasons for the oscillations in bacterial populations, potential interactions with populations of bacterial predators, in particular bacterial-feeding nematodes (BFN), were investigated (Chapter 6). In two microcosm experiments, soil bacteria (CFU's and microscopic counts of stained bacteria) and nematode populations in 22 families were monitored daily for 25 or 30 days after incorporation of clover + grass (CG) plant material into soil. Soil bacterial populations fluctuated significantly after incorporation of the plant material with 2 peaks within the first week and 3 or 4 smaller peaks thereafter. Total nematodes and BFN populations started to increase in the course of the second week after CG incorporation, but the proportion of BFN increased within one week. Inactive juvenile BFN (dauerlarvae) seemed to be activated after two days (as the percentage of Rhabditidae increased and dauerlarvae decreased), followed by step-wise increases in dauerlarvae every four days, indicating that there was a new generation every four days. There were significant wave-like fluctuations in daily population changes of BFN, but not in total nematode communities, over the duration of these experiments. These fluctuations had similar periods (5 days) as those of bacterial populations, but were shifted about 3 days relatively to the bacterial fluctuations.

In another microcosm experiment, dynamics of bacterial populations were monitored in response to gamma-irradiated plant material added to gamma-irradiated soil mixed with filtered bacterial suspensions and to non-irradiated soil. Gamma-irradiation of soil significantly increased the periods and amplitudes of bacterial oscillations compared to untreated field soil. Nematode populations were decimated in gamma-irradiated soils, but a small number of protozoa were accidentally introduced in the irradiated soil, and may have been partially responsible for the delayed regulation of bacterial growth. We conclude that fluctuations in bacterial populations were not directly related to similar fluctuations in populations of BFN, as expected from classical Lotka-Volterra equations for predator-prey relationships, but were related to changes in growth rates of BFN. An alternation in active and inactive stages in a synchronized predator community after a disturbance could allow periods of bacterial growth alternated with periods of death. Fluctuations in bacterial populations were dampened after a much longer period when the soil fauna was largely eliminated.

Findings of regular oscillations in bacterial populations and in the rate of change in numbers of bacterial predators after addition of fresh organic matter to soil stimulated the development of a simulation model to investigate potential mechanisms of those oscillations, and whether they were initiated by bacteria-

substrate interactions or predatory regulation of bacteria (Chapter 7). The model could also be used to investigate mineral nitrogen release during short-term organic matter decomposition. A substrate-based food web model was constructed with 3 plant residue and 5 soil organic matter compartments, 3 trophic groups of bacteria (copiotrophic, oligotrophic and hydrolytic), and two predatory groups (BFN and protozoa). Both carbon and nitrogen flows were modeled. Fluctuations in microbial populations in soil after plant residue incorporation could be reproduced with and without participation of predators. The first two peaks in bacterial numbers were mainly related to bacteria-substrate interactions, while predators (particularly protozoa) influenced bacterial dynamics during later stages of bacterial community development. Oligotrophic bacteria had a stabilizing effect on fluctuations of other trophic groups, and were the main source of nutrients for predators. A peak in soil ammonium occurred within one week after residue incorporation. Nitrate increased sigmoidally after a short lag phase. The final nitrate concentration was primarily determined by bacterial dynamics and to a lesser extent by protozoa and nematodes. This model emphasized the importance of substrate-consumer relations for regulation of populations at different trophic levels and nutrient release from fresh organic matter added to soil.

This research has given insight in potential mechanisms underlying oscillations in populations of soil bacteria and their predators after a disturbance. Despite the advances achieved in this thesis, there are still some problems to be solved. Precise regulation of substrate-consumer interactions and mechanisms that initiate growth and death cycles of soil bacteria have to be investigated in detail. Nevertheless, the “BACWAVE-WEB” model has good potential to predict responses of microbial communities to a disturbance, which could be used to characterize soil health. The model could be expanded to include denitrification and nitrate leaching, so that the extent of N losses after soil disturbance could be predicted.

Резюме

Колебания бактериальных популяций в природе и в эксперименте можно наблюдать, если определение численности бактерий путём прямого счёта или методом посева на среды производится в достаточно длинной серии образцов, взятых с достаточной частотой. Колебания численности во времени и в пространстве известны не только для бактерий, но и для других представителей почвенной биоты. Такие колебания особенно проявляются после нарушающих воздействий на почвенную систему, например, после вспашки, высушивания и последующего увлажнения, а также после внесения в почву питательных веществ, например, свежих растительных остатков. Однако, до сих пор данные, отражающие колебания численности бактерий, редко являлись объектом соответствующего статистического анализа, способного выявить в них значимые периодические составляющие (Глава 1). В настоящей диссертации представлены результаты исследования феномена волнообразных колебаний бактериальных популяций, полученных в краткосрочных экспериментах (длительностью 1 месяц). Колебания численности бактерий в контролируемых условиях были исследованы как в ризосфере, так и в неризосферной почве после внесения свежих растительных остатков. Была построена имитационная модель, воспроизводящая краткосрочную динамику колебаний численности бактерий, которая может служить инструментом, способным обеспечить существенный вклад в понимание причин и последствий колебаний численности бактерий после нарушающих воздействий на почвенную систему.

Для выявления пространственных колебаний популяций различных трофических групп бактерий (копиотрофов (КБ) и олиготрофов (ОБ)) и концентрации органического углерода (тотального водорастворимого органического углерода (ТВУ)) в ризосфере было проведено определение численности колоний образующих единиц (КОЕ) бактерий и содержания ТВУ вдоль корня растений пшеницы. Количество КОЕ и содержание ТВУ учитывали в образцах ризосферной и неризосферной почвы, взятых вдоль корня через каждые 2 см у 2-х, 3-х и 4-х недельных растений (Глава 2). В первом эксперименте при использовании почвы, содержащей значительное количество растительных остатков (1 % С в почве), был выявлен лишь умеренный ризосферный эффект, тогда как во втором эксперименте, с почвой, более бедной органическим веществом (0.7 % С), ризосферный эффект был значительным. Как в первом, так и во втором экспериментах вдоль корней, длиной 60 и 90 см соответственно, были выявлены существенные колебания численности КОЕ бактерий обеих трофических групп и содержания ТВУ. Наличие статистически значимых регулярных колебаний в распределении КОЕ и ТВУ вдоль корня было подтверждено с помощью гармонического анализа. Содержание ТВУ было максимальным у основания и у кончика корня и минимальным в средней части корня. Численности КБ и ОБ имели два максимума: один – ближе к кончику корня, а другой – ближе к основанию корня, или три максимума, один у кончика корня, другой у основания и третий в средней части корня. Фазы и периоды значимых гармоник, обнаруженных в распределениях КОЕ бактерий разных трофических групп вдоль корня растений одного и того же возраста, различались незначительно, однако положение максимумов и минимумов распределений КОЕ обеих групп бактерий вдоль корня заметно менялось от недели к неделе, в зависимости от

возраста растений, при этом величины численности КОЕ бактерий не коррелировали с концентрацией ТВУ или количеством боковых корней. Таким образом, традиционная точка зрения, что характер распределения бактерий в ризосфере вдоль корня растений однозначно определяется положением нескольких фиксированных зон эксудации корня и формирования ризодепозитов, по-видимому, не соответствует действительности. Представляется, что наблюдаемые волнообразные распределения численности бактерий вдоль корня являются следствием циклов роста и отмирания бактерий (в результате автолиза или выедания хищниками). Рассматривая кончик корня как движущийся источник питательных веществ, совокупность временных колебаний бактериальных популяций в каждой точке ризосферы можно представить как пространственную, вдоль корня, волну, перемещающуюся вслед за кончиком корня. Предполагается, что новая концепция функционирования бактериальных популяций в ризосфере может найти применение в исследованиях по улучшению условий роста растений и оценке «здоровья почвы».

Для поверки гипотезы, что основным механизмом волнообразного распределения бактерий вдоль корня являются циклы бактериального роста, отмирания, автолиза отмерших клеток и последующего возобновления роста, вызванные движущимся источником питательных веществ (кончиком корня), была построена имитационная модель, описывающая поведение популяций КБ (Глава 3). Данные пространственного распределения бактерий с учётом скорости роста корня были преобразованы в их условную временную динамику. Таким образом, модель, в которой был применён метод интегрирования Рунге-Кутта, позволила воспроизвести динамику биомассы КБ, соответствующей экспериментально определенному количеству КОЕ этой группы бактерий. В модели принято, что удельные скорости роста и отмирания КБ зависят от содержания субстрата так, что кривые скорости роста и отмирания пересекаются в области доступных концентраций субстрата. Первичным источником субстрата в модели является кончик корня, а дополнительным − образование питательных веществ при разложении органического вещества почвы и отмерших тканей корня. Некромасса бактерий также частично трансформируется в субстрат. Модель получила название “BACWAVE” - ‘бактериальные волны’. Полученная с помощью модели циклическая динамика бактерий была преобразована в пространственные колебания (волны), перемещающиеся вслед за движущимся источником питательных веществ (кончиком корня). Значения параметров модели были оценены на основе начальных концентраций субстрата и наблюдаемого распределения бактерий вдоль корня с помощью итерационного метода оптимизации. Найденные оценки кинетических параметров модели соответствуют литературным данным. Проверка адекватности модели была осуществлена с использованием независимой серии данных по распределению КОЕ в ризосфере вдоль корня пшеницы, выращенной на относительно богатой органическим углеродом почве. Рассчитанные с помощью модели величины бактериальной биомассы позволили воспроизвести пространственные колебания близко к экспериментальным значениям биомассы, полученным в результате пересчёта КОЕ. Концентрация легко утилизируемого бактериями субстрата (ЛУС), рассчитанная на основе динамики биомассы бактерий, не соответствовала содержанию ТВУ, который состоит не только из ЛУС , но также и из различных полимеров и гумусовых кислот. Таким образом, наличие перемещающегося

источника питательных веществ в почве и вызывающего возникновение циклов роста и отмирания бактерий, может служить объяснением наблюдаемого феномена бегущих волн бактериальных популяций вдоль корня растений. Настоящая модель является первой, с помощью которой удалось описать волнообразное распределение микроорганизмов вдоль корня как результат взаимодействия одной группы организмов и потребляемого ею субстрата.

Модель "BACWAVE", воспроизводящая волнообразную динамику КБ, была модифицирована в целях совместного описания динамики КБ и ОБ (Глава 4). Пространственное распределение численности КОЕ КБ и ОБ вдоль корня, определённое по 24 образцам почвы, взятым из ризосферы пшеницы, выращенной в почве с низкой концентрацией углерода, было преобразовано во временную динамику биомассы обеих трофических групп бактерий с учетом скорости роста корней и размеров клеток КБ и ОБ. Удельные скорости роста обеих групп бактерий увеличивались с ростом концентрации ЛУС согласно уравнению Моно с использованием соответствующих каждой трофической группе параметров. Удельная скорость отмирания КБ уменьшалась монотонно с увеличением концентрации ЛУС, в то время как удельная скорость отмирания ОБ сначала уменьшалась, а затем увеличивалась с возрастанием концентрации ЛУС. Оценки значений параметров модели были взяты из литературных источников, либо получены путём применения итерационного метода оптимизации. Значения начальной биомассы и кинетических параметров были ниже для ОБ, чем для КБ, и находились в пределах, известных из литературы. Модель была проверена на другой серии данных, полученной в аналогичном эксперименте с почвой, более богатой органическим углеродом, в этом случае скорость подтока ЛУС, формирующегося в результате разложения органического вещества почвы и начальные значения биомассы бактерий были увеличены, все остальные параметры модели были оставлены без изменений. Полученные с помощью модели расчётные значения удовлетворительно аппроксимировали экспериментальные данные. Настоящая модель бактериального сообщества ризосферы является первой, включающей в рассмотрение динамику популяций ОБ.

Для проверки гипотезы, что внесение в почву свежего органического вещества приводит к возникновению колебаний численности бактерий, было проведено несколько лабораторных экспериментов (Глава 5). Численность бактерий, содержание минерального азота, pH, и окислительно-восстановительный потенциал (ОВП) определяли в почве ежедневно в течение одного месяца после внесения смеси клевера и травы. Количество КОЕ и клеток, учитываемых под микроскопом при окрашивании флюоресцирующими красителями − ФДА (флюоресцеин-диацетатом) и ФИТЦ (флюоресцеин-изотиоцианатом), − увеличивалось сразу после внесения растительных остатков, снижалось через 2 дня, и затем колебалось до конца эксперимента. Гармонический анализ выявил наличие статистически значимых гармоник в колебаниях численности бактерий. Гармоники имели три или четыре пика в продолжение 1 месяца после внесения в почву смеси клевера и травы. Во всех экспериментах содержание аммония (NH_4^+) увеличивалось в начальный период и в дальнейшем снижалось с наступлением активной фазы нитрификации. Концентрация нитратов (NO_3^-) снижалась сразу после внесения растительного материала, а затем монотонно возрастала до конца наблюдений. В динамике NH_4^+ и NO_3^- не

было обнаружено заметных осцилляций, таким образом возникновение волнообразной динамики численности бактерий не могло быть следствием изменений концентрации минерального азота в почве. pH почвы сначала повышался, а позднее снижался в соответствии с изменением содержания NH_4^+ в почве. ОВП снижался в самом начале эксперимента, до того как концентрация NH_4^+достигала максимума, а затем возрастал , прежде чем содержание NH_4^+ падало. Поскольку в динамике ОВП также не было отмечено заметных осцилляций, это можно рассматривать как свидетельство, что временная нехватка кислорода не является причиной возникновения регулярных колебаний численности бактерий. Таким образом, впервые была экспериментально продемонстрирована волнообразная динамика численности бактерий в почве после нарушающего воздействия в виде внесения свежего органического вещества. Параллельное наблюдение за динамикой ряда почвенных параметров позволило исключить из дальнейшего рассмотрения несколько причин отмирания бактерий, как возможных факторов, способных вызвать колебаний бактериальных популяций.

Для уточнения роли одной из возможных причин возникновения колебаний численности бактерий была исследована взаимная динамика популяций бактерий с популяциями хищников, в частности, с популяцией бактериотрофных нематод (БТН) (Глава 6). В двух лабораторных экспериментах после внесения в почву свежих растительных остатков в виде смеси клевера и травы в течение 25 или 30 дней была прослежена ежедневная динамика бактериальных популяций (методами посева и прямого учёта ФИТЦ-окрашеных клеток под микроскопом) и динамика численности нематод 22 семейств.В динамике численности бактерий были выявлены значимые колебания, при этом первые 2 пика приходились на первую неделю эксперимента, и 3 - 4 меньших пика – на более поздний период. Общая численность нематод и численность БТН начала возрастать со второй недели эксперимента, однако доля БТН стала увеличиваться еще в течение первой недели. Неактивные молодые БТН (НМБТН (dauerlarvae)), по-видимому, активизировались уже через два дня (т.к. доля Rhabditidae от общего числа нематод увеличилась, а доля НМБТН уменьшилась), в дальнейшем происходило ступенчатое возрастание количества НМБТН (dauerlarvae) каждые четыре дня, указывающее на появление новой генерации каждые четыре дня. В каждом из экспериментов за период наблюдений были выявлены значимые колебания в ежедневном изменении численности именно БТН, но не общего числа нематод. Эти колебания при смещении по фазе на 3 дня имели тот же период (5 дней), что и колебания численности бактерий.

В другом лабораторном эксперименте была прослежена популяционная динамика бактерий в почве с внесённым растительным материалом, которые были предварительно простерилизованы радиоактивным излучением и затем инокулированы бактериальной суспензией, полученной путём пропускания нестерильной почвенной суспензии через крупнопористый мембранный фильтр. В другой серии этого эксперимента была использована смесь нестерилизованных почвы и растительного материала. Колебания численности бактерий в почве, подвергнутой стерилизации, имели существенно большие величины периодов и амплитуд по сравнению с нестерилизованной почвой. В стерилизованной почве нематоды практически отсутствовали, однако были обнаружены клетки простейших, случайно внесенные

в почву, которые могли оказать определённое влияние на рост бактерий на поздних стадиях эксперимента. На основании полученных результатов был сделан вывод, что вопреки уравнениям Лотки-Вольтерра, описывающим взаимодействия в системе хищник-жертва, колебания численности бактерий не связаны непосредственно с колебаниями численности БТН, а коррелируют с изменениями скорости роста БТН. Чередование неактивной и активной стадий в синхронизированной после нарушающего воздействия популяции хищников может создавать условия для чередования периодов роста и отмирания бактериального сообщества. Затухание колебаний бактериальных популяций наступало значительно позднее после почти полного удаления почвенной микрофауны.

Для дальнейшего исследования наблюдаемых после внесения в почву свежих растительных остатков колебаний численности бактерий и их хищников была построена имитационная модель. Создание модели было осуществлено в целях изучения потенциальных механизмов колебаний, а именно: являются ли они следствием взаимодействия бактерий и субстрата или следствием взаимодействия типа хищник-жертва. Такого рода модель могла бы быть использована и для исследования процессов минерализации азота на ранних стадиях разложения свежих растительных остатков в почве. Созданная модель включала: 3 компартмента, представлявших разные компоненты растительных остатков, 5 компартментов почвенного органического вещества, 3 трофические группы бактерий (КБ, ОБ и гидролитиков) и две группы хищников (БТН и простейших). В модели были описаны как потоки углерода, так и азота. Было выявлено, что колебания численности бактерий в почве после внесения растительных остатков могут быть воспроизведены как с участием хищников, так и без них. Согласно результатам моделирования, было установлено, что первые два пика в динамике численности бактерий возникают вследствие взаимодействия субстрат – потребитель, тогда как хищники (в основном, простейшие) оказывают влияние на динамику бактерий на более поздних стадиях развития бактериального сообщества. ОБ оказывали стабилизирующее действие, способствуя затуханию колебаний популяций других трофических групп, и являлись основным источником пищи для хищников. Максимальное выделение аммонийного азота наблюдалось в течение первой недели после внесения в почву растительных остатков. Содержание нитратов в почве увеличивалось сигмоидно после короткой лаг-фазы. Конечное содержание нитратов было обусловлено главным образом жизнедеятельностью бактерий и в значительно меньшей степени простейших и нематод. Результаты моделирования показали, что взаимодействие типа субстрат-потребитель является наиболее важным механизмом регуляции как численности организмов разных трофических уровней, так и поступления в почву питательных веществ при разложении свежего растительного материала.

Настоящие исследования вносят существенный вклад в понимание потенциальных механизмов возникновения колебаний численности бактерий и их хищников в почве после нарушающих воздействий. Вместе с тем, несмотря на достигнутые в настоящей работе определённые успехи, имеются проблемы, которые необходимо будет разрешить в дальнейших исследованиях. Предстоит, например, подробно изучить конкретные механизмы взаимодействий потребителя и субстрата, а также в деталях установить причины переключения фаз роста и отмирания в

микробных сообществах. Тем не менее, следует отметить, что представленная в настоящей диссертации модель “BACWAVE-WEB” обладает широкими возможностями и способна достаточно точно предсказывать реакцию микробных сообществ на нарушающие воздействия, что могло бы быть использовано для тестирования «здоровья почвы». Включение в настоящую модель описания процессов денитрификации и вымывания из почвы нитратного азота позволит использовать её для расчета возможных потерь азота в результате нарушающего воздействия. на почву.

Samenvatting

Oscillerende veranderingen in bacteriële populaties kunnen worden waargenomen mits er voldoende vaak en lang wordt bemonsterd voor directe tellingen met de microscoop of via uitplaten. Zulke veranderingen over tijd of in de ruimte zijn zowel waargenomen voor bacteriën als voor andere bodembewoners. Dergelijke veranderingen zijn vooral goed waarneembaar na een verstoring van de grond zoals bijvoorbeeld omploegen, drogen en herbevochtigen of toevoegen van nutriënten, bijvoorbeeld in de vorm van vers plantmateriaal. Tot dit proefschrift werden de bacteriële veranderingen echter meestal niet op de juiste wijze statistisch geanalyseerd om significante periodieke veranderingen uit de geanalyseerde data te halen (Hoofdstuk 1). Voor dit proefschrift werd het fenomeen van oscillerende bacteriële populaties bestudeerd in de rhizosfeer en in niet door planten beïnvloede bodem in kortlopende (1 maand) experimenten onder gecontroleerde omstandigheden. In de rhizosfeer werd substraat aangevoerd via de plantenwortel, in de onbegroeide bodem als vers plantenafval. De dynamica van zulke kortdurende oscillaties in de bacteriële populaties werd gesimuleerd met een mechanistisch model, dat significant zou kunnen bijdragen aan ons begrip van de oorzaken en gevolgen van bacteriële oscillaties na het toevoegen van een substraat aan grond.

Om de ruimtelijke verschillen in dichtheid van verschillende trofische groepen bacteriën (copiotrofen en oligotrofen) en in koolstofbronnen in de rhizosfeer te bepalen, zijn aantallen kolonievormende eenheden (*colony forming units*, CFU's) en de totale hoeveelheid oplosbaar organisch koolstof (*total organic carbon*, TOC) om de 2 cm bepaald zowel direct langs de wortel als in de omringende grond twee, drie en vier weken na planten (Hoofdstuk 2). In het experiment werd een klein rhizosfeer effect waargenomen in rijke grond met vers plantmateriaal (1% C in de grond) en een uitgesproken rhizosfeer effect in het volgende experiment met organisch armere grond (0.7% C). Zowel de beide trofische bacteriegroepen als TOC vertoonden golvende fluctuaties langs de gehele wortel (60 of 90 cm lang). Harmonische analyses toonden aan dat deze fluctuaties significant zijn. De TOC concentraties waren maximaal aan de worteltop en –basis en minimaal daartussenin. De populaties copiotrofe en oligotrofe bacteriën lieten twee of drie maxima zien; in de buurt van worteltop en –basis en bij langere series een derde in het midden. De fase en periode van de golven in populaties van de twee trofische groepen verschilden licht. De plaats en het patroon van de golven van bacteriën lieten een voortschrijdende verschuiving zien van week tot week en deze waren niet gecorreleerd met TOC concentraties of met de vorming van zijwortels. De traditionele visie dat verschillen in aantallen bacteriën direct gerelateerd zijn aan de plaatsen op het worteloppervlak waar exudatie of beschadiging plaatsvindt, zou dus niet juist kunnen zijn. De waargenomen oscillerende patronen in de bacteriële populaties zijn toegeschreven aan cycli van bacteriële groei en afsterven (door autolyse of predatie). Als men de worteltop als bewegende nutriëntenbron ziet, dan resulteren oscillaties over tijd in een lopende golf langs de wortel. Deze conceptuele verandering van bacteriële populaties in de rhizosfeer kan verstrekkende gevolgen hebben voor de bevordering van plantengroei en bodemgezondheid.

Om de hypothese te toetsen dat het belangrijkste mechanisme achter de oscillerende verdeling van bacteriën langs de wortel een cyclus is van groei, dood, autolyse en opnieuw groei van copiotrofe

bacteriën in reactie op een bewegende voedselbron (de worteltop), is een simulatiemodel gemaakt (Hoofdstuk 3). Na de transformatie van de waargenomen ruimtelijke gegevens naar aannemelijke temporele data gebaseerd op de groeisnelheid van een wortel, is een simulatiemodel gebouwd op basis van de Runge-Kutta integratie methode. Dit model simuleert de dynamiek van de kolonievormende bacteriële biomassa met relatieve groei- en sterftesnelheden afhankelijk van de hoeveelheid aanwezig substraat zodanig dat de curven elkaar kruisen bij een substraatconcentratie die binnen het gebied ligt van aannemelijke substraatbeschikbaarheid. De oorspronkelijke substraatbron was de worteltop, aangevuld met een achtergrondstroom (*background flux*, BGF) van substraat uit organisch materiaal in de bodem en van dode wortelcellen. Afgestorven biomassa van bacteriën werd gedeeltelijk teruggevoerd als substraat. Dit model werd "BACWAVE" genoemd naar de Engelse benaming "*bacterial waves*" voor bacteriële golven. Het model genereerde een cyclische dynamiek voor bacteriën die vertaald kon worden in verschuivende ruimtelijke golven achter een bewegende nutriëntenbron aan. De parameterwaarden werden geschat met een iteratieve optimalisatiemethode op basis van de berekende initiële substraatconcentraties en de waargenomen microbiële verdelingen langs graanwortels. Deze geschatte kinetische parameters vielen binnen de in de literatuur gerapporteerde waarden. Het model werd beproefd met een onafhankelijke dataset van bacteriën langs de wortels van graan in een relatief koolstofrijke grond. De berekende waarden voor de microbiële biomassa lieten vergelijkbare ruimtelijke verdelingen zien als die welke experimenteel verkregen waren aan de hand van aantallen kolonievormende eenheden. De concentraties makkelijk opneembaar substraat (*readily utilizable substrate*, RUS) berekend uit de biomassa dynamiek waren niet vergelijkbaar met de gemeten concentraties TOC, die niet alleen uit substraat bestaan maar ook uit verschillende polymeren en humuszuren. Een bewegende nutriëntenbron in de bodem kan dus resulteren in cycli van groei en afsterven van bacteriën en zo het waargenomen fenomeen van bewegende bacteriële golven langs groeiende plantenwortels verklaren. Dit was het eerste verslag over de oscillerende dynamiek van micro-organismen in de grond langs een plantenwortel als gevolg van de interactie van één enkele groep organismen en hun voedselbron.

Het "BACWAVE" model voor de oscillerende dynamiek van copiotrofe bacteriën (CB) werd uitgebreid om ook de dynamiek van oligotrofe bacteriën (OB) te beschrijven (Hoofdstuk 4). CFUs van OB en CB langs graanwortels (24 monsters) in een koolstofarme grond werden getransformeerd naar temporele biomassa variaties met behulp van de groeisnelheid van de wortel en gemiddelde celgroottes. Volgens Monod vergelijkingen werden de groeisnelheden van beide groepen bacteriën groter met de toename van de hoeveelheid makkelijk opneembaar substraat (RUS), maar elke groep had daarbij zijn eigen karakteristieke parameterwaarden. Het sterftecijfer van de CB nam gelijkmatig af met de substraat concentratie, terwijl het sterftecijfer van de OB eerst afnam en vervolgens juist toenam. De parameters voor het model werden geschat op basis van literatuurgegevens en met een iteratieve optimalisatiemethode. De beginwaarde van de biomassa en de kinetische parameters waren beide lager voor de OB dan voor de CB. Beiden vielen binnen de grenzen van de gevonden literatuurwaarden. Het model is gevalideerd met een onafhankelijke data set van bacteriën langs graanwortels in relatie tot een relatief koolstofrijke bodem op een dusdanige wijze dat de BGF en de initiële microbiële populaties groter waren maar de andere parameters in het model hetzelfde waren

voor beide datasets. De uit het model verkregen data beschrijven de experimentele data naar verwachting. Dit is het eerste rhizosfeer-model dat ook de oligotrofe bacteriën meeneemt.

Verschillende microcosmos-experimenten zijn uitgevoerd om de hypothese te testen dat een puls vers substraat in een bodem een oscillatie in de bacteriële populatie teweeg zou brengen (Hoofdstuk 5). Bodembacteriepopulaties, hoeveelheden minerale stikstof, pH en de redoxpotentiaal (ROP) werden dagelijks bepaald gedurende één maand na de incorporatie van een mengsel van gras en klaver (*clover-grass*, CG) in de grond. De aantallen kolonievormende eenheden (CFUs) en de aantallen microscopisch direct waarneembare FDA- en FITC-gekleurde bacteriën namen direct toe na de incorporatie van het plantmateriaal, namen af binnen twee dagen en fluctueerden daarna verder. Harmonische analyses lieten zien dat er significante oscillerende fluctuaties optraden met drie of vier significante maxima gedurende één maand na het inwerken van het gras-klavermateriaal. De hoeveelheid ammonium (NH_4^+) nam toe vanaf de start van het experiment totdat de nitrificatie begon. De hoeveelheid nitraat (NO_3^-) nam onmiddellijk na het inwerken van het plantmateriaal af om vervolgens tot het eind van het experiment gestaag toe te nemen. Er waren geen golvende verschuivingen in de hoeveelheden NH_4^+ of NO_3^- waar te nemen zodat de bacteriële fluctuaties niet toegeschreven konden worden aan afwisselende perioden van stikstofschaarste en –overvloed. pH-waarden veranderden met de NH_4^+ concentraties mee. De redoxpotentiaal (ROP) zakte net voordat de NH_4^+ concentraties toenamen en nam toe net voordat deze concentraties lager werden; er waren geen regelmatige fluctuaties waar te nemen in de ROP zodat de waargenomen fluctuaties in de bacteriële populaties misschien niet toe te schrijven zijn aan tijdelijke zuurstoftekorten. Zo werd voor het eerst beschreven hoe bacteriële populaties een regelmatig oscillerende dynamiek vertonen na een verstoring van de grond in de vorm van het toevoegen van vers organisch materiaal en hoe verschillende mogelijke redenen voor de afstervingsfases in deze fluctuaties konden worden geëlimineerd.

Om andere mogelijke oorzaken van de oscillaties in aantallen bacteriën te achterhalen, zijn de mogelijke interacties met populaties predatoren van bacteriën, met name bacterie-etende nematoden (BFN) onderzocht (Hoofdstuk 6). In twee microcosmos-experimenten zijn de bodembacteriën (CFUs en directe microscopische tellingen van gekleurde bacteriën) en de populaties van 22 families nematoden gedurende 25 of 30 dagen na het inwerken van gras en klavermateriaal (CG) in de bodem geobserveerd. De populaties bodembacteriën fluctueerden significant na het inwerken van het plantmateriaal met twee pieken gedurende de eerste week en 3 of 4 kleinere pieken in de periode daarna. De totale aantallen nematoden en de aantallen bacterie-etende nematoden begonnen in de tweede week na het inwerken van CG toe te nemen, het aandeel bacterie-eters nam echter binnen een week al toe. Inactieve juveniele BFN (dauerlarvae) leken te worden geactiveerd in twee dagen tijd (het aantal Rhabditidae nam toe en het aantal dauerlarvae nam af), gevolgd door een stapsgewijze toename in het aantal dauerlarvae elke vier dagen, wat er op wijst dat er elke vier dagen een nieuwe generatie was. Er waren significante golvende fluctuaties waar te nemen in de dagelijkse veranderingen in aantallen BFN, maar niet in de totale aantallen nematoden gedurende de experimenten. De periode van deze fluctuaties in dagelijkse veranderingen in BFN waren vergelijkbaar met fluctuaties in bacteriepopulaties (5 dagen), maar waren drie dagen verschoven ten opzichte van deze bacteriële fluctuaties. In een ander microcosmos-experiment werd de dynamiek van de bacteriële populaties

gevolgd als reactie op het inwerken van gamma-bestraald plantmateriaal in eveneens gamma-bestraalde grond waaraan een gefilterde bacteriesuspensie was toegevoegd en in een niet-bestraalde grond. De bestraling leidde in vergelijking tot de onbehandelde grond tot significant langere perioden en grotere amplitudes in de fluctuaties in bacterieaantallen. De nematoden in de gamma-bestraalde grond waren uitgeschakeld, maar een klein aantal protozoën zijn per ongeluk geïntroduceerd in de bestraalde grond en zíj zijn mogelijk verantwoordelijk voor de vertraagde regulatie van de bacteriegroei. We concluderen dat de veranderingen in de bacteriepopulaties niet direct gerelateerd zijn aan vergelijkbare fluctuaties in de BFN populaties, zoals verwacht volgens de klassieke Lotka-Volterra berekeningen voor predator-prooi verhoudingen, maar gerelateerd zijn aan veranderingen in de groeisnelheid van de BFN. Een verschuiving in verhouding tussen actieve en inactieve stadia in een synchrone predatorpopulatie kan na een verstoring cycli van bacteriegroei en –afsterven verklaren. De fluctuaties in bacterieaantallen werden later afgezwakt toen de bodemfauna grotendeels geëlimineerd was.

De ontdekking van regelmatige oscillaties in bacteriële populaties en predatoren van bacteriën na het toevoegen van vers organisch materiaal aan de bodem, stimuleerde de ontwikkeling van een simulatiemodel om de mogelijke mechanismen achter deze fluctuaties te achterhalen en na te gaan of ze geïnitieerd worden door interacties tussen bacteriën en substraat of door de regulatie door bacterie-etende predatoren (Hoofdstuk 7). Dit model kan ook gebruikt worden om het vrijkomen van minerale stikstof te bestuderen tijdens kortstondige afbraak van organisch materiaal. Een voedselwebmodel gebaseerd op substraat werd gemaakt met 3 componenten van plantenresten en 5 van bodem organisch materiaal, 3 trofische groepen bacteriën (copiotroof, oligotroof en hydrolytisch) en 2 groepen predatoren (BFN and protozoën). Zowel koolstof- als stikstofstromen werden gemodelleerd. Veranderingen in de microbiële populaties na de incorporatie van plantenresten konden worden gereproduceerd met en zonder de invloed van predatoren. De eerste twee pieken in aantallen bacteriën waren vooral gerelateerd aan de interacties tussen bacteriën en substraat, terwijl de predatoren (met name de protozoa) de dynamiek van de bacteriën vooral tijdens de latere stadia van de ontwikkeling van de bacteriële gemeenschap beïnvloedden. Oligotrofe bacteriën hadden een stabiliserend effect op de fluctuaties binnen de andere trofische groepen en bleken de belangrijkste voedselbron voor predatoren. Een maximum aan ammonium werd een week na de incorporatie van plantenresten waargenomen. De hoeveelheid nitraat was korte tijd stabiel en nam daarna sigmoïdaal toe. De uiteindelijke nitraatconcentratie werd vooral bepaald door de bacteriële dynamiek en in mindere mate door protozoën en nematoden. Dit model benadrukte het belang van de relaties tussen substraat en consument in de regulatie van populaties op verschillende trofische niveaus en in het vrijkomen van nutriënten uit aan grond toegevoegd, vers, organisch materiaal.

Uiteindelijk heeft dit onderzoek inzicht verschaft in de potentiële mechanismen achter de oscillaties in de bodembacteriepopulaties en hun predatoren na een verstoring. Ondanks alle vorderingen die met dit proefschrift gemaakt zijn, zijn er nog steeds een aantal problemen om op te lossen. Zo moeten de fijnregulatie van de substraat en consument relaties en de mechanismen die de cycli van groei en afsterven van bacteriën initiëren nog in detail bestudeerd worden. Desondanks is het “BACWAVE-WEB” model goed in staat om te voorspellen hoe microbiële gemeenschappen zullen reageren op een

verstoring, wat op zich gebruikt kan worden om de bodemgezondheid te karakteriseren. Het model kan verder worden uitgebreid met de processen van denitrificatie en het uitlekken van nitraat om de grootte van de stikstofverliezen in de bodem na een verstoring in te schatten.

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Vladimir V. Zelenev
Wageningen, December 17, 2003

Biography

Vladimir Viktorovich Zelenev was born on September 1, 1961 in Moscow, Russia. After high school, he studied Soil Science and Agrochemistry at Moscow State University, Faculty of Soil Science, and received an MSc Diploma in Pedology in 1983. Besides his study in Soil Science, he took extensive courses in mathematics and computer science.

He worked first as Post-graduate Student and later as Junior Researcher at Moscow State University, Faculty of Soil Science, from 1983 to 1990. In this period he worked primarily on problems of soil formation, soil quality and nutrient cycling in natural and agricultural soils. His research interests then moved towards soil microbiology, and he was a Junior Researcher at the Institute of Microbiology of the Russian Academy of Science (RAS) from 1990 until 1999. During this period he worked primarily on evaluation of greenhouse gas emission from European and Siberian wetlands, together with among others Dr. Alexander M. Semenov. In 1995 he spent three months in the International Institute for Applied System Analysis, Laxenburg, Austria, to assess annual methane flux from Russian soils.

In the Spring of 1998 and again in 1999, he was a Post-Graduate Researcher in the Department of Plant Pathology, University of California at Davis, to analyze data on microbial fluctuations along the total length of wheat roots (Chapter 2 of this thesis) and along the path of an artificial nutrient source pulled through soil. He also initiated the construction of a simulation model for the spatial distribution of bacteria along a moving nutrient source in soil (Chapter 3 of this thesis).

In 1999, he moved from the Institute of Microbiology, RAS, to the Russian Academy of Medical Sciences, Institute of Vaccines and Serums, in the Laboratory for Modeling of Microorganisms Cultivation Processes, where he became a scientific researcher (his current position).

In 2000, he went to the Biological Farming Systems Group of Wageningen UR for 9 months to carry out several microcosm experiments on microbial fluctuations after clover-grass incorporation into soil. In the following two years, he spent 3 months each year at the Biological Farming Systems Group to continue with the microcosm experiments and the simulation model for bacterial fluctuations in the rhizosphere along wheat roots. In 2003 he spent 6 months at Wageningen UR to write the substrate-based food web model “BACWAVE-WEB” and finalize his PhD thesis.

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