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Seasonal fluctuations in soil microbial biomass carbon, phosphorus, and activity in no-till and reduced-chemical-input maize agroecosystems

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Summary. The soil microbial biomass contains important labile pools of C, N, P, and S, and fluctuations in its size and activity can significantly influence crop productivity. In cropping systems where fertilizer use is reduced or eliminated and green-manure legumes are used, nutrient availability is more directly linked to C-cycle dynamics. We observed the fluctuations in microbial biomass C and P, and in microbial biomass activity over three cropping seasons in continuous maize and 2-year maize–wheat–soybean rotation agroecosystems under no-till and reduced-chemical-input management. We estimated the concentrations of microbial C and P using fumigation–incubation and fumigation–extraction techniques for the surface 20 cm of Cecil and Appling series soils (clayey, kaolinitic, thermic, Typic Kanhapludults). There were significant seasonal fluctuations in microbial C and P under all cropping systems. Generally, the magnitude of fluxes and the quantity of microbial C and P tended to be higher in reduced-chemical-input systems due to tillage and incorporation of crop, weed, and legume residues. Over 3 years, the means for microbial C were 435 under reduced-input maize; 289 under no-till maize; 374 und the reduced-input crop rotation; and 288 mg kg^{-1} soil under the no-till rotation. The means for microbial P were 5.2 under reduced-input maize; 3.5 under no-till maize; 5.0 under the reduced-input rotation; and 3.5 mg kg^{-1} soil under the no-till rotation. Estimates of microbial activity, derived from CO_2-C evolution and specific respiratory activity (mg CO_2-C per mg biomass C), suggest that reduced-chemical-input management may cause a larger fraction of the biomass to be relatively "inactive" but may also increase the activity of the remaining fraction over that in no-till. Thus in these specific systems, the turnover of C and P through the microbial biomass with a reduced chemical input to the soil may be higher than under a no-till system.

Key words: Microbial biomass – No-till agrosystem – Maize – Fumigation – Extraction method

The cycling of nutrients in soils of agricultural ecosystems is, to varying degrees, dependent on the energy supply to and through the soil biota. The soil microbial biomass is an important labile pool of C, N, P, and S, and fluctuations in its size and activity can significantly influence crop productivity (Rosswall and Paustian 1984; McGill et al. 1986). Coleman et al. (1983) observed that the microbial biomass, while comprising a relatively small pool of N, P, and S, may cycle these nutrients perhaps 8–10 times per year. The microbial biomass may typically comprise 1%–3% of the total C and 0.5%–24% of total P in mineral soils (Brookes et al. 1982, 1984). Recently the increase in extratable C, N, P and S following chloroform fumigation of soil has been used to estimate microbial C (Ladd and Amato 1988; Sparling and West 1988; Ross 1990), N (Brookes et al. 1985; Amato and Ladd 1988), P (Brookes et al. 1982, 1984), and S (Strick and Nakas 1984).

Fresh organic material added to soil supplies energy, which stimulates the microbial biomass. In annual agroecosystems, above-ground crop residues generally provide a single pulse of energy, while the below-ground components (roots and root exudates) contribute variable, yet continuous additions to the soil. The response of the microbial biomass to variations in the concentration and type of added C has been shown to be a key indicator of short- and long-term changes in soil biological properties (Doran 1980; Carter and Renni 1984; Carter 1986). Various studies have established that biomass C increases in the surface of no-till soils compared with tilled soils, and in soils under crop rotation management compared with continuous monoculture (Doran 1980; McGill et al. 1986; Granastein et al. 1987).

Unfortunately, the conclusions drawn from most of these studies have been based on a single season or specific sampling dates, and few data describe the flux in the

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size and activity of the microbial biomass with time and management. Nannipieri (1984) suggested that we cannot reasonably determine biomass and activity at any one time, and that at least a full year of data is required to average the effect of environmental conditions in the field. Ross (1987, 1988, 1990) has studied seasonal fluctuations and noted significant relationships between soil temperature and moisture and the size of the microbial biomass.

In cropping systems where fertilizer use is reduced or eliminated and green manure legumes are used, nutrient availability is more directly linked to C cycle dynamics. The size and activity of the microbial biomass become critical determinants of crop productivity. Any management system which increases the total crop C accumulation per unit area per year should increase the size and activity of the microbial biomass. Bolton et al. (1985) observed a larger biomass and greater enzyme activities in soils with winter legume crops in comparison with a conventionally managed winter fallow system in the Pacific Northwest. In a study in the Piedmont region of North Carolina, Gauger (1987) determined that microbial C was greater in a legume–maize system than in a fallow–maize system following legume incorporation in the spring.

Differences in cropping systems, tillage practices, soil types, climate, as well as the assay methodology make it difficult to compare results from field studies. Lynch and Panting (1980a) assayed intact soil cores in a humid temperate climate and observed that seasonal fluctuations in the size of the microbial biomass corresponded to the development and activity of wheat (*Triticum aestivum* L.) root systems and additions of straw after a grain harvest. Conversely, Granastein et al. (1987) used sieved samples in a Mediterranean climate, and observed little change or difference in the microbial biomass under different cereal–legume rotations until late season rains or additions of fresh crop residues.

Cole et al. (1978) observed, in laboratory microcosms, that bacteria quickly assimilated and retained much of the labile soil inorganic P as C substrates were metabolized. Chauhan et al. (1979) observed that microbial P was affected only slightly by the addition of organic residues and/or fertilizer P in a Mollisol high in available P. Conversely, Chauhan et al. (1981) found a rapid rate of microbial P uptake from the labile inorganic P fraction following C additions and incubation. They observed that the addition of P to two different soils lowered microbial C:P ratios, while additions of cellulose-C without P increased C:P ratios. Where C was added with P, the labile inorganic P pool increased significantly.

We are not aware of any published field studies involving sequential field sampling and assays of microbial C and P in maize (*Zea mays* L.) agroecosystems of any temperate region. The primary objective of the present study was to describe the fluxes in size and acitivity of microbial biomass C and P in no-till and reduced-chemical-input maize agroecosystems in the Piedmont region of North Carolina. We were most interested in the influence of legume additions and rotation on microbial dynamics during the summer growing season, and also during the fall and early spring.

Materials and methods

Study site description

All soil samples were taken from a long-term crop rotation and fertility management experiment being conducted at the North Carolina State University Research Unit 9 at Raleigh. The plots are imposed on Cecil and Appling series soils (clayey, kaolinitic, thermic, Typic Kanhapludults), which are typical of the southeastern U.S. Piedmont. The site is rolling, with maximum slopes not exceeding 10%. Temperatures in the area range between a mean of 26 °C in July to 6 °C in January, while the mean annual precipitation is 1168 mm. Soil properties in the various plots of this study were quite variable due to significant past erosion. The ranges of selected physical and chemical properties were: sand ($500-800\ g\ kg^{-1}$), silt ($140-200\ g\ kg^{-1}$), clay ($60-320\ g\ kg^{-1}$), gravel ($50-370\ g\ kg^{-1}$), pH (5.5–6.2), total organic C ($7.3-13.7\ g\ kg^{-1}$), and Mehlich 3-extractable P ($55-328\ \mu g\ cm^{-3}$ soil).

We collected samples from four replications of each of four cropping treatments; continuous maize managed with no-till (N fertilization, pesticides) or with reduced chemical input [chisel plow and disk tillage, N supplied by crimson clover (*Trifolium incarnatum* L.), with cultivation for weed control]; and 2-year maize–wheat–soybean (*Glycine max*) rotation managed with no-till or with reduced chemical input. Details of annual field operations have been described elsewhere (Buchanan and King, in preparation).

Sampling

We sampled soils at varying intervals between May 1987 and July 1989. In 1989, we collected samples approximately every 10 days until mid-July. In each crop system replicate, we took 24 cores (30 mm diameter, 0- to 20-cm depth). The cores were pooled, coarsely sieved (<6 mm), mixed in the field immediately after a plot was sampled, and placed on ice in darkness until we returned to the laboratory. The samples were then picked clean of roots and extraneous organic residues prior to analysis. In most cases, the soils were "fresh", as no more than 3 h had passed before the assays were begun. Occasionally, soil samples had to be stored in darkness at 4 °C for up to 24 h.

Microbial C estimates

Microbial C was assayed by two methods over the three cropping seasons of the research period. Data for 1987 and for the winter of 1988 were developed from a fumigation–incubation method. Beginning in the spring of 1988, we developed and calibrated a fumigation–extraction method for estimating microbial C (Buchanan and King 1992). However, we continued to use the fumigation–incubation technique through 1989, both for continued calibration and verification and for measurements of CO_2-C evolution. In sonification experiments using laboratory cultured microorganisms, Sparling and West (1988) have found that most of the C extracted after fumigation is likely to be derived from cytoplasmic material. The fumigation–extraction procedure overcomes errors associated with soils that have had recent or large additions of organic matter.

Fumigation–incubation. We weighed triplicate soil samples to 25 g (fresh weight) in 100-ml glass beakers. These replicate samples (non-fumigated controls) were placed in vacuum desiccators lined with moist paper towels and stored in the dark for 24 h at 23 °C. A second triplicate set was weighed to 25 g and placed in vacuum desiccators lined with moist paper towels, with a beaker containing ~40 ml alcohol-free $CHCl_3$. The desiccators were evacuated until the $CHCl_3$ boiled, then sealed and held in the dark for 24 h. Following the fumigation period, the chloroform reservoir was removed and the residual $CHCl_3$ removed from soil by repeated evacuation of the desiccators.

The beakers were then placed in incubation jars with a small vial of distilled water and a beaker with 10 ml 1 *N* NaOH to serve as a CO_2 trap. If necessary, based on gravimetric moisture analysis, soils were adjusted to approximately 60% water-holding capacity before they were placed in the incubation jars. The jars were sealed and incubated in the

dark at 23 °C for 10 days. Following the incubation period, the CO_2 traps were removed and the $NaHCO_3$ formed by the alkali reaction with CO_2 was precipitated by the addition of 3 N $BaCl_2$. The remaining NaOH was titrated to the phenopthalein endpoint with a standardized 1 N HCl titrant.

Microbial C (M_c) was calculated from the relationship:

$$M_c = \frac{(CO_2\text{-C fumigated soil}) - (CO_2\text{-C non fumigated soil})}{0.41} \quad (1)$$

The k_c factor (0.41), determined by incubations at 22 °C by Anderson and Domsch (1978), was chosen over the value (0.45) determined at 25 °C by Jenkinson and Powlson (1976), since ambient temperatures in our laboratory were 23 °C.

Fumigation–extraction. We used the same field-moist samples and prepared them concurrently with those used for the incubation method. Triplicate sets of non-fumigated soils were weighed to 10 g in 50-ml polycarbonate centrifuge tubes. The labile organic C extracted immediately by shaking on a reciprocating platform with 25 ml 0.5 M K_2SO_4 for 30 min. Following the extraction, the suspensions were clarified by centrifugation. Soil was also subjected to a 24-h fumigation under vacuum with $CHCl_3$. Following removal of the fumigant, triplicate sets of 10-g samples were weighed into centrifuge tubes, then extracted and clarified as above. All extracts were filtered through a 0.45 µm membrane using a syringe filtration system, and if necessary, frozen until analysis for extractable organic C.

In 1988, organic C in the extracts was determined with a modified Mebius procedure (Nelson and Sommers 1982). A 5-ml aliquot of the filtered extract was digested for 30 min at 150 °C in the presence of 5 ml 1 N $K_2Cr_2O_7$ and 10 ml concentrated H_2SO_4. Following the digestion, the remaining oxidized Cr^{6+} was titrated with 0.25 N $Fe_2SO_4 \cdot 7H_2O$ to the phenanthroline end-point. In 1989, organic C in the extracts was determined with a Perkin Elmer Total Carbon Analyzer. A correction factor k_{EC}) for the conversion of the extractable organic C to microbial C was determined in another study (unpublished data). Microbial C was calculated from the relationship:

$$M_c = \frac{E_c \text{ fumigated} - E_c \text{ non fumigated}}{k_{EC}} \quad (2)$$

where M_c is microbial C, E_c is extractable organic C, and k_{EC} is 0.38.

Microbial P estimates

We weighed triplicate field-moist samples to 5 g in 50-ml polycarbonate centrifuge tubes. The non-fumigated samples were extracted immediately by shaking them on a reciprocating platform with 25 ml 0.5 M $NaHCO_3$ for 30 min. Following the extraction, the suspensions were clarified by centrifugation. Soil was also subjected to a 24-h fumigation under vacuum with $CHCl_3$. Following fumigant removal, triplicate sets of 5-g samples were weighed into centrifuge tubes, then extracted and clarified as above. All extracts were filtered through Whatman no. 41 paper, and if necessary, frozen until analysis for inorganic P. The inorganic P in extracts was determined with a molybdate–ascorbic acid procedure (Olsen and Sommers 1982). Microbial P was calculated as the difference between inorganic P in fumigated samples and inorganic P in non-fumigated samples. No correction factors were derived or assumed for this measure of microbial P.

Results and discussion

Microbial biomass C

As expected, estimates of microbial C fluctuated with time in all of the management treatment. Despite the differences in crops and management, flux patterns were similar over the period (Fig. 1). We generally observed that microbial C was significantly higher on most sampling days in our reduced-chemical-input systems, where winter legume growth and incorporation, along with intensive tillage, incorporated more plant C per unit or volume of soil. The overall means (mg kg^{-1} soil) were maize 435 and rotation 374 for the reduced-chemical-input system, and maize 289 and rotation 288 for the no-till system (coefficient of variation 56.9%). Crop management systems that increase the input of C by applying winter green manure crops, rotations, or additions of organic waste have been shown to increase microbial biomass and activity more than systems that rely on fertilizer inputs (Bolton et al. 1985; Granastein et al. 1987).

A significant spring peak occurred in all cropping systems each year, whether following maize planting (no-till), legume incorporation reduced chemical input, or at the anthesis stage for wheat (both systems). In all years continuous reduced-chemical-input maize management stimulated the growth of microbial biomass to a greater degree than any other system. As our sampling did not begin until the end of May in 1987, we probably missed the maximum peak in all cropping systems for that year. The spring peaks in 1989 (367–764 mg kg^{-1}) were much lower than those observed in 1988 (981–1300 mg kg^{-1}). We believe that some of this difference arises from reduced C accumulation by the legume crops before they

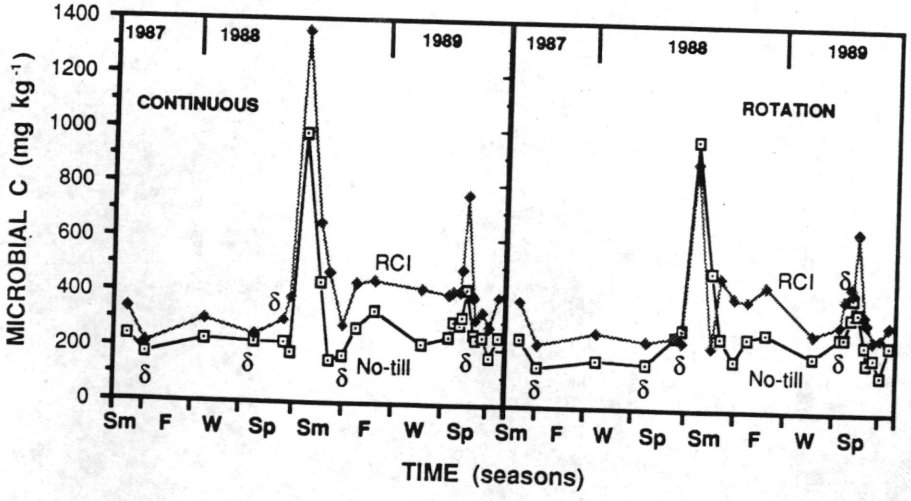

Fig. 1. Fluxes of microbial C with continuos maize and a maize–wheat–soybean rotation under no-till and reduced-chemical-input (*RCI*) management. δ, non-significant differences ($P > 0.05$). *Sp*, spring; *Sm*, summer; *F*, fall; *W*, winter

were incorporated into the soil in 1989 and some from the often saturated soil conditions which may have caused short-term anaerobiosis. A second significant peak occurred in the reduced-chemical-input rotation in late spring of 1988, following the wheat harvest and incorporation of the residue.

We believe that part of the observed increases in microbial C in the no-till and reduced-chemical-input systems during the spring and summer seasons reflected root growth and activity, and increasing soil temperatures. Lynch and Panting (1980a,b) concluded that sharp increases in biomass C are observed only in response to the input of C substrates from exudates, decomposing roots, and incorporated crop residues. In a single sample-date study, Srivastava and Singh (1969) observed a positive relationship between total plant biomass or root biomass and estimates of microbial C, N, and P. Microbial biomass formation is, in part, affected by the magnitude of root turnover (Merckx et al. 1985). Buyanovsky and Wagner (1987), using ^{14}C labelling, showed that "rhizodeposition" accounted for about 60% of the total C deposition in soil in a wheat ecosystem. This would explain the large increases in microbial C seen in the rotation systems (in wheat) in the late spring of 1988, and under soybeans at anthesis in October of 1988. We assume that that the large rooting density under the wheat crops increased the labile C supply via root death and exudates, thus causing larger increases in biomass C.

In July of 1987 and 1988 microbial C declined significantly. It is probable that these declines were related to reductions in the labile C pool and the hot/dry weather conditions that occurred during the summers of 1987 and 1988 (Figs. 2, 3). In a separate study (unpublished data), we observed that labile organic C, as measured by extraction with 0.5 M K_2SO_4, declined in the summer months. Other workers have noted declines in water-extractable C under summer conditions or following spring peaks in biomass C accumulation and activity (Cogle and Saffigna 1989; Sarathchandra et al. 1989). In the early fall, biomass C consistently increased with maize crops following the harvest in 1987 and 1988. In the reduced-chemical-input continuous-maize system this was likely due to fall tillage operations and the partial incorporation of above-ground residues. Both rotation systems also exhibited biomass C increases in the fall of 1988 during the pre- and anthesis stage for soybeans. These brief in-

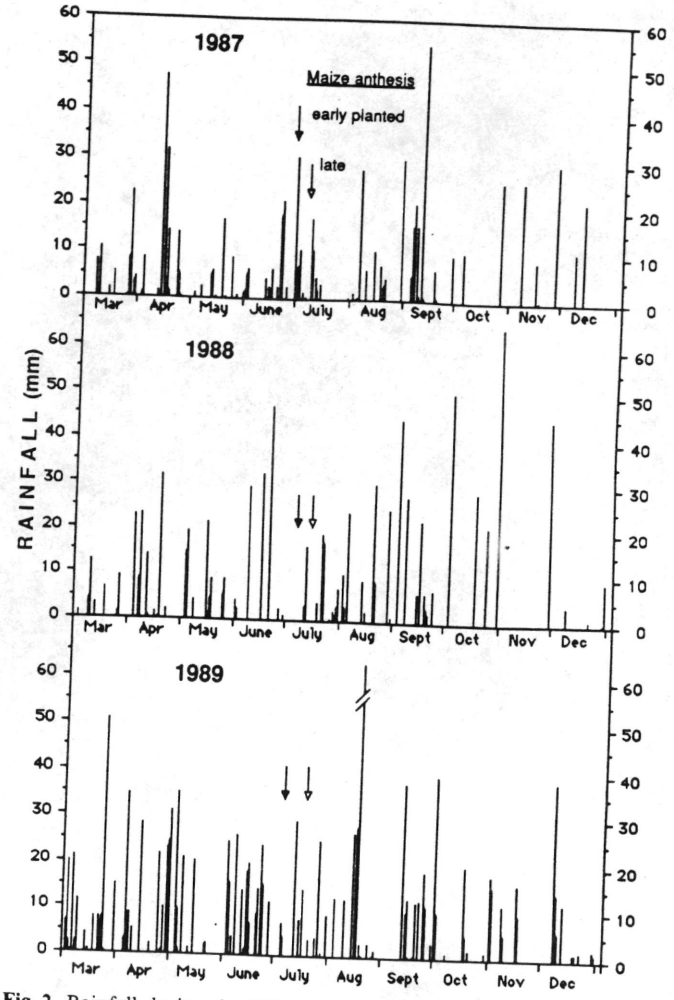

Fig. 2. Rainfall during the 1987–1989 cropping seasons

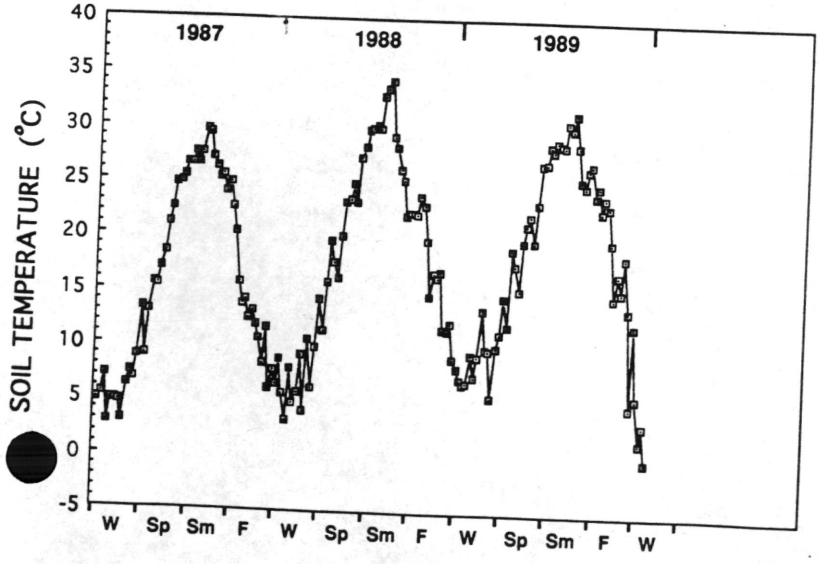

Fig. 3. Mean weekly soil surface temperatures for 1987–1989

creases, occurring in the fall after the maize harvest in 1987 and 1988, were also likely related to increased rainfall and more favorable soil temperatures. In 1987, long dry periods were combined with very high temperatures. In 1988, soil moisture was more adequate, but daily maximum temperatures were even high than in 1987. Midday surface soil temperatures as high as 42 °C were measured during 1988. In late August of each year, rainfall increased as temperatures declined. The increase in decomposition related to microbial activity and growth following rewetting of dry soils has long been recognized (Bottner 1985; Van Veen et al. 1985). A flush of weed growth occurred in response to these climatic changes.

Conversely, microbial C tended to decline and remain relatively constant in the winter and early spring in all systems. During these periods, microbial C was consistently higher in the reduced-chemical-input systems than in the no-till systems. Decreases in microbial C during the late fall and winter are most affected by low soil temperatures. However, they are also affected by the lower quantities of simple, labile C substrates and the presence of more complex, resistant substrates in the form of dead lignified roots and crop residues.

Microbial biomass P

Estimates of microbial P also fluctuated with time and management (Fig. 4). Overall, the estimates for microbial P also tended to be higher in the reduced-chemical-input systems. The overall means (mg kg^{-1})were maize 5.2 and rotation 5.0 for the reduced-chemical-input system, and maize 3.0 and rotation 3.5 for the no-till system (coefficient of variation 40.0%). We chose not to use a correction constant for the measurements of microbial P (Brookes et al. 1982; McLaughlin and Alston 1986), because of uncertainties in the universal validity of such correction factors. Thus our estimates of microbial P are likely quite low. For example, we calculated generally higher values for C:P ratios from our data (up to 395) compared to other studies (Chauhan et al. 1981; Brookes et al. 1984). It is more appropriate to define our measures as chloroform-labile P.

The apparent immobilization of P in the biomass was generally greatest in the spring months, and was followed by significant declines in late spring and summer. Generally, fluctuations in chloroform-labile P were greater with reduced-chemical-input management than with no-till management. In the no-till rotation chloroform-labile P tended to be relatively constant over time, perhaps due to the lack of soil disturbance during the study period. We observed that chloroform-labile P tended to decline or remain constant in soils under no-till management during the fall and winter months. In both reduced-chemical-input systems there were slight or dramatic increases in chloroform-labile P in the fall and winter months of 1987 and 1988. Overall, the reduced-chemical-input systems held higher and more variable quantities of P in the biomass during these periods.

Sarathchandra et al. (1989) estimated from laboratory incubations that about 25 kg P ha^{-1} might be released in late spring as microbial P declined in soils under pasture. Similar declines were observed in our field studies in the late spring or summer of each year. For example, the decline in chloroform-labile P in the reduced-chemical-input continuous of P (up to 29 kg P ha^{-1}) was released into soil during the main cropping period. If this were all available to plants it would represent slighthly more P than was taken up by any maize crop during the study period. Death and autolysis of the microbial biomass, or death and lysis due to microfaunal grazing will eventually release some microbial-derived P into the soil solution. It is possible that greater microbial activity, concurrent with increased microbial P and turnover, could lead to increased P uptake by plants. Lee et al. (1990) demonstrated that increased microbial activity (measured by CO_2-C evolution), following the addition of C to the soil, reduced the sorption of inorganic P, maintained inorganic P in solution and labile pools, and increased microbial P in a Piedmont region Ultisol.

In contrast to the patterns observed for microbial C, no consistent trends among the cropping systems were noted for chloroform-labile P. Only the reduced-chemical-input maize system exhibited similar patterns to the fluxes observed in microbial C, although a regression of

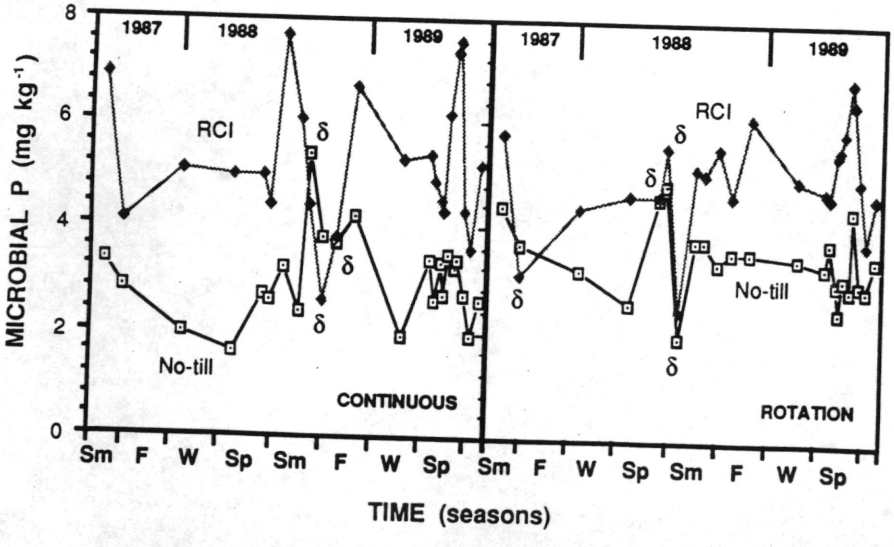

Fig. 4. Fluxes of microbial P (chloroform-labile P) with continuous maize and a maize–wheat–soybean rotation under no-till and reduced-chemical-input (*RCI*) management. For other explanations, see Fig

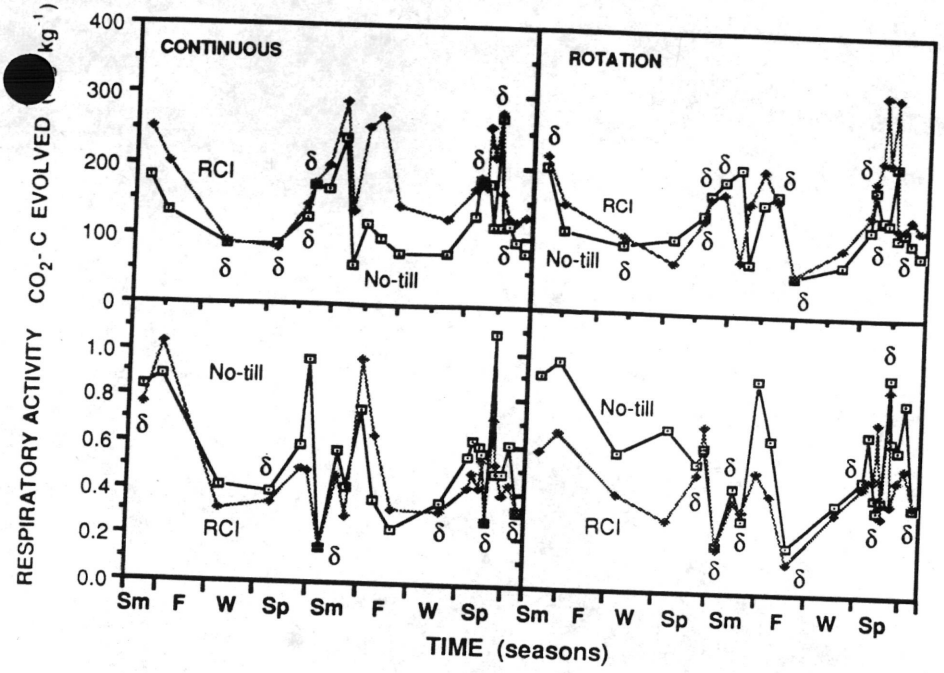

Fig. 5. Specific respiratory activity and CO_2 evolution from microbial biomass with continuous maize and a maize–wheat–soybean rotation under no-till and reduced-chemical-input (*RCI*) management. For other explanations, see Fig. 1

chloroform-labile P against C did not account for much of the variation ($R^2 = 0.09, P < 0.001$). Under simulated temperature regimes, Sarathchandra et al. (1989) also noted trends in microbial C and P that were similar to our field observations. Microbial C was higher under autumn to late spring conditions and declined during the winter, while microbial P increased during winter and early spring. Chauhan et al. (1981) also failed to observe a correlation between microbial C and P fluxes over a 9-month laboratory incubation.

Microbial activity

We calculated two measures of microbial activity from the data derived from microbial C estimates and CO_2-C evolved from non-fumigated soils during the 10-day incubation. Specific respiratory activity was calculated as mg CO_2-C evolved per mg biomass C during 10 days of incubation (Schnurer et al. 1985). In Fig. 5 we compare these two parameters for each cropping system with time. Microbial activity, as assayed by the CO_2-C evolved over 10 days at 23 °C, tended to be higher in the reduced-chemical-input systems. In 1987, a general mid-to-late season decline occurred in both measures of microbial activity in all cropping systems. In 1988, two peaks of activity were observed in all systems, irrespective of the cropping pattern. In 1989, with more intensive sampling, we noted a great deal of fluctuation in microbial activity during the moist and temperate early growing season.

Among all systems, a significant but poor relationship was found between the specific respiratory values and CO_2-C evolution ($R^2 = 0.22, P < 0.0001$). Similar analyses indicated a better, although still weak, relationship between these parameters in the reduced-chemical-input systems but not the no-till systems. Thus we are left with conflicting assessments of microbial activity in the different cropping systems. Conversely, Schnurer et al. (1985) found a good correlation between measures of specific respiratory activity and CO_2-C evolution.

Despite the lack of agreement between these measures a pattern is suggested by the often inverse relationship between CO_2-C and specific respiratory activity calculations. Bottner (1985) determined that soil drying destroyed 1/4 to 1/3 of the microbial biomass, and that after each rewetting, the biomass progressively returned to approximately the original size. In the absence of additional C-substrate inputs, the regenerating biomass tended to be less active with each drying-wetting cycle. These data tend to suggest that an increasing fraction of the biomass is becoming "inactive", while a decreasing fraction is remaining in an "active" state. It is possible to interpret the discrepancies in our measured CO_2-C evolution and specific respiration, in the light of these data. On a number of sampling days, it seems that a larger fraction of the biomass in reduced-chemical-input systems was "inactive", shown by a significantly lower level of specific respiratory activity. However, the "active" proportion of the biomass remaining in these systems may have been more metabolically active than the larger portion in the no-till systems.

Conclusions

We assume that tillage and the increased amount of C added to the soil by incorporating the winter legume and weeds had a significant effect on microbial dynamics in the reduced-chemical-input systems through the main cropping periods. Tillage can affect the supply and availability of C substrates to the microbial biomass through residue incorporation and comminution, root pruning, and soil aggregate disruption. In our reduced-chemical-input systems tillage probably had a great influence on the fluxes and magnitude of microbial P (measured as

chloroform-labile P). However, few workers have considered the influence of sequential tillage operations (e.g., weed control) on microbial C and P dynamics. Tillage effects on the short-term dynamics of the microbial biomass require further study. We believe that there is a significant potential for improved nutrient management and efficiency of use through a more comprehensive knowledge of microbial biomass dynamics. The efficiency of nutrient use could be optimized by manipulating the microbial immobilization of nutrients during non-cropping cycles, and death, release, and/or mineralization processes during a cropping period.

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