Microbial Dynamics in Maize-Growing Soil under Different Tillage and Residue Management

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Abstract

Microorganisms are involved in the fertility-related processes of agricultural fields. The long-term impact of tillage and residue management on soil microorganisms was studied over the growing season, in a sandy loam to loamy sand soil of southwestern Quebec. Tillage and residue treatments had been first imposed in fall 1991, on a maize (*Zea mays* L.) monoculture. Treatments consisted of no till, reduced tillage, and conventional tillage with crop residues either removed from (-R) or retained on (+R) experimental plots, laid out in a randomized complete block design. Soil microbial biomass carbon (SMB-C), soil microbial nitrogen (SMB-N) and phospholipid fatty acid (PLFA) concentrations were measured four times over the 2001 growing season i.e., in May 7 (preplanting), June 25, July 16, and September 29 (prior to corn harvest). The effect of time was larger than those of tillage or residue treatments. While SMB-C showed little seasonal change (160 µg C g^{-1} soil), SMB-N was responsive to post emergence mineral nitrogen fertilization, and PLFA analysis showed an increase in fungi and total PLFA throughout the season. The effect of residue was more pronounced than that of tillage, with increased SMB-C and SMB-N (61% and 96%) in +R plots compared to –R plots. This study illustrated that measuring soil quality based on soil microbial components must take into account seasonal changes in soil physical and chemical conditions.

Introduction

The importance of soil microorganisms in natural and agricultural ecosystems is widely recognized. Microorganisms mineralize organic matter allowing the recycling of nutrients in plants, nitrify and denitrify, mechanisms through which soil mineral nitrogen is lost to the atmosphere, and transforme micronutrients such as Fe or Mn, which alters in this way their availability in soil. In fact, most soil processes are microbially driven (Paul and Clark, 1989). Furthermore, some microorganisms such as mycorrhizal fungi and several nitrogen fixing bacteria improve plant productivity through symbiotic associations formed between them and their host plants.

Agricultural soils are managed under cropping systems that disturb the habitat within which soil microbial processes take place. There is no doubt that cropping practices influence microbial dynamics in soil and, hence, soil processes. For example, conventional soil tillage inverts the sod up to 20 cm and buries crop residues, a major source of microbial food in agricultural soils.

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Fertilization stimulates plant development, modifies microbial interactions (Sundareshwar et al., 2003), and modifies plant-microsymbiont relationships in N_2-fixing legumes (Alexander, 1977) as well as in mycorrhizal crops (Smith and Read, 1997). All these impacts of cropping on soil microbial communities take place within the framework of seasonal fluctuations, which are induced by plant development and climatic variables in particular, and which most likely interact with the effect of cropping practices.

While one foresees impacts of cropping systems on soil microbial communities, no one knows much about the nature and relative magnitude of these impacts. The objective of this study, therefore, was to find out how residue management and tillage intensity influence soil microbial dynamics under corn monoculture, in eastern Canada, along the growing season. The working hypothesis subtending this work was that tillage intensity and crop residue addition modify the soil microbial community under a corn crop. Impacts on the soil microbial community were assessed during the growing season through the determination of (a) SMB-C and -N, a measure of microbial community size and quality, and (b) soil extracted phospholipid fatty acid (PLFA) profile analysis, to assess the relative proportion of fungal to bacterial components in this SMB, as well as microbial diversity.

Materials and Methods

Long term plots located at the E. Lods Agronomic Research Station of McGill University, Montreal Canada (45° 30' N, 73 ^{o} 35' W), were used for this study. Mean annual temperature is 7.4 ^{o}C and, on average, there is 1062 mm of precipitation per year at this site, 529 mm of which falls during the growing season (May to October). The experiment had a factorial design with two levels of residue (corn stalk left [+R] or removed [-R] after harvest) and three levels of tillage (conventional [CT], fall plowing to 20 cm and spring offset disking to 10 cm; reduced tillate [RT], fall and spring disking to 10 cm; and no tillage [NT]) arranged in a complete randomized block fashion within three blocks. Treatments combinations had been applied year after year on the same 80 m x 18.5 m plots, for 10 years prior to this study. The site consists of 2.4 ha of St-Amable sand to loamy sand with areas of Courval sandy loam. Thirty six kg N ha^{-1} and 9.2 kg P_2O_5 ha^{-1} applied in a band as monoammonium phosphate and ammonium nitrate at seeding, and of 140 kg N ha^{-1} as ammonium nitrate and 69 kg K_2O ha^{-1} as muriate of potash applied at the 6-leaf stage of corn, on June 16. Corn was seeded May 8 and 9, 2001.

Soil was sampled four times during the season: (1) pre-planting, May 7, (2) where mycorrhizal effect are normally seen, six weeks after planting, June 25, (3) during the hot and dry days of summer, July 16, and (4) at crop maturity, September 29. At each date, ten 2-cm diameter cores were taken in each plot to the plowing depth i.e., 20 cm, and separated into halves to produce 0-10 cm and 10-20 cm segments. Cores were immediately put on ice. In the laboratory, a subsample of each composite sample was taken, stored at -20 ^{o}C, and used for lipid extraction at a later date. The rest of the samples were placed at 4°C.

SMB-C and SMB-N were extracted after chloroform fumigation (Voroney et al., 1993) using a soil to extractant ratio of 3:1, within three days from sampling. Organic C in the extracts was measured with a Shimadzu Total Organic Carbon Analyzer and N was measured colorimetrically (Lachat Instruments, QuickChem method 10-107-04-1C) after persulphate oxidation (Williams et al., 1995). K_2SO_4 extractable C and N from non-fumigated samples were used as measures of soluble extractable C (Rochette and Gregorich, 1998) and dissolved organic C (Calderon et al., 2000). Soil moisture was determined gravimetrically after drying soil samples at 105 ^{o}C for one day. Soil

organic matter was determined by the dichromate redox method (Tiessen and Moir, 1993). Eight grams of each freeze-dried soil sample was extracted in an Automated Solvent Extractor (ASE 200, Dionex) using methanol and chloroform (2:1, vol:vol) at 80 $^{\circ}$C and 8.28 Mpa for 5 min., after which two 15-min static extraction cycles occurred. Lipid-class separation and fatty acid methyl ester creation were performed according to White and Ringelberg (1998). PLFAs were analyzed in split mode (50:1) with a gas chromatograph (Hewlett Packard 6890) equipped with a Simplicity Wax column (Supelco 2-4326) and flame ionization detector. Oven temperature was 60 $^{\circ}$C initially, then raised by 3 $^{\circ}$C per minute to a final temperature of 230°C which was held for 20 min. Inlet and detector were at 200 $^{\circ}$C and 250 $^{\circ}$C. C19:0 was used as an internal standard. Peak identification was based on comparison of retention times to known standards (Supelco 37 Component FAME Mix #47885-U and Supelco Bacterial Acid Methyl Esters #47080-U). The abundance of individual PLFAs was normalized to C16:0 (Drijber et al., 2000).

Analysis of variance (ANOVA) was used to detect significant differences between the main experimental effects of management (treatment) and sampling date. ANOVA was conducted on plot means by repeated measures in proc Mixed, SAS Version 8 (Statistical Analysis System Institute Inc., 2000). Analyses were on plot means within each depth category (0-10 cm, 10-20 cm, 0-20 cm). A 5% probability level was used for accepting or rejecting the Null hypotheses. Bonferroni's test was used for comparison of means with $P<0.05$ used for detecting significant differences. Residuals were tested for normality via Shapiro-Wilks' test and non-normal data transformed using log scale where required. Correlation and stepwise discriminant analysis (DA) were conducted using Statistica 5.1 (StatSoft, 1998).

Results and Discussion

The corn-growing soils under different tillage intensity and residue management systems under study were clearly dominated by the effect of time (Table 1). Tillage and residue treatment effects were only seen at the 0-10 cm soil depth. Time effect was also more frequent at this depth. At 0-10 cm depth, in fact, only soil extractable C and SMB-C were not significantly influenced by sampling date. Therefore, the scope of this presentation is limited to the 0-10 cm layer of soil.

Table 1: Significance of Long Term Effects of Tillage and Residue Treatments, and Sampling Period Effects at Two Soil Depths on Soil Moisture (S.Moist.), Extractable C (Ext C), Dissolved Organic N (DON), Soil Microbial Biomass C (SMB-C), Soil Microbial Biomass N (SMB-N), Total Phospholipid Fatty Acids (Total PLFA), Fungi to Bacteria PLFA ratio (Fungi:Bacteria), Fungal PLFA, and Shannon-Weaver Biodiversity Index (S-W Biodiv.).

Source of variation	0-10 cm								
	S.Moist.	Ext C	DON	SMB-C	SMB-N	Total PLFA	Fungi: Bacteria	Fungal PLFA	S-W Biodiv.
Tillage	ns	ns	ns	**	ns	ns	ns	ns	ns
Residue	**	ns	ns	**	**	ns	ns	ns	ns
Sampling Time	**	ns	**	ns	**	**	**	**	**
Block	**	ns	ns	ns	ns	**	ns	ns	**
Tillage x Residue	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage x Time	ns	ns	ns	ns	ns	ns	ns	ns	ns

Source of variation									
Residue x Time	ns	ns	ns	ns	ns	ns	ns	ns	ns
TillagexResiduexTime	ns	ns	ns	ns	ns	ns	ns	ns	ns

Source of variation	10-20 cm								
	S.Moist.	Ext C	DON	SMB-C	SMB-N	Total PLFA	Fungi: Bacteria	Fungal PLFA	S-W Biodiv.
Tillage	ns	ns	ns	ns	ns	ns	ns	ns	ns
Residue	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sampling Time	**	ns	**	**	ns	**	ns	**	ns
Block	**	ns	**	ns	ns	ns	ns	ns	ns
Tillage x Residue	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage x Time	ns	ns	**	ns	ns	ns	ns	ns	ns
Residue x Time	ns	ns	ns	ns	ns	ns	ns	ns	ns
TillagexResiduexTime	ns	ns	ns	ns	ns	ns	ns	ns	ns

**, statistically significant at $P<0.05$; ns, not statistically significant at $P<0.05$.

Tillage had a very limited effect on soil microbial properties (Table 1). CT plots had lower SMB-C than RT plots at 0-10 cm (Fig.1). SMB-C might have been lower under CT because, in this system, residues are incorporated to 20 cm in the fall. In contrast, residues are mixed within the 0-10 cm soil layer by disking under RT. Interestingly, there was no significant difference between NT and CT plots. It is commonly believed that tillage exposes microsites rich in substrates, a situation which can lead to a burst in microbial activity and growth. This enhancing effect of RT on SMB-C may compensate for the disruptive effect of tillage on fungal mycelium. Kabir et al. (1999) have shown that soil disturbance in spring has much less impact on fungal mycelium viability and mycorrhizae formation than soil disturbance in fall where the disruptive effect of tillage is compounded by several months of absence of plant roots. No other significant changes in properties measured in the 0-10 cm soil layer were induced by tillage treatments.
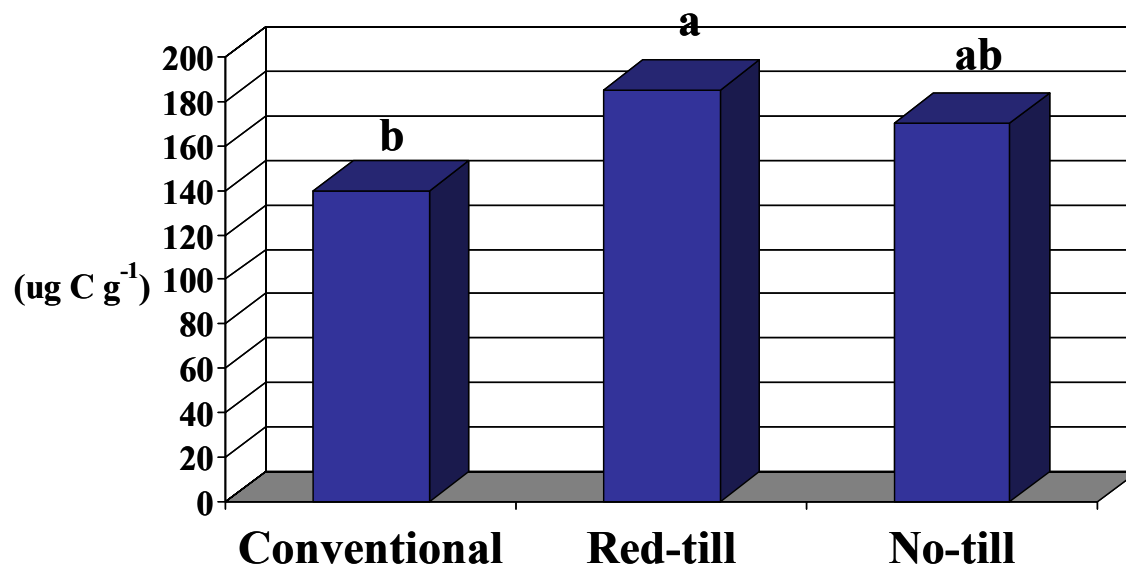
Figure 1. Long term effect of tillage on SMB-C in the 0-10 cm soil layer. Bars with the same letter are not significantly different at the 5% probability level, according to a Bonferroni's test.
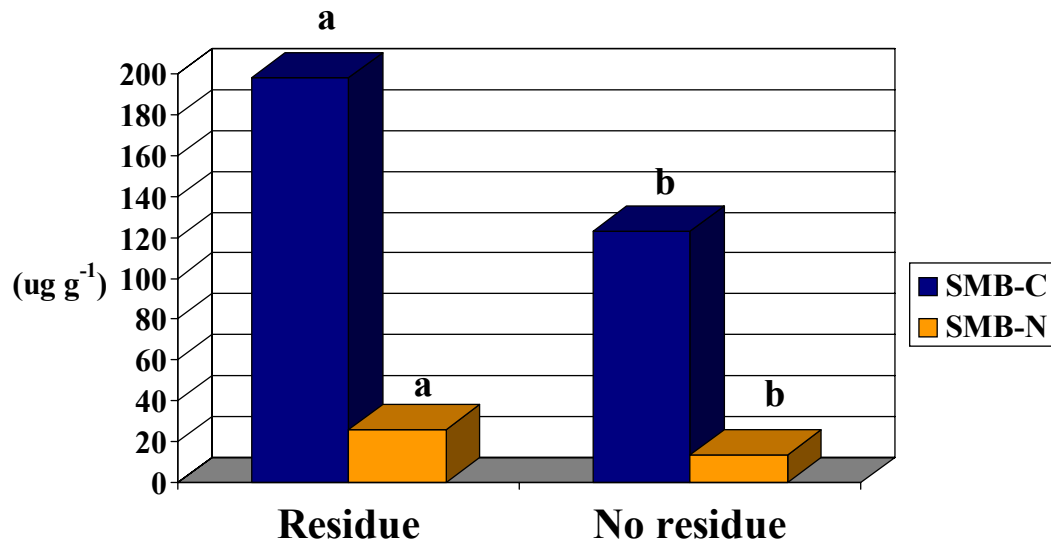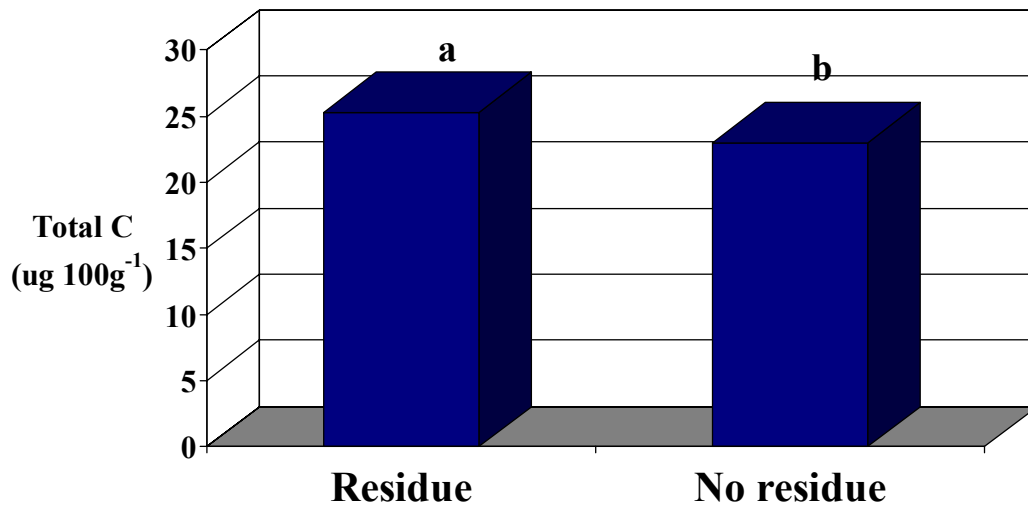

Figure 2. Long term effect of residue treatments on SMB-C and SMB-N in the 0-10 cm soil layer. Bars with the same letter are not significantly different at the 5% probability level, according to a Bonferroni's test.

Figure 3. Long term effect of residue treatments on soil organic matter content in the 0-10 cm soil layer. Bars with the same letter are not significantly different at the 5% probability level, according to a Bonferroni's test.

The 0-10 cm soil layer of +R plots contained significantly more SMB-C and SMB-N than that of no residue plots (Fig. 2). The 61% increase in SMB-C and 96% increase in SMB-N measured in residue amended plot soil is considerably higher than the 4% increase in SMB-C and the 30% measured in SMB-N measured when residues were added to Canadian prairie soils (Bremer and Van Kessel, 1992).

Previous research on this site has shown that 6 to 8.2 Mg ha^{-1} of crop residue remains within +R plots each year compared to 1.5 to 3 Mg ha^{-1} in –R plots (Mehdi et al., 1999). The addition of corn residues for 10 years at this site has had a positive effect on total carbon content in the soil (Fig. 3). Residues left on the soil surface also improve water infiltration by reducing runoff, maintaining cooler soil temperatures and reducing evaporation by providing soil cover that reduces sunlight intensity. Results show that, at 0-10 cm, leaving residues within the soil system increases soil moisture content (Table 1; data not shown). Increased soil organic matter and possibly higher soil moisture could both be involved in the positive effect of residue addition in this study. There was no evidence indicating that soil microorganisms were water-limited in this ecosystem, however.

The pattern of seasonal variation in soil moisture level did not match any of the seasonal variation patterns found for the microbial variables monitored in this study and this, in spite of the occurrence of an unusually dry mid-summer period. Different patterns of seasonal variation were found among measured microbial variables. While SMB-C did not vary significantly throughout the growing season (Fig. 4a), SMB-N was temporaly variable (Fig. 4b). Others have found similar seasonal trends in SMB-C (Rochette and Gregorich, 1998) and SMB-N (Joergensen et al., 1994). Changes in the microbial biomass pools coincide with changes in the availability of nutrients or in soil conditions. Seasonal shifts in SMB-C and SMB-N have been attributed to inputs of N (Ross et al., 1995) either from plant residues or fertilizer. In our case, application of 140 kg N ha^{-1} as ammonium nitrate on June 16th, lead to increased levels of dissolved organic nitrogen at the June 25th sampling and to increases in SMB-N in the July 16th samples. While SMB-N increased, SMB-C stayed the same suggesting the occurrence of a shift in soil microbial population in favor of species with elevated N content. We know that release of fertilizer N during fumigation may interfere with biomass N determinations (Petersen et al., 2002), but it has also been shown that the presence of nonhydrolyzed urea or insoluble ammonium in the soil a week after fertilization is very unlikely (Cabrera et al., 1991). PLFA analysis revealed an enrichment in fungi during the July drought. The C:N ratio of fungi is higher than that of bacteria and, hence, an enrichment of the microbial community with fungi does not explain the increase in SMB-N measured. It seems that the observed increase in SMB-N pool, then, could be attributed to a luxury consumption of N by soil organisms, to the shift of the microbial community in favour of high N-containing bacteria and fungi, or to a change in physiological status (Bardgett et al., 1999). Eiland et al. (2001) have found that some fungi present in composts with high amounts of added nitrogen had a high potential to degrade cellulose and hemicellulose. It seems that N fertilization could enhance C cycling through a shift in soil microbial populations.

The previous description of the dynamics of fungal abundance under maize through direct microscopic observation, at a nearby site (Kabir et al., 1997), supports our conclusion of an increase in fungi abundance in July (Fig. 5a). Fungal PLFA and soil moisture content (data not shown) were negatively correlated. Although fungi are generally more tolerant to water (Paul and Clark, 1989) and osmotic stress (Griffiths et al., 1999) than bacteria, the observed increase in relative fungal
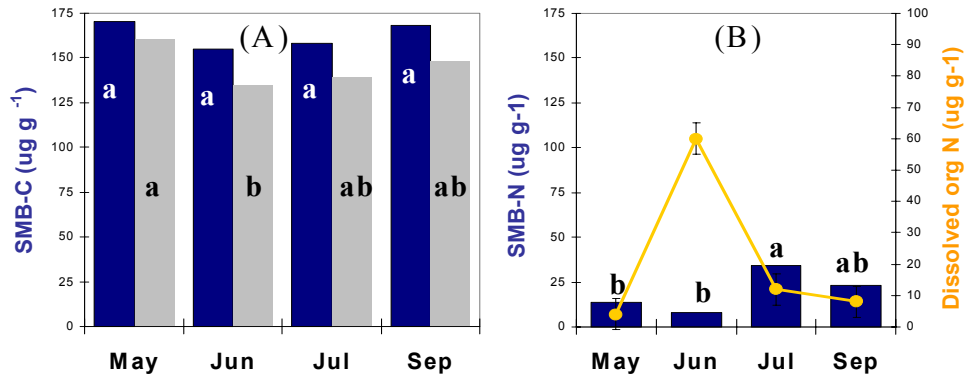
Figure 4. Effect of time on (A) SMB-C at 0-10 cm (dark) and 10-20 cm (pale) soil depths and on (B) SMB-N and dissolved organic N, in the 0-10 cm layer of the soil. Bars with the same letter are not significantly different at the 5% probability level, according to a Bonferroni's test. One standard deviation is indicated above and below data points showing extractable C and dissolved organic N values.
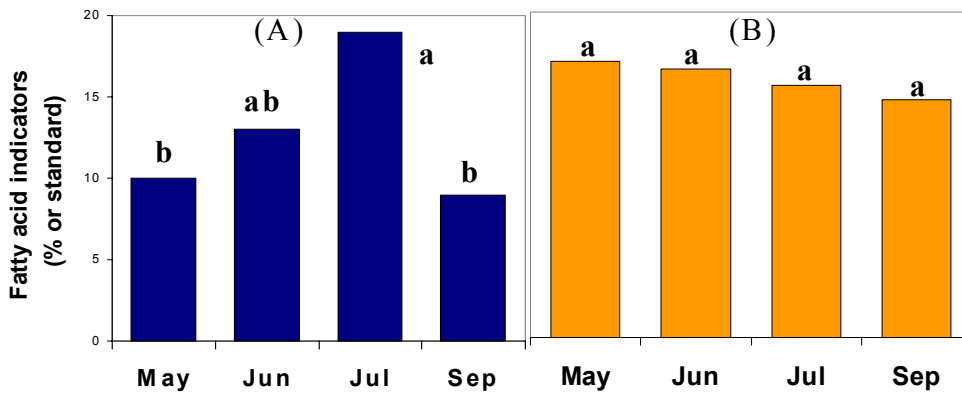

Figure 5. Effect of time on the amount of (A) fungal and (B) bacterial PLFA markers, in the 0-10 cm layer of the soil. Bars with the same letter are not significantly different at the 5% probability level, according to a Bonferroni's test.
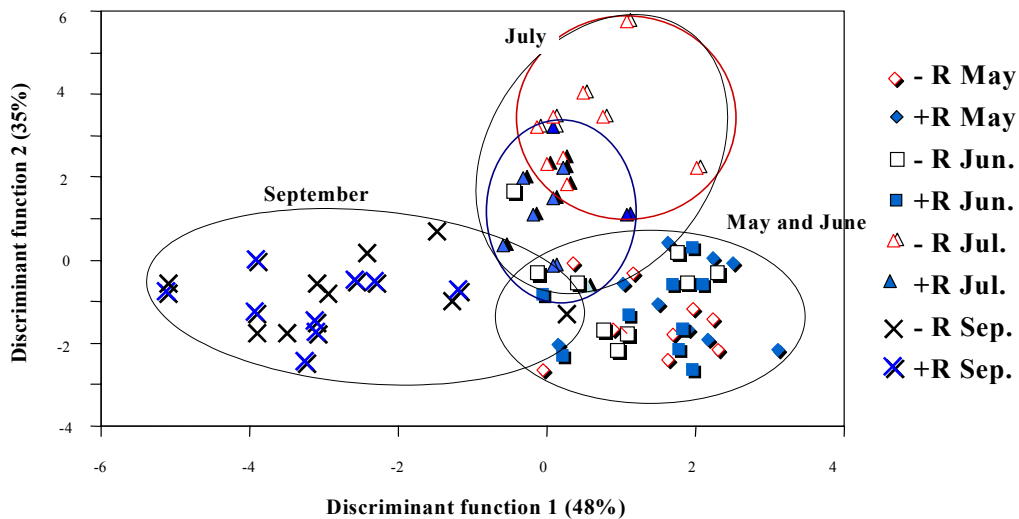

Figure 6. Effect of long term crop residue addition to soil and time effect on soil microbial community composition according to a Canonical discriminant analysis of the PLFA profiles.

abundance in July, when moisture contents were at their lowest, is more likely due to seasonal establishment of mycorrhizal fungi and growth of their hyphal networks, as previously described by Kabir et al. (1997), than to any beneficial influence of drier conditions. The abundance of bacterial PLFA did not vary significantly over the growing season (Fig. 5b).

Unlike other studies that have shown decreases in microbial biomass due to drying (Gunpala and Scow, 1998), in this study, SMB-C and extractable C remained constant despite decreasing moisture contents. Similarly, residue incorporation in RT plots in spring did not cause a detectable increase in SMB-C. The lack of variability in SMB-C may suggest several things: (1) that there was sufficient C from crop residue addition at the beginning of the season to sustain microbial C demand throughout the growing season, (2) that biomass turnover provided a sufficient supply of C or (3) that the increasing input from the growing plant roots with time maintained soil microbial biomass while the contribution of the previous crop's root and shoot residues decomposition diminished. Root inputs, which develop first in the 0-10 cm layer of soil, would explain why SMB-C remained unchanged through time at this depth, while it decreased at the end of June in the 10-20 cm layer (Fig. 4a). There is no doubt that root growth can influence SMB-C. For example, Angers et al. (1992) reported that high levels of N fertilization could contribute to increased SMB-C through increased root biomass. Carter and Rennie (1984) have attributed increases in microbial biomass to increases in C additions from plant roots.

Root development was not monitored in our study. This prevents us from concluding with certainty to any root effects. However, it seems likely that microbial biomass was affected by the cumulative effect of: (1) early season residue inputs, (2) rhizodeposition throughout the growing season and (3) the presence of crop roots at the end of season. This is supported by previous reports which state that SMB-C reflects the cumulative rather than immediate input of carbon in the soil (McGill et al., 1986; Rochette and Gregorich, 1998).

It is clear that seasonal variation in the microbial community structure is important. Canonical discriminant analysis on PLFA profiles indicated that there was more variability in PLFA profiles due to time than to tillage or residue management I(Fig. 6). Within a sampling date, PLFA based community profiles showed little variation between residue or tillage treatments. This is in agreement with Bardgett et al. (1999) who found that, while the variation in SMB-C could largely be attributed to management (fertilization and drainage), variation in total PLFA was attributable rather to temporal variability. Grayston et al. (2001) found much more temporal variation in microbial community structure than in SMB-C. Bossio et al. (1998) found that changes in community structure over time were of greater magnitude than those associated with management regime (fertilizer type and crop rotation).

Shannon-Weaver biodiversity index was calculated from the PLFA profiles obtained from soil extracts. Soil microbial biodiversity increased with time in the season and was significantly larger in September than in May or June (data not shown). The successive change in microbial community structure with time in the season, which was revealed by PLFA profile analysis and SMB-N, suggests the successive raise and fall of some microbial groups as the season progress. Microbial successional communities contain species of organisms that are momentarily favored by the conditions of the environment. Some species from each successional community could have

persisted in time up to the fall, explaining soil microbial biodiversity increases throught the growing season.

Conclusion

This study suggests that the largest influence on soil microbial community under corn is that of seasonal fluctuations that could be induced by several factors. Among these factors, N fertilization appeared to be an important one. In contrast, soil moisture did not seem to influence soil microorganisms in our study. Moisture did not seem to be limiting soil microorganisms in spite of the unusually dry mid-summer conditions encountered in this study. Ten years of tillage treatment had little influence on soil microorganisms. Ten years of corn residue addition increased soil microbial biomass concurrently with total soil organic matter but, apparently, did not influence microbial community structure. Biodiversity was highest in fall.

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