Geoderma, 56 (1993) 265–276
Elsevier Science Publishers B.V., Amsterdam

Synlocation of biological activity, roots, cracks and recent organic inputs in a sugar beet field [α]

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(Received December 4, 1991; accepted after revision July 16, 1992)

ABSTRACT

Van Noordwijk, M., De Ruiter, P.C., Zwart, K.B., Bloem, J., Moore, J.C., Van Faassen, H.G. and Burgers, S.L.G.E., 1993. Synlocation of biological activity, roots, cracks and recent organic inputs in a sugar beet field. In: L. Brussaard and M.J. Kooistra (Editors), Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships. Geoderma, 56: 265–276.

Soil tillage does not lead to homogeneously mixed soil layers. Depending on the interaction between the physical condition of the soil and the tillage implement, a repetitive, layered pattern of accumulated crop residues or other freshly added organic matter can often be observed. Such a non-homogeneous pattern may affect subsequent biological activity, the rates of decomposition, and the chances for root development to occur close to the sites of mineralization in the next growing season.

Patches of organic matter, soil cracks and roots were mapped on clear plastic (polythene) sheets on a soil profile wall. Maps were analysed for spatial correlation of the various mapped items. Small samples of the patches of crop residues and freshly added organic matter, and of surrounding (control) soil were analysed for the abundances of bacteria and protozoa.

A statistical test for synlocation of roots and cracks was developed, on the basis of measured root densities as a function of the distance to the nearest crack. A significant increase of root density (by a factor two) close to the crack was found. For organic matter patches a similar synlocation with cracks was indicated.

Patches with freshly added organic matter contained twice as many bacteria and five times as many protozoa as the control soil. Patches with recent crop residues contained four times as many protozoa, but equal densities of bacteria as control soil. Only a small part of the spatial variability in the abundance of soil organisms, encountered when using a random sampling scheme, is accounted for by distinguishing these patches. Nevertheless, knowledge of the location and activity of these "hot spots" may help in understanding the process of decomposition. The present data indicate a certain degree of synlocation of roots and sites of increased n mineralization, but its effect on plant nutrition and on N use efficiency is probably small.

[α]Communication No. 46 of the Dutch Programme on Soil Ecology of Arable Farming Systems.
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INTRODUCTION

Restricted accessibility of resources to organisms, even when located close together, is a focus of interest in soil ecology. The structure of the soil largely determines the accessibility of food to organisms, and of organisms to their predators. Most attention in this respect has been directed at the accessibility of fine pores, as determined by the body size (diameter) of soil organisms. However, differences in accessibility might exist also at a larger scale. Soil organisms differ widely in their mobility and "home range", and therefore differ in the scale of the patchiness of resource supply to which they can respond, either as individuals or as populations. Colonization of and ecological succession on recent organic inputs by soil organisms, leading to decomposition and mineralization, may thus depend on the location and distribution of such inputs in the soil.

Soil tillage does not lead to a homogeneously mixed plough layer. Rather, depending on the interaction between the physical condition of the soil and the tillage implement, a layered pattern may develop, with accumulations of crop residues or other freshly added organic matter in a repetitive pattern. Such a non-homogeneous pattern has the potential to affect the subsequent rate of decomposition through effects on biological activity, and may also affect the chances that roots occur close to the sites of mineralization in the next growing season. Quantification of such effects is needed.

Branch root development may also respond to local patches of mineral nutrients (Wiersum, 1958; Drew and Saker, 1975; De Jager, 1982), of organic acids (Wiersum, 1974) or of loose soil around cracks. If roots were located preferentially close to recent organic inputs (synlocation) they would benefit more directly from N-mineralization. A statistical test of spatial correlation of roots with patches of organic matter, or with cracks can be based on a comparison of point densities (number per unit surface area) at increasing distances from the objects of interest (Fig. 1). Such comparison can be made with computer image analysis methods (Van Noordwijk et al., 1993), where the area within a certain distance of the crack can be identified and measured, as well as the number of roots for each distance class.

Although heterogeneous distribution of roots and soil organisms is relevant in its own right, it also contributes to "sampling error", if average densities are to be measured. The coefficient of variation for root weight and root length per sample of 385 cm^3 is normally between 0.3 and 0.5 for relatively homogeneous agricultural soils (Van Noordwijk et al., 1985). Sampling schemes acknowledging different positions in row crops, especially in the top layers and in young plants, may help to reduce sampling error (Van Noordwijk et al., 1985). At the start of the Dutch Programme on Soil Ecology of Arable Farming Systems, similar sampling schemes were devised for the various types of soil organisms, in the expectation that at least some organisms might be

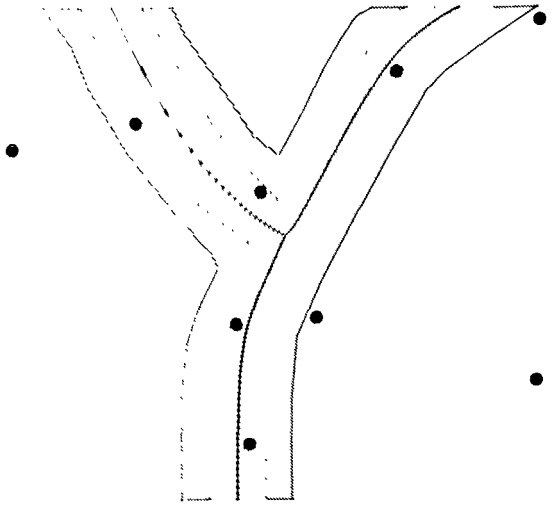

Fig. 1. Tests of spatial correlation of roots (●) and cracks (lines or irregularly shaped area) can be based on a classification of the map area by distance to the cracks and measurement of both surface area and number of points for each distance class.

spatially correlated with roots (Brussaard et al., 1990). Bacteria and protozoa appeared to be normally distributed in the soil with a coefficient of variation per composite sample of ten 100 cm^3 subsamples of ~10% (Bloem et al. 1992a; Zwart, unpublished). We expected that at a smaller scale, densities of soil organisms might correlate with concentrations of recent organic inputs. Moreover, the distribution of patches of organic inputs might be related to the tillage system (Staricka et al., 1991) rather than being randomly distributed in the plough layer. If locally high densities were to occur in macroscopically identifiable patches of organic matter, sampling schemes should account for this type of spatial variation.

We tested whether biological activity differed between macroscopically identifiable subsamples of top soil, representing (i) the bulk soil, (ii) recognizable crop residues and (iii) a recently incorporated, organic amendment. Spatial correlations of cracks, organic matter and roots were tested on the basis of maps made of a transect of the plough layer.

METHODS

The test site is located at the Lovinkhoeve Experimental Farm in the Noordoostpolder (Marknesse, The Netherlands). The soil is a calcareous silt loam of pH (KCl) 7.5, and was reclaimed in 1942. Annual precipitation at the site is usually between 600 and 950 mm.

The results presented in this paper are from two management practices:

integrated and conventional. Integrated differs from the conventional practice in that (i) N-fertilizer application rates are reduced to 50–65% of those recommended in conventional practice (viz. from 130–285 to 65–170 kg per ha per year, depending on crop), (ii) pesticide application is reduced, as is (iii) depth of soil tillage (20–25 cm plough in conventional, 12–15 cm cultivator in integrated). Due to a different soil management in the past, the organic matter content of the upper 20 cm of the conventional plot is 2.1% while that of the integrated plot it is 2.7%.

Since 1985, the four-year crop rotation on both plots has been: winter wheat, sugar beets, barley and potatoes. The data presented here are from the 1989 sugar beet crop. In the soil profile stubble and straw residues of the winter wheat, harvested in August 1988, could still be identified. Their original C/N ratio was ~120. In the autumn of 1988 an organic waste product of commercial mushroom production, "champost", had been applied to the integrated system, before soil tillage, at a rate of 30 t/ha. This product had a dry matter content of 35% and on a dry weight basis it had a C concentration of 28%, a total N concentration of 2.0% and thus a C/N ratio of 14.

The distribution of crop residues and organic matter accumulations ("champost" and others) relative to cracks and gaps was mapped on clear plastic (polythene sheets), from within a trench made perpendicular to the tractor orientation during soil tillage operations. In September 1989, root distribution in the mature sugar beet crop was mapped on polythene sheets; cracks and organic matter accumulations were mapped as well.

Maps were digitized and root density was measured as a function of distance to cracks (Van Noordwijk et al., 1993), using a Quantimet 570 image analyzer. As a statistical test of spatial correlation, a model was fitted of the form:

$$N_D = A_D[c + \exp(b_0 + b_1 \times D)]$$

where N_D is number of roots, A_D is sample area, both as a function of distance, D, the coefficient c represents the average point density not influenced by distance to the crack, b_0 determines the point density at distance 0, and b_1 the rate of change with distance. The best fit of this model, assuming that point densities have a Poisson distribution, was derived using a Genstat 5 (Payne et al., 1987). If b_1 differs from 0 in a two-sided test, we may conclude that roots and cracks are spatially correlated. A negative value for b_1 indicates a positive correlation (synlocation), a positive value indicates a negative correlation (avoidance).

On three sampling dates (April 14 , May 30, June 8, 1989) small samples were carefully taken from patches which visually contained either high concentrations of (i) fine organic material (on the integrated plot this was mostly "champost") or (ii) high concentrations of plant residues (recognizable plant structures, mostly wheat stubble and straw). As a control, samples were taken

from the surrounding soil. In the laboratory, the soil samples were analyzed for numbers of bacteria and protozoa. Bacterial numbers were determined by epifluorescence microscopic counts in soil smears after staining with Europium chelate, as described by Bloem et al. (1992b). Protozoa were determined using the Most Probable Number technique (Darbyshire et al., 1974). An index for bacterial activity was obtained by counting the frequency of dividing–divided cells [FDDC; for method see Davis and Sieburth (1984) and Bloem et al. (1992a, b)].

RESULTS

Root distribution profile maps

Figure 2 shows a map of a profile wall, sampled in six adjacent "windows" in May. In the pattern of major cracks the effect can be recognized of ploughing, with a plough blade of about 40 cm. Organic matter patches appear to be associated with these cracks, but their number is too low for a statistical test. Figure 3 gives part of a root map for the plough layer in September on the same field. Figure 4 shows root densities and occurrence of patches of recent organic matter as a function of distance to the nearest crack for the map of Fig. 3. For the roots, an acceptable fit was obtained with the model:

$$N_D = A_D[0.00058 + \exp(-6.6 - 0.10D)]$$

where the three parameters are all significantly different from 0. Distance D is measured in cm and roots were represented as a single pixel on a map where one pixel is about 0.5 mm in reality. For the organic matter patches a similar, or slightly stronger, decrease of point density with distance to the crack was found as for the roots, but the statistical model is not applicable here.

Biological activity

On the sampling dates in May and June the crop residue samples had a C content of about 28%, indicating considerable admixture of soil particles (due to internal slaking of the soil?). The C/N ratio of the retrieved residues was about 30:1, which was considerably less than that of the material worked under in Autumn 1988.

Analysis of variance showed no significant interaction between the factors place and time, so only main effects are discussed here. The factor place had a significant effect ($p < 0.01$) on the densities of protozoa and bacteria. In the stubble patches, densities of bacteria and protozoa (number per g dry soil) were higher than in the control soil (for bacteria a factor of 2; for protozoa a factor of 5; Table 1). In patches with high concentrations of organic matter, the number of protozoa was significantly higher than in the control soil (a factor of 4), whereas the numbers of bacteria were not significantly

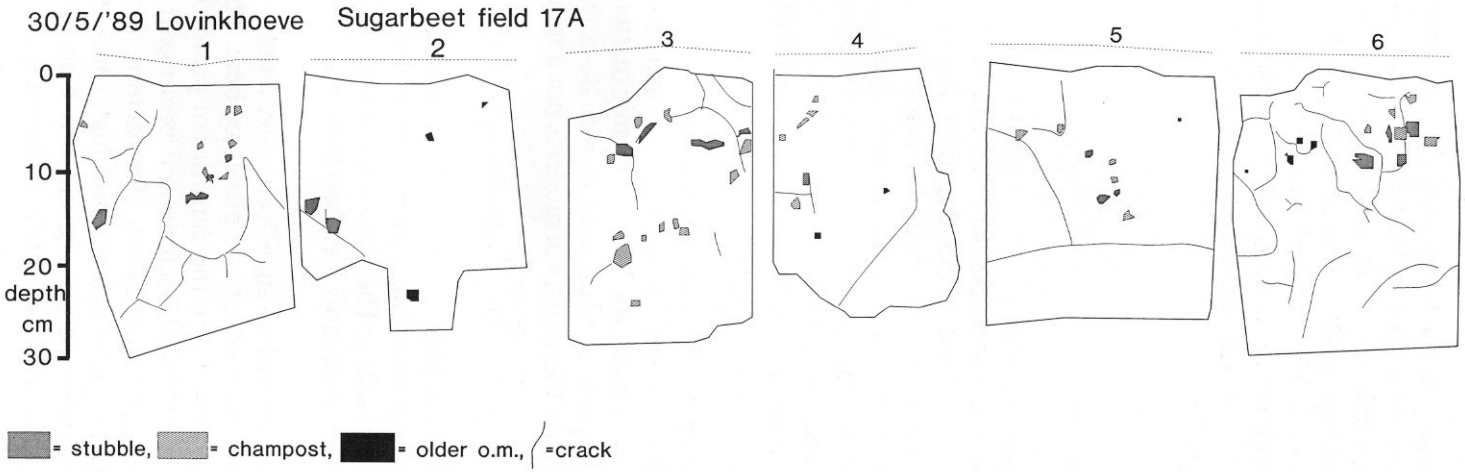

Fig. 2. Map of cracks (lines) and patches of recent organic inputs, macroscopically identifiable crop residue and older organic matter patches on vertical profile walls of the plough layer in a sugar beet field, May 30, 1989; the maps were drawn 1:1 scale on transparent plastic sheets.

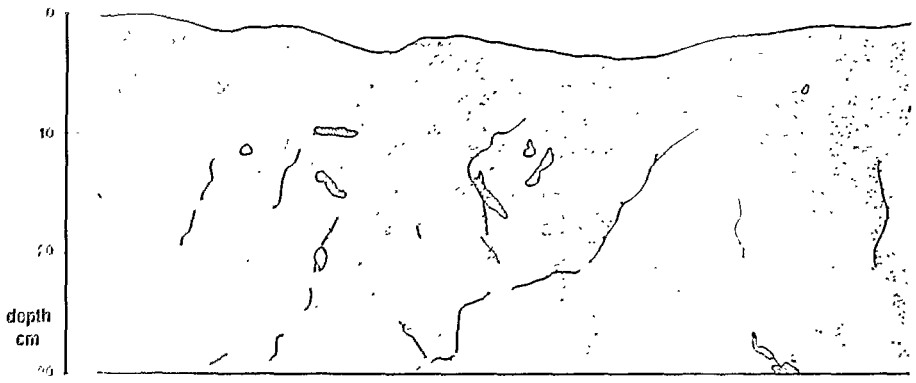

Fig. 3. Map of cracks (lines), roots (dots) and patches of recent organic inputs in the same sugar beet field as Fig. 2, on September 5, 1989.

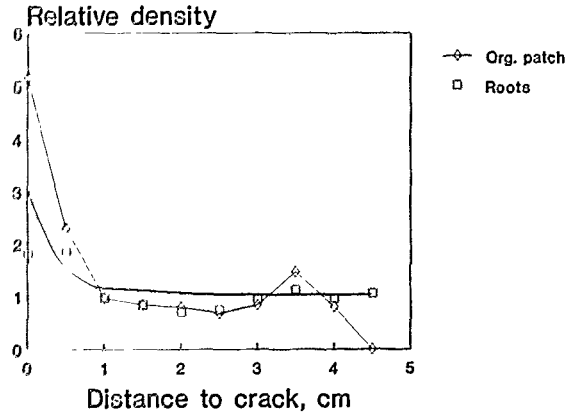

Fig. 4. Root density and occurrence of patches of recent organic inputs as a function of distance to large cracks in the profile, based on the map of Fig. 3; average point density was used to scale the results.

different. Relative growth rates of bacteria, expressed as the frequency of dividing cells (FDDC), did not differ significantly between the different types of soil samples. Bacterial density was significantly higher in May than in April and June.

Decomposition rates of organic matter (i.e. feeding rates of bacteria in μg C day^{-1} g^{-1} soil) in the different types of soil samples were calculated assuming steady-state conditions (Hunt et al., 1987), i.e., feeding rates were assumed to balance energy losses due to natural death and predation. The feeding rate of bacteria (F_{bact}) becomes:

$$F_{bact} = \frac{D_{bact} B_{bact} + P_{bact}}{e_{bact}} \quad (1)$$

where D_{bact} is the specific natural death rate (day^{-1}), B_{bact} is the bacterial biomass ($\mu g\ C\ g^{-1}$ soil), P_{bact} is the death rate due to predation, e.g., by protozoa ($\mu g\ C\ day^{-1}\ g^{-1}$ soil), and e_{bact} is the production:consumption ratio of bacteria.

The predation rate by protozoa can be calculated:

$$P_{bact} = F_{prot} = \frac{D_{bact} B_{bact}}{e_{prot}} \quad (2)$$

Taking a specific death rate of protozoa (D_{prot}) of 0.02 day^{-1} (Hunt et al., 1987), and a production efficiency (e_{prot}) of 0.4 (Zwart and Darbyshire, 1992), the feeding rate of protozoa becomes 0.685, 0.515 and 0.125 μg C $day^{-1}\ g^{-1}$ in soil organic matter patches, in plant residue patches, and in control soil, respectively. Taking a natural death rate of bacteria (D_{bact}) of 0.002 day^{-1} and a production efficiency (e_{bact}) of 0.30 (for references see De Ruiter et al., in prep), the feeding rates of bacteria become 2.5, 1.8 and 0.5 μg C $day^{-1}\ g^{-1}$ soil in organic matter patches, in plant residues patches, and control soil, respectively. This indicates that, as compared to the control bulk soil, the potential decomposition rate in organic matter patches is five times higher, and in patches with plant residues it is three times higher. Following this method of calculation, the specific growth rates of bacteria [nominator eq. (1) per bacterial biomass] will amount to 0.08, 0.10 and 0.03 day^{-1} for organic matter patches, plant residues patches, and control soil, respectively. These differences in specific growth rates are, however, not confirmed by the FDDC values.

TABLE 1

Bacterial and protozoan biomass and frequency of dividing–divided cells (FDDC) in patches with high concentrations of organic matter (o.m.), high concentration of crop residues (stubble) and control soil

	Place				Time			
	p	O.M.	Stubble	Control	*p*	April	May	June
Bacterial								
No. ($\times 10^9$/g soil)	0.002	1.04[a]	0.55[b]	0.49[b]	<0.001	0.56[b]	1.09[a]	0.44[b]
Biomass (μg C/g soil)	0.002	33.4[a]	17.6[b]	15.7[b]	<0.001	18.0[b]	34.8[a]	13.9[b]
FDDC (%)	0.82	7.8	7.2	8.4	0.01	6.6[ab]	11.8[a]	4.9[b]
Protozoan								
No. ($\times 10^6$/g soil)	<0.001	0.083[a]	0.063[a]	0.017[b]	0.06	0.097	0.040	0.027
Biomass (μg C/g soil)	<0.001	13.4[a]	10.27[a]	2.48[b]	0.06	15.0	6.86	4.34

Mean values and *p*-values are given for the factors place and time (sampling date), as no significant interactions between these factors were found; analysis of variance was based on the logarithm of densities. Any difference between values followed by the same letter is not statistically significant.

DISCUSSION

The differences in biomass of bacteria and protozoa between the different types of soil samples indicate strongly that biological processes occur at different rates in the different patches. However, the estimated decomposition rates, based on the biomass data, were not consistent with the FDDC values. The FDDC is an index of the specific growth rate (μ). Thus, although the specific growth rate seems to be similar in control soil and in organic matter, in the latter the cell production ($\mu \times$numbers) will be larger due to the higher cell numbers.

The fact that in patches with high concentrations of organic matter, the biomasses of bacteria and protozoa per unit sample weight were relatively high, indicated that the availability of food is an important environmental factor regulating their densities, especially those of the protozoa. Maps of the soil profile indicated that the patches of recently applied organic matter and plant residue were few in number and small in size. The biomasses of bacteria and protozoa were relatively high in these patches, but only by factors of ~ 2 and 4 for bacteria and protozoa, respectively. Kanazawa and Filip (1986) reported much greater differences (by factors of 10 to 100) in bacterial numbers between organic fractions and mineral soil particles fractionated from an arable brown soil by wet sieving. They used dilution plate counts and found 10^7–10^9 bacteria g^{-1} dry wt. in organic particles, about 10^6 bacteria g^{-1} dry wt in mineral particles of sizes between 0.05 and 0.5 mm, and about 10^7 bacteria g^{-1} dry wt. in the silt–clay fraction <0.050 mm. The greater differences in their study, compared to our present data, may have been caused by the use of dilution plate counts for enumeration of bacteria, whereas we used direct microscopic counts. Because the results of plate counts depend on the growth medium used, they are selective and do not represent total bacterial biomass (Gray, 1990). Nevertheless, differences in plate counts between organic and mineral soil particles may indicate qualitative differences between bacterial populations which are not revealed by microscopic counts of total numbers. Another reason for the smaller differences in bacterial numbers between bulk soil and organic particles in our study may be the soil type. Kanazawa and Filip (1986) found about ten times more bacteria in the silt clay fraction $<$ 0.050 mm than in the coarser fractions of mineral particles. Since in our silty loam soil more than 70% (v/v) of the soil particles are smaller than 0.050 mm (Kooistra et al., 1989) smaller differences in bacterial numbers between bulk soil and organic particles may be expected than in sandy soils.

It must be noted that the two- and four-fold higher numbers of bacteria and protozoa per gram of organic matter may be caused by a lower bulk density, and thus a larger volume per gram, compared to control soil. The difference in organism density may well have disappeared if sample volume rather than

sample weight had been used as the basis of comparison. No reliable estimates of bulk densities exist for the sampled patches to allow expression of organism densities in this way. As sample weight is the normal basis of reference, the results obtained suggest that for protozoa some improvement of sampling efficiency might be obtained from analyzing the stratum, "recent organic inputs", separately from the bulk soil. In a small part of the soil (roughly 2–5% of the soil volume) biological activity is about five times higher than in the "background" soil. This might be a basis for using a "stratified" sampling scheme, but a quantitative analysis of the expected gains of applying such a scheme is desirable. Cochran (1963) gave three situations when stratified sampling will produce large gains in precision: (1) strata differ in frequency; (2) strata differ in mean value; and (3) a good estimate of stratum frequency is available. The possible advantage of stratified sampling consists of a more efficient use of a limited sampling capacity (smaller variance for the estimated mean), especially if the strata differ in internal variability. For unlimited sampling intensity no advantage will be obtained; in fact, stratified sampling carries the danger of introducing a bias in the results if an unreliable estimate of the weights of the various strata is used. If no previous knowledge on strata frequencies (in our case: the volume frequency of "hot spots" in the soil) is available, stratification is only useful if a simple and reliable estimation procedure is available. It may be questioned whether the present method of mapping "hot spots" on a profile wall meets this criterion. The option of pooling (a large number of) subsamples before analysis, which does not exist in a sociological survey, and a situation where analyzing rather than taking samples is a rate limiting step, is more attractive than stratification if we want an efficient (in terms of reliability per unit effort) estimate of the mean. For a better understanding of the processes of decomposition, however, stratification may be valuable.

The differences in flow rates, assuming steady-state conditions, were of the same order of magnitude as the differences in protozoan densities. The small area fraction suggests that the contribution to the C- and N-flows of these macroscopically identifiable patches of recent organic inputs is relatively small, or even negligible, compared to the overall C- and N-flow in the soil. This conclusion is based only on the sampling period in spring, half a year after addition of these organic inputs to the soil, but may be valid in other periods of the year too.

A significant spatial correlation between roots and cracks was found in the plough layer, although root density only doubled for zones close to the cracks. Spatial correlation between recent organic inputs and cracks (mainly tillage induced) was indicated, although for a statistical test larger sample areas would be needed, due to the low densities. By combining these effects, a certain degree of synlocation of roots and sites of increased N-mineralization is indi-

cated, but its effect on plant nutrition and on N-use efficiency is probably small.

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