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Effects of Decomposing Maize Litter on Community Level Physiological Profiles of Soil Bacteria

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ABSTRACT

Microbial biomass, basal respiration, and community level physiological profiles (CLPP) based on substrate utilization were studied during the decomposition of maize litter under different simulated soil management systems. Laboratory experiments were conducted on agricultural soil samples from Denmark, Germany, and Italy. Maize litter was either placed on soil surface (mulched) or mixed into soil (incorporated) to simulate two soil management types: tillage and no-tillage. Control samples lacking maize litter were also investigated. All soil samples were incubated at 14°C for 52 weeks. Microbial parameters were assessed after 2, 4, 8, 16, 32, and 52 weeks of incubation. During incubation, we found a significant decrease in microbial biomass C in the soils amended with litter. For all treatment types, there was a clear shift in the CLPP during decomposition; during incubation, the relative utilization of carbohydrates decreased and the usage of amino acids increased respectively. After 52 weeks of incubation, the CLPP from all treatment types were very similar.

Introduction

Application of organic residues is commonly practiced in agricultural systems to replenish soil organic matter and to supply major nutrients [1]. The composition of soil microbial communities may affect the decomposition of added organic residues. Several studies have reported relationships between residue quality, composition of decomposer community, and decomposition rate [4, 10, 15]. Holland and Coleman [10] found that the straw decomposition rate is slower in surface litter treatments than in incorporation treatments, and the nutrient losses can be minimized by retaining the straw on the soil surface rather than mixing it in. Neely et al. used substrate-induced respiration (SIR) to study the relative contribution of bacterial and fungal components during litter decomposition [15]. More recently, Scheu and Parkinson reported changes in microbial nutrient status during litter decomposition [17]. Using the respiratory response to glucose and nutrient (N and P) additions, they analyzed changes in the limiting element and nutrient demands of microorganisms during litter decay. They found that, during the early stages of decay, microbial growth is limited by N, and, in later stages, by C. The authors concluded that this change in the chemical composition of aging organic material may be responsible for the change in the

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Table 1. Site characteristics and soil physical and chemical properties

Site	Soil type (FAO)	Mean annual temperature (°C)	Mean annual precipitation (mm)	pH	Soil texture (%)			C_{org} (%)	Total N (%)
					Sand	Silt	Clay		
Denmark	Luvisol	8.4	675	6.6	47	43	10	1.52	0.15
Germany	Luvisol	7	833	6.4	17	62	22	1.26	0.15
Italy	Andosol	16.8	415	6.3	37	39	24	1.24	0.13

composition of microbial communities during the decomposition process. Further, the change in the substrate chemistry during litter decomposition may also alter the functional abilities of microorganisms. However, this important aspect of microbial ecology has rarely been addressed in litter decomposition studies.

Garland and Mills [7] were the first to use CLPPs to characterize microbial communities from different ecosystems. CLPPs have since been used effectively to measure functional diversity [22], to study the functional abilities of the microbiota during composting of manure [12], to detect effects of genetically engineered microorganisms [20], and to assess the effects of hydrocarbon pollution [21]. The use of 95 different carbon sources on CLPP plates provides a distinct community-level Biolog physiological profile [8] that can be used to differentiate among microbial communities.

Short-term changes in CLPP may indicate long-term changes in ecosystem functioning that may impair sustainability of agricultural systems. In this study, we used CLPPs to differentiate microbial communities during the decomposition of maize litter that was either mixed into the soil (incorporation), or placed on the soil surface (mulching). Soils from different climatic regions of Europe were analyzed. Besides CLPP, we also measured microbial biomass C and basal respiration. The metabolic quotient (qCO_2) was calculated from microbial biomass and basal respiration. The main objectives of our study were: (i) to follow the shift in the CLPP during maize litter decomposition in soils from different climatic regions and under different treatments, and (ii) to determine whether CLPPs change in response to differences in substrate availability during the decomposition of litter material.

Materials and Methods

Soils, Sites, and Experimental Design

Soils from a north-south transect through Europe (Denmark, Germany, and Italy) were used for the study. A description of the sites and the main physical and chemical properties of the soils are given in Table 1.

After sampling, soils were passed through a 2-mm mesh sieve. Before starting the experiment, the water content was adjusted to 40% of the maximum water holding capacity (and maintained throughout the incubation), and the soils were preincubated at 14°C for 2 weeks. The soil columns used for the incubation were 7 cm in length, and had a diameter of 10 cm. Each column was filled with 700 g rewetted soil (594 g DW, 586 g DW, and 586 g DW for Danish, German, and Italian samples, respectively). The columns were set up with enough replicates to allow destructive sampling of 3 replicates for each sampling date.

Three different treatments were analyzed: Control—no application of maize litter; Incorporation—maize litter mixed into soil; and Mulch—maize litter placed on soil surface.

Litter addition (7.8 g per column) corresponded to 1 kg litter m^{-2}. The soils were then incubated for up to one year at 14°C. The samples were analyzed after 2, 4, 8, 16, 32, and 52 weeks.

For analysis of the mulch treatment, the top (0–1.5 cm) and lower (1.5–7 cm) layers were mixed prior to being measured. Samples of the top and lower layers were also analyzed separately. These sample types are referred to as mulch-litter: top (0–1.5 cm) layer of mulch treatment; and mulch-soil: lower (1.5–7 cm) layer of mulch treatment.

Microbial Biomass, Respiration, and Metabolic Quotient

Microbial biomass (C_{mic}) was determined by substrate-induced respiration (SIR) [2]; 10 mg glucose g^{-1} dry soil was added to obtain maximum initial respiratory response. Microbial respiration was measured at 22°C, using a continuous flow infrared gas analyser [9]. Readings were taken hourly. Basal respiration was calculated as the mean of the 15–20 h period after attachment to the analyzer. The metabolic quotient (qCO_2) was calculated [3] from biomass and basal respiration.

Soil Extraction and Inoculation of Microtiter Plates

Microorganisms were extracted from the soil using a standard extraction method. Ten g soil was mixed with 90 ml 0.85% NaCl solution, and shaken for 1 h on a rotary shaker at 250 rpm. After sedimentation of coarse soil particles, the supernatant was used for further dilutions. Each well of the microtiter plate was inoculated with 125 µl of diluted suspension containing approximately 2.0–3.2 $\times$ 10^8 cells ml^{-1} (acridine orange direct count, AODC). Three

replicate plates for each sample were incubated at 14°C for 6–7 days. Color formation was measured at 592 nm, with a microtiter plate reader (SLT SPECTRA, Grödig, Austria), every 12 h. Plates which showed an average absorption of 1.0 ± 0.1 were selected for analysis. To eliminate the variation due to differences in inoculum density, the data were transformed by calculating average well color development (AWCD; Garland and Mills [7], modified by Insam et al. [12]).

Statistics

Principal component analysis (PCA) and discriminant analysis (DA) were performed on the CLPP dataset. Discriminant scores 1 and 2 were each analyzed using one way analysis of variance (ANOVA), with treatment as the factor. Tukey's HSD tests were performed to determine significant differences among treatment types during early and late stages of decomposition (significance level 0.05). Microbial biomass, basal respiration, and qCO_2 were also compared, using an ANOVA followed by Tukey's HSD test. For all statistical analysis, the SPSS statistical package [18] was used.

Results

Microbial Biomass, Basal Respiration, and Metabolic Quotient

The initial values for microbial biomass were much higher for samples receiving litter than for control and mulch-soil samples. Two weeks after litter amendment, microbial biomass of the Danish and German soils doubled, and the C_{mic} of the Italian soil increased five-fold. After the initial enhancement, a subsequent decline in C_{mic} was observed for all litter-amended soils (Fig. 1).

The litter amendment enhanced basal respiration for all soils, most notably for the Italian soil (Table 2). With litter incorporation, the basal respiration increased to 2.22, 2.67, and 3.11 µg CO_2 g^{-1} soil h^{-1} for Danish, German, and Italian soils, respectively. It then declined gradually. The response was even more pronounced for mulch-treated soils. Two weeks after the amendments, the highest value for basal respiration was found for the Italian samples (5.02 µg CO_2 g^{-1} soil h^{-1}). For the Danish and the German soils, basal respiration reached its peak after 8 and 16 weeks, respectively.

The qCO_2 of all three control samples decreased from its initial value during the incubation. Mulch treatment caused a higher qCO_2 than incorporation of the litter. At the end of the experiment, the Italian soil had a lower qCO_2 than the Danish and the German soils.

We found no significant differences in microbial parameters between incorporation and mulch treatments during the incubation period. At the early stage of decomposition,

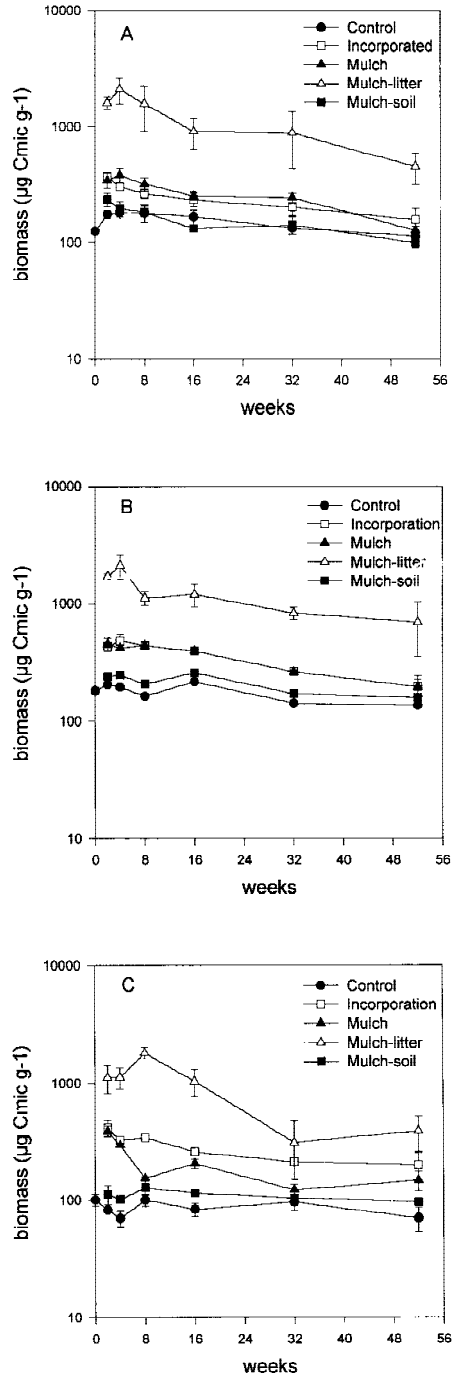


Fig. 1. Microbial biomass for a Luvisol soil from Denmark (**A**), a Luvisol from Germany (**B**), and an Andosol from Italy (**C**). (Bars indicate standard deviation, n = 3.)

both mulch and incorporation treatments had significantly higher ($P < 0.05$) microbial biomass, basal respiration, and qCO_2 than the unamended samples. After 52 weeks, however, these differences shrank. For the German soil, the differences in microbial biomass, basal respiration, and qCO_2 among the treatment types at the end of the incubation were

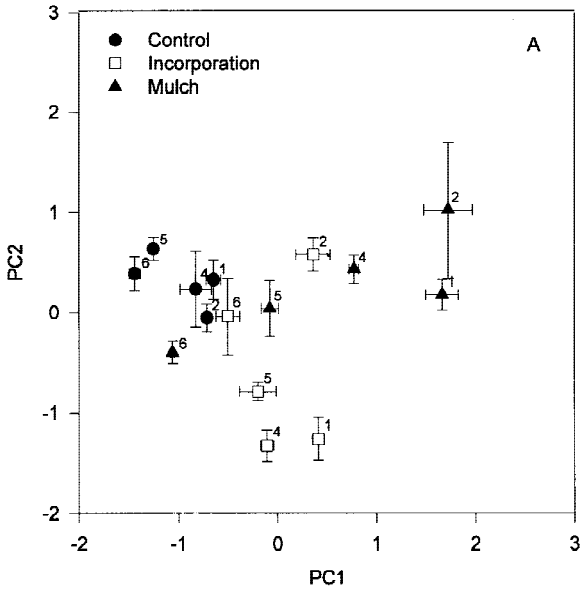
Table 2. Basal respiration and qCO_2 of soils from Denmark, Germany, and Italy during a one-year laboratory incubation at 14°C

		Danish soil		German soil		Italian soil	
Treatment	Weeks	Basal respiration (μg CO_2 g^{-1} soil h^{-1})	qCO_2 (μg C g^{-1} C_{mic} h^{-1})	Basal respiration (μg CO_2 g^{-1} soil h^{-1})	qCO_2 (μg C g^{-1} C_{mic} h^{-1})	Basal respiration (μg CO_2 g^{-1} soil h^{-1})	qCO_2 (μg C g^{-1} C_{mic} h^{-1})
Control	0	0.6 ± 0.2	1.2 ± 0.5	0.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	2.1 ± 0.6
	2	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.4 ± 0.2
	4	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	1.8 ± 0.1
	8	0.6 ± 0.2	0.9 ± 0.2	0.3 ± 0.0	0.4 ± 0.1	1.5 ± 0.2	4.1 ± 0.2
	16	0.1 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.3	1.3 ± 0.8
	32	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	0.7 ± 0.2
	52	0.4 ± 0.1	0.9 ± 0.3	0.4 ± 0.1	0.9 ± 0.3	0.2 ± 0.1	0.6 ± 0.3
Incorporation	2	2.2 ± 0.1	1.6 ± 0.2	2.7 ± 0.7	1.7 ± 0.5	3.1 ± 1.0	2.0 ± 0.4
	4	2.2 ± 0.1	2.0 ± 0.0	2.6 ± 0.2	1.5 ± 0.3	2.1 ± 0.1	1.7 ± 0.0
	8	2.0 ± 0.2	2.0 ± 0.4	2.3 ± 0.3	1.4 ± 0.2	3.3 ± 0.1	2.6 ± 0.1
	16	1.5 ± 0.2	1.8 ± 0.1	2.0 ± 0.3	1.4 ± 0.2	1.3 ± 0.0	2.4 ± 0.1
	32	2.1 ± 0.1	2.9 ± 0.3	1.6 ± 0.1	1.7 ± 0.1	1.3 ± 0.3	1.7 ± 0.3
	52	1.3 ± 0.1	2.4 ± 0.4	1.0 ± 0.4	1.6 ± 1.0	0.9 ± 0.5	1.2 ± 0.3
Mulch	2	2.6 ± 0.2	2.1 ± 0.2	1.5 ± 0.2	0.9 ± 0.0	5.0 ± 0.7	3.5 ± 0.1
	4	3.7 ± 0.7	2.6 ± 0.2	2.4 ± 0.2	2.2 ± 0.1	4.3 ± 0.5	3.9 ± 0.3
	8	3.4 ± 0.1	3.0 ± 0.4	3.1 ± 0.5	2.2 ± 0.2	4.9 ± 0.6	8.7 ± 0.9
	16	2.1 ± 0.2	2.3 ± 0.4	3.5 ± 0.2	2.3 ± 0.1	2.0 ± 0.2	2.6 ± 0.1
	32	2.3 ± 0.5	2.6 ± 0.3	1.9 ± 0.2	2.1 ± 0.2	1.4 ± 0.3	3.1 ± 0.8
	52	1.4 ± 0.5	2.1 ± 0.9	1.4 ± 0.4	1.8 ± 0.3	0.8 ± 0.4	1.4 ± 0.5
Mulch-litter	2	19.1 ± 0.5	3.3 ± 0.4	17.6 ± 1.1	2.8 ± 0.3	12.0 ± 4.0	2.9 ± 0.2
	4	36.0 ± 12.7	4.6 ± 0.6	33.0 ± 9.6	4.2 ± 0.2	22.8 ± 3.6	5.3 ± 0.3
	8	22.6 ± 8.3	4.0 ± 0.3	11.1 ± 1.9	2.7 ± 0.2	38.1 ± 3.9	5.8 ± 0.1
	16	6.4 ± 3.6	2.0 ± 1.1	14.5 ± 0.2	3.5 ± 0.8	14.0 ± 6.7	3.5 ± 0.9
	32	15.7 ± 12.6	4.3 ± 1.4	12.1 ± 1.6	4.0 ± 0.4	2.7 ± 0.5	3.1 ± 1.9
	52	7.2 ± 1.1	4.6 ± 0.8	6.8 ± 3.9	2.6 ± 0.3	1.5 ± 0.1	1.1 ± 0.3
Mulch-soil	2	0.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.3 ± 0.2
	4	0.7 ± 0.2	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.2 ± 0.4
	8	0.7 ± 0.2	1.0 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	0.4 ± 0.2	5.3 ± 0.8
	16	0.6 ± 0.1	1.3 ± 0.2	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.4	1.5 ± 0.9
	32	0.6 ± 0.3	1.0 ± 0.5	0.8 ± 0.4	1.9 ± 0.7	0.9 ± 0.5	2.4 ± 1.3
	52	0.6 ± 0.2	1.7 ± 0.5	0.6 ± 0.4	1.0 ± 0.7	0.4 ± 0.2	1.0 ± 0.5

not significant. For the Danish soil, the basal respiration and qCO_2 were still significantly higher, compared to the other samples. For the Italian soil, after 52 weeks, only the microbial biomass of the mulch sample was significantly higher than the control.

CLPP

In Figs. 2–4, ordinate plots of a principal component analysis based on the complete dataset of each country are shown. Clear shifts in CLPPs were found during the incubation of soils from all three climatic regions (Figs. 2, 3, and 4). These shifts were more pronounced for the Danish and German samples (Fig. 2) than for the Italian (Fig. 4). During the incubation, a gradual shift in CLPP was observed for mulch-litter and mulch treatments for all climatic regions (Figs. 2, 3, and 4). The ordinate plot obtained after PCA of the Danish soil showed a distinct shift in microbial communities, with time along the axis of PC1 (the first two PC factors explained 33% of the variance). The shifts were considerably less pronounced for control and mulch-soil samples than for mulch-litter and mulch treatments (Figs. 2A and 2B). Incorporation treatments were intermediate between these two groups. At the earlier stages of incubation, control and mulch-soil treatments had negative PC1 scores; Mulch and mulch-litter treatments had positive PC1 scores. However, towards the end of the incubation (32 and 52 weeks), the litter-amended samples had negative PC1 scores similar to the unamended samples. Somewhat similar results were obtained for the German soil (Figs. 3A and 3B; the first two PC



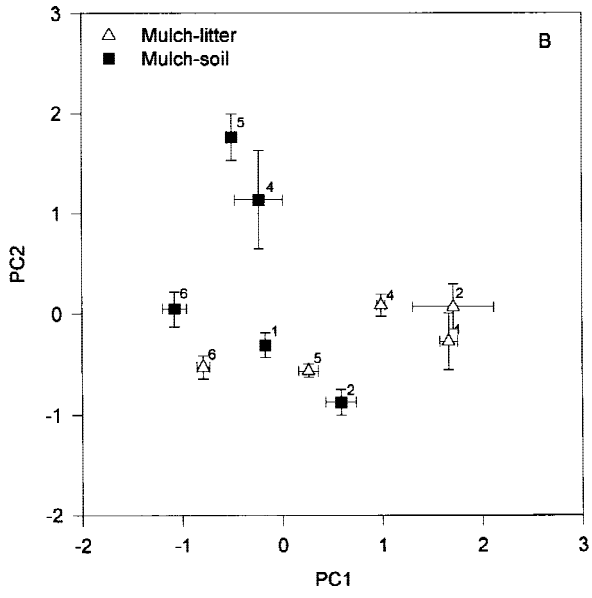
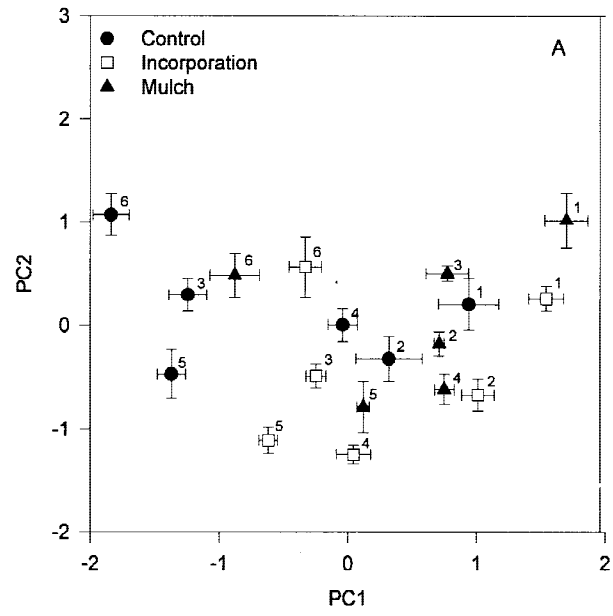


Fig. 2. Ordinate plots after principal component analysis based on all data from 6 sampling dates during a 1-year incubation of Danish soil. Soil samples include control, incorporation, and mulch **(A),** mulch-litter and mulch-soil **(B).** Each point represents the mean and standard errors of 9 replicate plates. The number next to each symbol indicates the sampling date: 2 weeks (1), 4 weeks (2), 8 weeks (3), 16 weeks (4), 32 weeks (5), and 52 weeks (6).



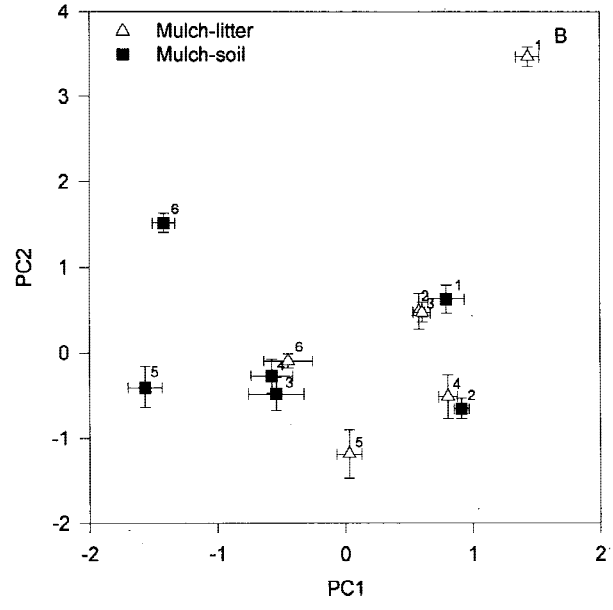


Fig. 3. Ordinate plots after principal component analysis based on all data from 6 sampling dates during a 1-year incubation of German soil. Soil samples include control, incorporation, and mulch **(A),** mulch-litter and mulch-soil **(B).** Each point represents the mean and standard errors of 9 replicate plates. The number next to each symbol indicates the sampling date: 2 weeks (1), 4 weeks (2), 8 weeks (3), 16 weeks (4), 32 weeks (5), and 52 weeks (6).

factors explained 28.7% of the variance). Although similar trends were seen in the Italian soil, the shifts were less distinct than for the soils from the other two climatic regions (Figs. 4A and 4B; the first two PC factors explained 22.7% of the variance).

Specific carbon sources responsible for the differences among samples are presented in Table 3. Most of the carbohydrates showed a strong positive correlation with PC1 for the Danish and German soils. In contrast, strong negative correlations were observed between amino acids and

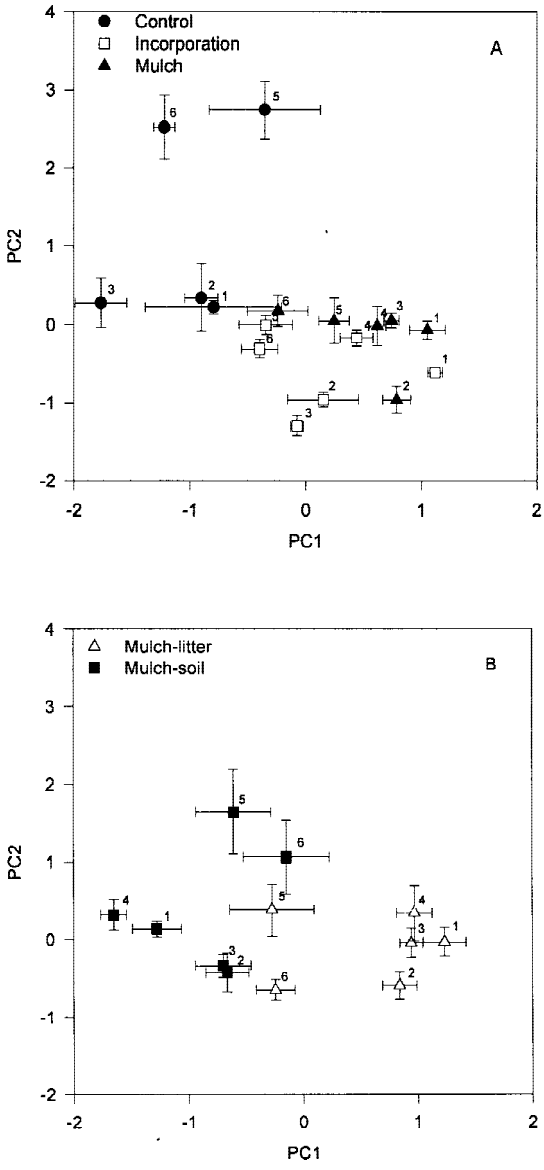


Fig. 4. Ordinate plots after principal component analysis based on all data from 6 sampling dates during a 1-year incubation of Italian soil. Soil samples include control, incorporation, and mulch **(A),** mulch-litter and mulch-soil **(B).** Each point represents the mean and standard errors of 9 replicate plates. The number next to each symbol indicates the sampling date: 2 weeks (1), 4 weeks (2), 8 weeks (3), 16 weeks (4), 32 weeks (5), and 52 weeks (6).

PC1. However, no specific correlations were seen for the Italian soil.

Discriminant Analysis

Discriminant analyses (DA) were performed on the entire CLPP dataset to determine the effects of different amendments during the decomposition process. At the early stage (after 2 weeks), all treatments, except for mulch and mulch-litter, had significantly different scores ($P < 0.05$) for discriminant function 1 (DF1), which explained 75% of the variance (Fig. 5A). The scores for DF2 (which explained 11% of variance) were not significantly different among litter-amended samples. At the later stages of decomposition (after 52 weeks), only control samples were distinctly separate on the axis of DF1 (which explained 59% of the variance), and only mulch-soil treatment had significantly different scores for DF2 (which explained 25% of the variance; Fig. 5B).

Substrate Groups

We divided the substrates on microtiter plates into four main groups: carbohydrates, carboxylic acids, amino acids, and others (polymers, amines and amides, aromatic compounds, and phosphorylated compounds). Their relative contributions during decomposition were examined. We found a strong contribution of carbohydrates and amino acids throughout the decomposition period. The average absorption value of carbohydrates declined, with simultaneous increase in the average absorption value of amino acids. For the Danish soil, the ratio of carbohydrates to amino acids (CH/AA) decreased significantly for mulch, mulch-litter, and control treatments (Fig. 6A). For the German soil, the decrease in CH/AA ratio was significant for all treatments (Fig. 6B). For the Italian soil, however, no significant change in CH/AA ratio was detected (Fig. 6C).

Discussion

Litter amendments enhanced C_{mic} and activity in soils from all three climatic regions. Microbial biomass declined significantly in litter-amended samples during incubation, which may be attributed to the depletion of readily available substrates. For control and mulch-soil samples, which received no fresh plant material, the biomass remained quite stable, albeit at a low level, throughout incubation. Under constant temperature and moisture conditions, the autochthonous community is able to survive for a long period. However, after easily available substrate is depleted, a microbial biomass in a dynamic state (after the addition of fresh organic matter) tends to decrease rapidly until a constant biomass level is reached [13]. Thus, after 52 weeks of incubation, the soils with different treatments become very similar.

Table 3. Correlation of carbon sources with the first principal component (PC1) in treated soils from Denmark, Germany and Italy during a one-year incubation

Denmark	PC1[a]	Germany	PC1	Italy	PC1
Carbohydrates		Carbohydrates		Carbohydrates	
α-D-Lactose	0.83	Cellobiose	0.73	i-Erythritol	0.66
Lactulose	0.83	β-Methyl-D-glucoside	0.71	Cellobiose	0.62
Cellobiose	0.82	D-Melibiose	0.69		
L-Fucose	0.82	Gentiobiose	0.66		
Xylitol	0.81	L-Fucose	0.65		
N-Acetyl-D-galactosamine	0.80	*N*-Acetyl-D-galactosamine	0.65		
i-Erythritol	0.80	D-Raffinose	0.62		
Gentiobiose	0.79	L-Rhamnose	0.62		
β-Methyl-D-glucoside	0.74				
Adonitol	0.72				
D-Melibiose	0.69				
L-Rhamnose	0.69				
D-Raffinose	0.67				
D-Psicose	0.67				
Amino acids		Amino Acids			
L-Histidine	−0.86	L-Alanine	−0.65		
L-Serine	−0.80	L-Histidine	−0.62		
L-Aspartic acid	−0.75	L-Serine	−0.62		
Hydroxy-L-proline	−0.79				
γ-Aminobutyric acid	−0.72				
L-Pyroglutamic acid	−0.63				

Holland and Coleman [10] reported that surface placement of straw can reduce the organic matter and nutrient losses, compared to straw incorporation. However, we did not find any significant differences in C_{mic} between incorporation and mulch treatments. The increase in C_{mic} in the mulch sample may be attributed to an increase of C_{mic} in the top 1.5 cm (mulch-litter), where the microbial biomass was about 10 times greater than the control for the Danish and German soils, and about 14 times greater than the control for the Italian soil. Our results could not support the theory that mulching would generally result in a soil organic matter conservation compared to litter incorporation, because no significant changes between these two treatments were observed for all soils. However, for conclusive evidence, field trials will be necessary.

Like other microbial parameters, CLPP separated the litter amended soils from unamended ones. A clear shift in CLPP during the incubation indicated that the composition or functional abilities of the microbial communities changed. This may be due to a change in substrate availability during the decomposition process. Several studies have reported changes in substrate chemistry during litter decomposition, with N limiting growth during early stages of decay, and C limiting growth during the later stages [11, 17]. In the present study also, a decrease was found in the C/N ratio during decomposition [19]. A decline in the CH/AA ratio during incubation (for Danish and German samples) may be related to the change in available substrates. Apparently, during the early stage of decomposition, the litter compounds, which were basically carbohydrates, were more abundant. In later stages, proteins mainly of microbial origin, were relatively abundant. This is supported by the positive correlation of carbohydrates with PC1 and negative correlation of amino acids with PC1 for the Danish and German soils. This suggests that carbohydrate utilization was more important during early stages of decomposition, while amino acid utilization was increasingly important in the later stages. This change was most pronounced for the Danish samples, which showed the clearest differentiation by treatment and incubation time. The following carbohydrates were correlated to PC1 ($r > 0.6$) in all soils: *cellobiose, β-methyl-D-glucoside, D-meliobiose, gentiobiose, L-fucose, N-acetyl-D-galactosamine, D-raffinose, L-rhamnose*.

Recently, Moorhead et al. [14] reported a change in community composition associated with litter decay, based on CLPP. They found that, during incubation, the utilization of polymer compounds increased relative to carbohydrates, and that the community composition on the first and last dates of their decomposition experiment (2 and 31 weeks) were most similar to each other. In our study, we did not

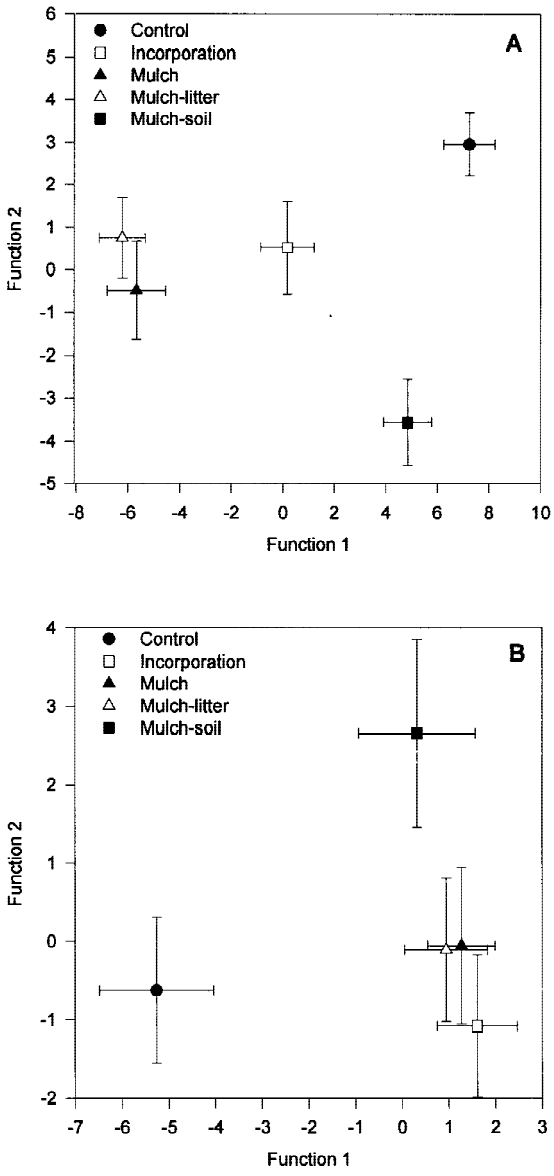


Fig. 5. Ordinate plots after discriminant analysis on the entire data set, including all sampling dates, from Danish, German, and Italian soils. Samples analyzed after 2-week **(A)** or 52-week **(B)** incubation. Each point represents the mean and standard deviation.

find any significant change in the utilization of polymers during incubation. However, we found a clear difference in the CLPP between first and last dates (2 and 52 weeks) of incubation, even for the control samples. Like other microbial parameters, the CLPPs of the different treatment types became very similar after 52 weeks of incubation, although shortly after litter amendment, the amended samples were clearly separated from the samples without litter (Fig. 5A). This may have been due to the absence of readily available substrate in the very late stages of decomposition, even when litter had been added in the beginning.

Soil origin also had an effect on microbial parameters and CLPP. The soils from Denmark and Germany were more similar than the soil from Italy. Microbial biomass and basal respiration in the Danish and the German soils were significantly higher than in the Italian soil. At the first sampling date (2 weeks), the litter amendment had increased the C_{mic} of the Italian soil about 5 times the control value, whereas, the control value of C_{mic} of Danish and the German samples doubled after litter amendment. Although the qCO_2 fluctuated during the incubation, at the end of the experiment, the qCO_2 of the Italian soil was lower than that of the Danish and German soils. This suggests either a more efficient C use by the microbial community in the Italian soil, or that a large part of the microbial community was dormant at the later stages of decomposition. All results indicate that the microbial community of the Italian soil behaved differently than that of the Danish and German soils. A more rapid C_{mic} increase in the Italian samples indicates that the microbiota are adapted to react quickly, given favorable conditions.

It is still unclear if the change in the CLPP during the incubation, and the differences among treatments and soils, can be attributed to a change in functional abilities only, or also to a change in community composition. Phospholipid fatty acid (PLFA) patterns suggest that changes in the community composition were involved [16]. Our results suggest that the change in utilization pattern of carbohydrates and amino acids was largely responsible for the shift in CLPP. However, as pointed out by Garland [6, 8], the functional relevance of substrate utilization profiles should be interpreted with caution. Garland et al. [8] found that the addition of a specific carbon source to a bioreactor caused significant changes in the overall substrate utilization profile, but no significant increase occurred in the corresponding well on the microtiter plates. Further studies, perhaps combining the substrate utilization profile with a biomarker (such as PLFA) or genetic techniques may substantially improve the interpretation of CLPP in relation to the C source availability in the habitat.

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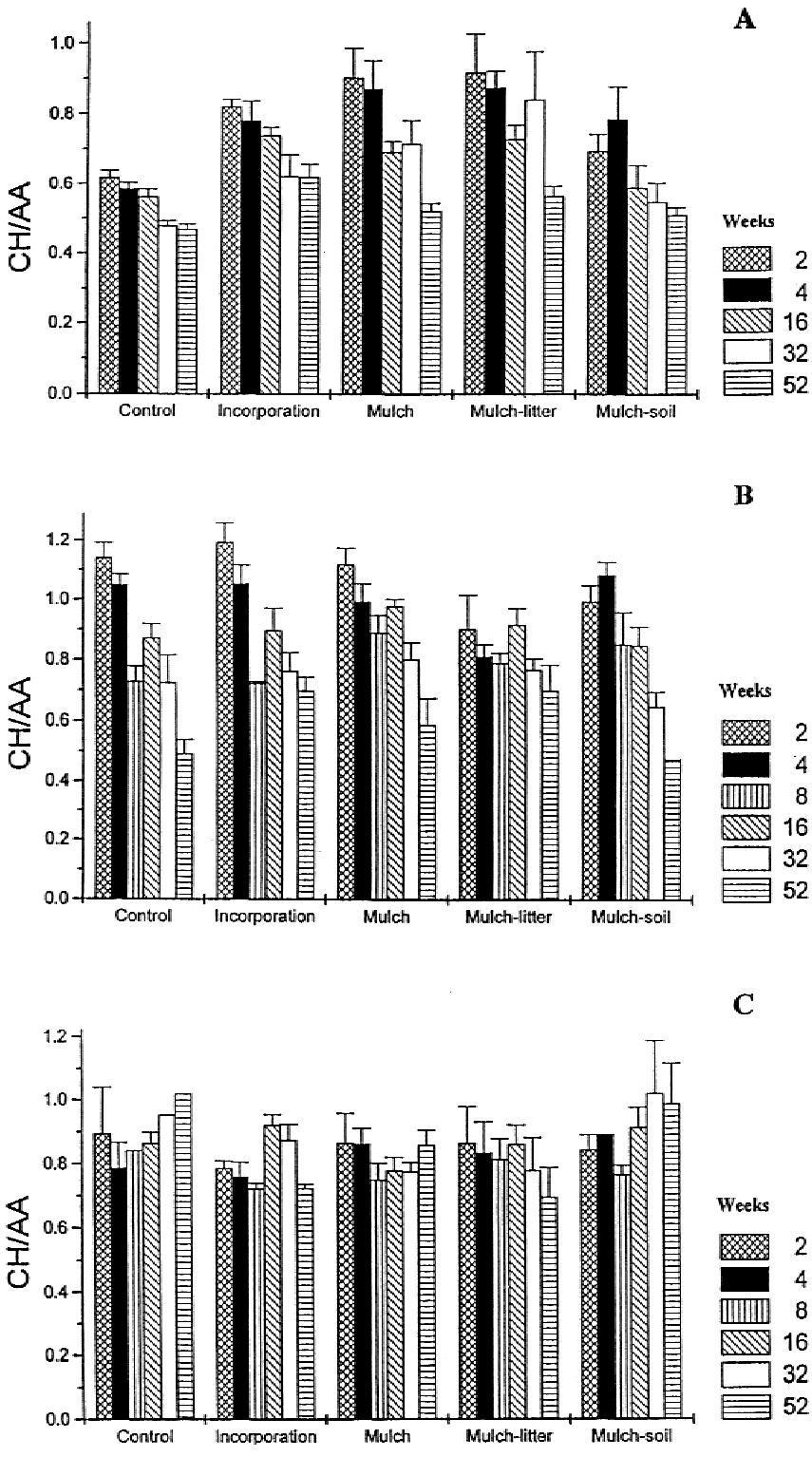

Fig. 6. The ratio of carbohydrate to amino acid–utilization (CH/AA) for soils from Denmark **(A),** Germany **(B),** and Italy **(C).**

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