Microbial indicators for assessing biological fertility status of soils

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Micro organisms respond rapidly to changing environmental conditions so that they are sensitive indicators of soil health and commonly used for soil status monitoring. The aim of this study was the characterization of three differently managed agricultural soils by using microbial indicators to assess soil biological fertility status. The study was carried out in Pavia Province, in Italy. The managements involved a soil defined as biodynamic (Biodynamic); a soil characterized by periodic application of stable cattle sludge and chemical fertilizer (Manure); a soil characterized by ten years of depurated and stabilized organic sludge amendment (Sludge). Samples were taken four times during a year, at two depths: 0-15 and 15-30 cm. An extensive characterization of soil organic matter was carried out for all soils. Biochemical parameters included metabolic quotient, mineralization quotient and microbial quotient. Community level physiological profile analysis (CLPP) was used to investigate functional diversity of soil bacteria. Total amounts of fungi and bacteria were determined by direct microscopy. Indicators related to labile and humic organic matter fractions suggest significantly lower soil fertility and lower sustainability in the Sludge amended treatment. Differences between the Biodynamic treatment and Manure treatment were small.

1. Introduction

The concept of “soil quality” is generally understood as the capacity of soil to function as a living system able to fulfil all its function, to sustain biological productivity, to promote the quality of air and water environments and to maintain plants, animals and humans health (Doran and Parkin, 1994). In particular nowadays it’s commonly accepted the concept of “soil fertility” as the capacity of soil to sustain biological production and all humans and natural factors affecting this production could be rightly considered as fertility factors (Sequi, 1989).

Being a very complex concept, attempts to carry a global evaluation of soil fertility result very hard and this is why fertility factors are generally included in three distinct categories on the base of their nature: physic, chemical and biological. Only the complex interaction of these three aspects makes up agronomic or integral fertility of soil, from which productivity depends.

Chemical fertility refers to the sum of available nutrients to plants while physical fertility concerned soil structure and texture. Biological fertility, instead, include metabolic expression of soils. Soil metabolic activity can be defined as the overall of reaction, both biotic and abiotic, that can ensure soil fertility. Since biotic reaction are essentially microbial ones, it’s possible to confuse soil metabolic activity as soil microbial activity, but while microbial activity is a term to indicate the wide range of activities carried out by micro organism in soil, biological or metabolic activity of soil reflects not only microbial activities but also the activities of the other organisms in the soil, including for example plant roots (Nannipieri et al., 1990). Although the two terms are conceptually different they are often confused.

Microbial fraction represents a really important component in soil fertility whose failing could become soil as a simple mechanical support for plants. Micro organisms, more than other organisms, are highly adaptable to varying conditions and respond rapidly to them (Hargreaves et al., 2003). For this reason they can be considered as sensible indicators of soil health and this is why they are usually used for soil status monitoring (Yakovchenko et al., 1996). In particular, measurements of microbial activity are actually included as indicators in a lot of national and international monitoring programs on soil quality.

At the end it’s likely to affirm that a better estimate of soil biological fertility is possible by using a lot of biological indicators. Usually an important criterion for an indicator is that it should respond promptly and accurately to perturbations (Holloway and Stork, 1991). No individual measurement is enough as a single index of soil quality. However, examination of several (or even the ratios between them) may provide useful information as in this case, on management-induced effects on soil fertility.

The aim of this study was to characterize soil microbial activity of three differently managed soils by using microbial indicators in order to better understand soil biological fertility status.

2. Materials and Methods

2.1 Soils

Soil samples were taken in Lombardy region at the north of Italy, in the area of Pavia Province. The study sites have been identified in the district of Corteolona and Bereguardo (about 35 km of distance one to each other).

First site (Biodynamic) was in Bereguardo. It was a meadow grass cultivation characterized by 25 years of biodynamic management with no fertilizer or manure application on soil, no herbicides and pesticides use, and no ploughing since 2002.

Second site (Manure) was also in Bereguardo. It was again meadow grass cultivation but the difference was the periodic application on soil of stable cattle sludge (150 kg manure/year /hectare) and 15N-15P-15K fertilizer. It was no ploughing since 1999.

Third site (Sludge) was in Corteolona. It was a maize cultivation characterized by ten years of depurated and stabilized organic sludge amendment.

2.2 Sampling

Sampling took place four times during a year on September 2004, and January, March and July 2005 for microbial indicators and CLPP analysis. Biomass of fungi and bacteria, potentially mineralizable nitrogen and hot water extractable carbon were determined only in September and March, and only in the upper soil layer. In each site a study plot (20m x 20m) has been identified and sampling involved (0-15) cm and (15-30) cm layers considering that microbial biomass decrease according to available organic matter decreasing as depth increase.

Within each plot five bulked soil samples were collected. Since it is not desirable that natural field variations should influence the results of biological indicators interfering with effects of interests such as long-term agricultural practices, standardized environmental factors in laboratory tests have been carried out for biochemical measurements and CLPP analysis, as field variability, water tension and temperature, have the advantage to allow the comparison of soils (Schloter et al., 2003). For these reasons samples was stored at 4°C and pre-conditioned (60% of water holding capacity) at 30°C for a week until starting analyses according to indication for Mediterranean area.

In addition to the analyses by the Italian laboratory, a limited number of samples were analysed by Alterra (the Netherlands) for fungal and bacterial biomass, potentially mineralizable nitrogen and hot water extractable carbon. This was done only in the September and March samples and only in the upper soil layer. The samples were kept cool during transport by courier, and after receipt stored at 12°C for 1 (September) or 2 weeks (March).

2.3 Analytical Methods

Qualitative and quantitative characterization of soil organic matter has been carried out. Total organic carbon contents, C_{org}, were determined according to the Springer and Klee method (1954). Total extractable carbon, C_{ext}, humic and fulvic carbon fraction, C_{HAFA}, and humification parameters (DH, humification degree, and HR, humification rate) were determined by using Ciavatta et al. method (1990).

Extraction of the soil organic matter was carried out by 0.1N NaOH and 0.1N $Na_4P_2O_7$ at 65°C for 48 hrs in N_2 atmosphere. Humic acids (HA) were precipitated by acidification (pH<1.5) of the extract and fulvic acid (FA), which remained in solution, were purified on a polyvynilpyrrolidone column and then recollected to the humic portion. Total extractable carbon, C_{ext}, and humic plus fulvic acid carbon, C_{HAFA}, were determined by dichromate oxidation method, according to Ciavatta et al. (1990). Humification parameters were calculated according to Ciavatta et al. (1990), as follows:

HR (%) = 100 x C_{HAFA} / C_{org}

DH (%) = 100 x C_{HAFA} / C_{ext}.

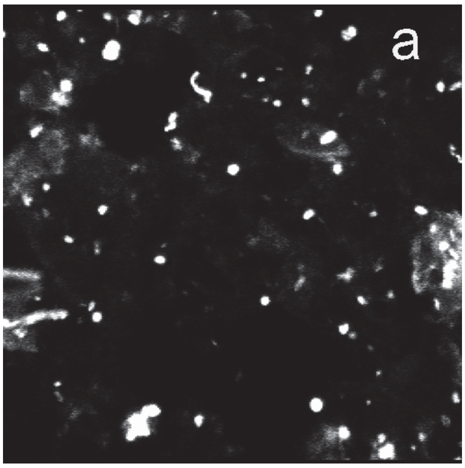
Biochemical determinations concerned metabolic quotient (qCO_2), representing specific activity as CO_2 evolution per unit of microbial biomass (Anderson and Domsch, 1990) and mineralization quotient (qM), defining as total microbial activity as CO_2–C evolution respect to total organic carbon (Dommergues, 1960). They were determined by classical measures of total organic carbon, C_{org}, microbial biomass carbon, C_{mic} (Vance et al., 1985) and respiration of soil (Isermayer, 1952) considered as basal respiration at 7^{th} days, corresponding to carbon mineralization value in field condition.

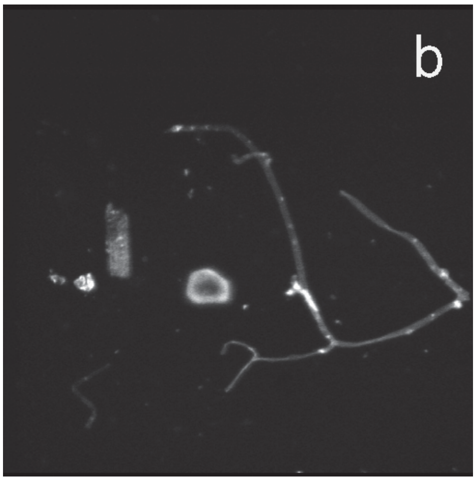
Organic matter decomposition processes have been also investigated as cumulative mineralization curves, considering total amount of mineralized carbon in laboratory conditions, C_{cum}, as daily CO_2-C evolution during 14 days of analysis. A non-linear regression square analysis was used to calculate kinetic parameters as mineralization kinetic constant, k_{MIN}, and potentially mineralizable carbon, C_0, from average cumulative data of C-mineralization (Riffaldi et al., 1996). The first order kinetic model of organic matter decomposition was $C_{cum}=C_0\ (1-\exp^{(-k_{MIN}*t)})$ (StaSoft Italia 6.0).

Since soil is generally substrate limited under natural conditions (Stotzky, 1997), community level physiological profile analysis, CLLP, (Garland and Mills, 1991) was used.

Potential soil microbial activity was investigated by integration (I_g) of typical logistic density-dependant equation as proposed in Guckert et al. (1996) by using $I_g=\int^t_0 AWCD_0/(1+\exp^{-r(t-s)})dt$. The calculation needs kinetic parameters obtained from Lindstrom model (Lindstrom et al., 1998) as potential average wells colour development, $AWCD_0$, and potential rate of microbial communities increase, k_{CLPP} (StaSoft Italia 6.0).

Bacteria were measured by confocal laser scanning microscopy and automatic image analysis (Bloem et al., 1995), after staining of soil smears with DTAF, a fluorescent dye which binds to proteins (Bloem and Vos, 2004). From the number and cell volumes bacterial biomass was calculated and expressed as μg C/ gram soil.





Microscopic image of soil bacteria (a) and fungal hyphae (b) (from Bloem et al., 1997).

Fungi in soil smears were stained with differential fluorescent stain, a mixture of two stains: fluorescent brightener which binds to cell walls (polysaccharides) and europium chelate which binds to nucleic acids (DNA and RNA). The total amount of fungal hyphae in soil was determined by measuring hyphal length under the microscope. The total hyphal length was used to calculate fungal biomass in terms of μg C/ gram soil (Bloem and Vos, 2004).

Potentially mineralizable nitrogen was measured by incubation of a soil sample under water (in slurry) for 1 week at 40°C (Keeny en Nelson, 1982; Canali en Benedetti, 2006). These warm and anoxic conditions are optimal for a quick mineralization of organic matter by anaerobic bacteria. The lack of oxygen prevents conversion of released NH_4 to NO_3 (nitrification) and uncontrolled N losses by denitrification can not occur. The amount of mineral nitrogen (NH_4-N) released is a measure of the quality (N-content and decomposability) of the organic matter, and thus for biological soil fertility.

Hot water extractable carbon was determined as the amount of dissolved organic carbon that is released during incubation of a soil sample in hot water during 16 hours at 80°C (Gani et al, 2003). This is a measure of easily decomposable (labile) organic carbon. This fraction is important food for bacteria and fungi and is also correlated with soil aggregate stability (formation of clay-humus complexes).

3. Results and Discussions

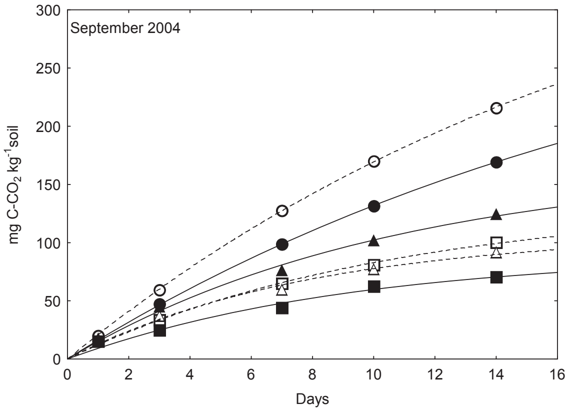
Results of organic matter characterization in soil are shown in *Table 1* as average content of all sampling because of no differences were found between them.

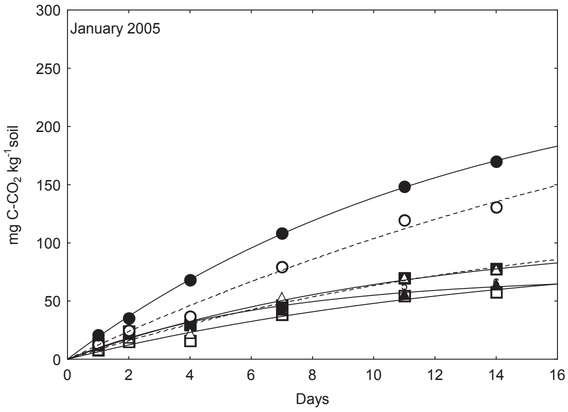
It's possible to notice a consistent similarity in total organic carbon values, C_{org}, for all managements while the extractable, C_{ext}, and humic and fulvic fraction, C_{HAFA}, of organic matter are significantly lower in both layer of Sludge treatment.

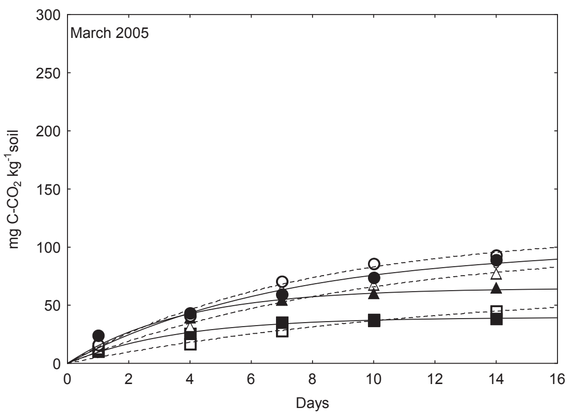
Table 1. Organic matter characterization. C_{org} = total organic carbon, g C 100g^{-1} soil; C_{ext} = total extractable carbon, g C 100g^{-1} soil; C_{HAFA} = humic and fulvic fraction of organic carbon g C 100g^{-1} soil; DH = humification degree, %; HR = humification rate, %. For each parameter different letter indicate significant differences (LSD test).

Site	Depth (cm)	C_{org}	C_{ext}	C_{HAFA}	DH	HR
Biodynamic	0-15	1.09	0.85	0.45	53.7	38.9
	15-30	1.05	0.81	0.46	56.1	45.3
Manure	0-15	1.14	0.87	0.45	47.0	45.5
	15-30	1.05	0.83	0.37	41.6[a]	40.2
Sludge	0-15	1.06	0.73[a]	0.33[a]	37.6[a]	38.0
	15-30	1.03	0.70[a]	0.34[a]	41.2[a]	40.7

Humification parameters indicate a better situation in Biodynamic and Manure treatments (0-15) cm layer compared to Manure (15-30) cm layer and Sludge treatment where values of humification degree (DH) are significantly lower in both layer then other soils. This fact can reveal a better conservation of organic matter in Biodynamic management and a sink function of soil. More intensive agricultural practices in Sludge management affect humification processes in soil. No significant differences were found in humification rate (HR).







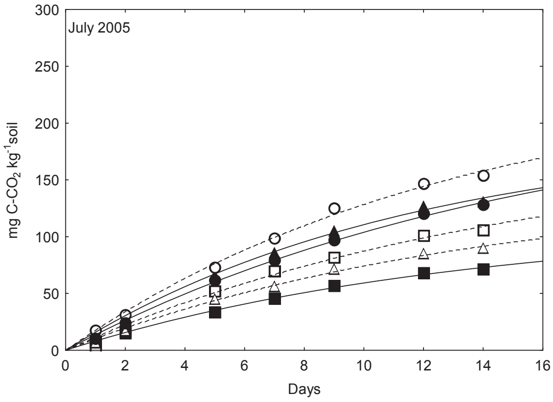


Figure 1. Soil respiration kinetic as cumulative CO_2-C evolution. (Ball = Biodynamic; Triangle = Manure; Square = Sludge. Full symbol and continue line indicate (0-15) cm layer; empty symbol and dotted line represent (15-30) cm layer). (StaSoft Italia 6.0)

Figure 1 shows soil respiration as CO_2-C evolution. Biodynamic management presents the maximum values of kinetic curves and maximum potentially mineralizable carbon (C_0 in *Table 2*) in all samplings respect to other managements. March 2005 sampling put in evidences the lowest curves and lowest microbial activity for all managements. Organic matter decomposition rates show low mineralization kinetic constant values, k_{MIN}, in Biodynamic management respect to Manure and Sludge treatments for all sampling, with the exception in July 2005 sampling. In the same time it's possible to observe in each treatment higher values of k_{MIN} in March sampling (*Table 2*).

Table 2. Kinetic parameters of organic matter decomposition processes: k_{MIN} = mineralization kinetic constant (1/days); C_0 = potentially mineralization carbon (mg CO_2-C kg^{-1} soil). (StaSoft Italia 6.0). For each parameter different letter indicate significant differences (LSD test).

Site	Depth	September 2004		January 2005		March 2005		July 2005	
	(cm)	k_{MIN}	C_0	k_{MIN}	C_0	k_{MIN}	C_0	k_{MIN}	C_0
Biodynamic	0-15	0.048[a]	344[a]	0.077	259[a]	0.223[a]	80	0.063	222[a]
	15-30	0.051[a]	424[a]	0.037[a]	334[a]	0.123	119[a]	0.076	242[a]
Manure	0-15	0.094	168	0.148[b]	71[b]	0.274[a]	64	0.087	190
	15-30	0.127	108	0.063	135	0.140	84	0.073	143
Sludge	0-15	0.107	91	0.088	106	0.266[a]	40[b]	0.075	112
	15-30	0.095	135	0.067	98	0.119	46[b]	0.065	182

Low mineralization curves in March 2005 sampling (*Figure 1*) could be explain considering natural competition for nutrients and energy substrate in spring months between micro organism and crops (grassland in Biodynamic and Manure treatments and maize crop in Sludge management). Besides, similarly between management, results indicate a brief and intensive activity of microbial populations in organic matter decomposition corresponding to the sampling of March 2005 as showed by high kinetic constants values, k_{MIN}. In January 2005 microbial activity is characterized by

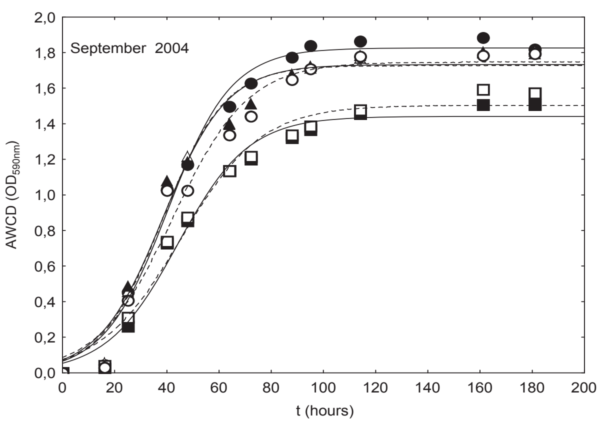
lower values of k_{MIN}, according to very low temperature characterizing cold months.
On the contrary highest mineralization curves in September 2004 are well representative of typical situation in Mediterranean area, where maximum microbial activity is expected in mild autumn but not during hot-sultry summer (Gallardo et al., 1991). In the same time this fact explain also the high curves in July sampling when the irrigation generates dry-rewetting effect of soil with a characteristic flush in microbial activity (Riffaldi et al., 2003).

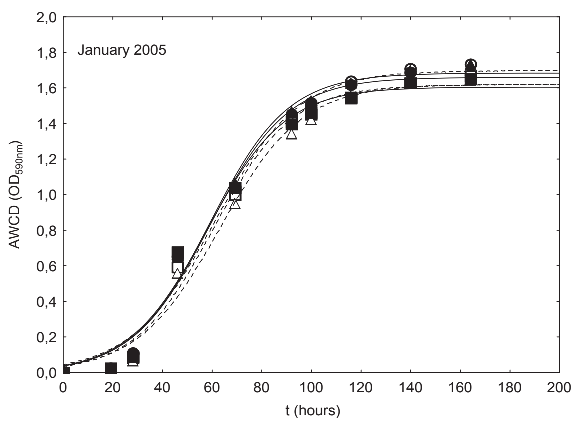
Values of qCO_2 are not too high (*Table 3*). In fact, metabolic quotient, qCO_2, shows an average good situation for all sampling and managements with the exception of March 2005, especially in deeper layer of Sludge treatment. This result, according to mineralization curves, can be due to the competition for nutrients during spring months and put in evidence a more stressed microbial community respect to other sampling.

Table 3. Eco-physiological indexes. qCO_2 = metabolic quotient, CO_2-C evolution per 100 g of microbial biomass (g CO_2-C 100 g^{-1} C_{mic} h^{-1}); qM = mineralization quotient, total microbial activity as CO_2-C evolution respect to total organic carbon (g CO_2-C 100 g^{-1} C_{org}). For each parameter different letter indicate significant differences (LSD test). No letter indicates similarity between values.

Site	Depth	September 2004		January 2005		March 2005		July 2005	
	(cm)	qCO_2	qM	qCO_2	qM	qCO_2	qM	qCO_2	qM
Biodynamic	0-15	0.26	1.23[a]	0.32	1.16	0.19	0.55	0.29	0.82
	15-30	0.19	1.44[a]	0.23	1.24	0.55[a]	0.95[a]	0.34	1.08[a]
Manure	0-15	0.10[a]	0.70	0.08[a]	0.36[a]	0.27	0.44	0.18[a]	0.81
	15-30	0.24	0.65	0.45[b]	1.11	2.00[b]	0.63	0.14[a]	0.55
Sludge	0-15	0.09[a]	0.46	0.15	0.58[a]	0.58[a]	0.37	0.27	0.51
	15-30	0.32	0.75	0.18	0.73	0.20	0.42	0.23[a]	0.79

On the contrary mineralization quotient values, qM in *Table 3*, show a good situation only in Biodynamic treatment and also in this case with the exception of March sampling. In fact, as reported in Dommergues (1960) organic matter addition on soil implies a decreasing of qM values because of the promotion of microbial activity, as can be observed in Manure and Sludge treatments where qM values put in evidence an elevated mineralization activity respect to total organic matter availability.
Scientific evidences demonstrated how the quality of organic matter added to soil can affect microbial C-use efficiency. In particular mineralization processes of high quality organic matter take more time (lower values of k_{MIN}) respect to mineralization time requested for low quality organic matter (Benedetti and Sebastiani, 1996; Alianello and Benedetti, 1994). Infact too quickly mineralization processes (high k_{MIN} values) could make available excessive amount of nitric and ammoniac nitrogen. Besides the application on soil of organic sludge causes a phenomenon named "priming effect" that result by stimulation of microbial activity processes. As consequence micro organisms consume more carbon than that one added on soil (Benedetti, 2004).





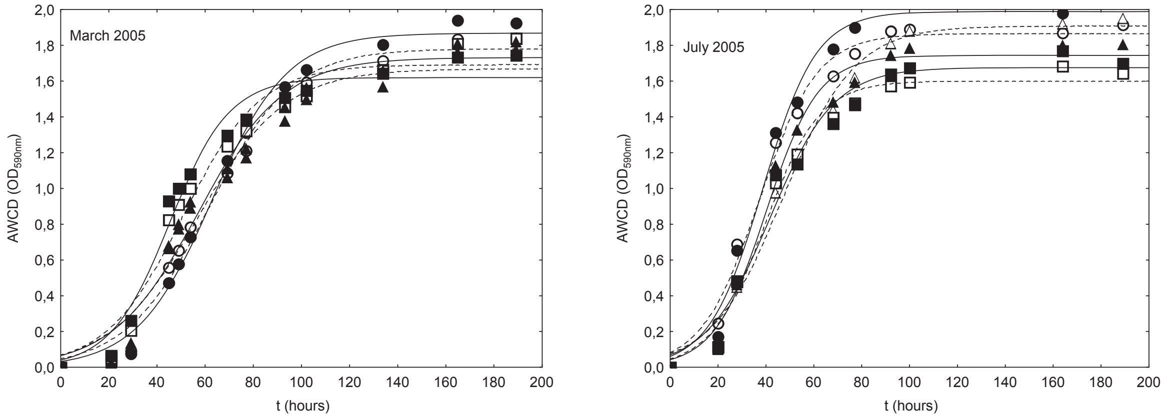


Figure 2. Community level physiological profile. (Ball = Biodynamic; Triangle = Manure; Square = Sludge. Full symbol and continue line indicate (0-15) cm layer; empty symbol and dotted line represent (15-30) cm layer). (StaSoft Italia 6.0)

Table 4. Kinetic parameters of microbial potential activity growing curves (community level physiological profile). kCLPP = potential rate of increase of growing curve (1/hours); I_g = potentially microbial activity as growing curve integration (OD_{590nm} h^{-1}). (StaSoft Italia 6.0). For each parameter different letter indicate significant differences (LSD test).

Site	Depth (cm)	September 2004		January 2005		March 2005		July 2005	
		kCLPP	Ig	kCLPP	Ig	kCLPP	Ig	kCLPP	Ig
Biodynamic	0-15	0.064	272.4	0.064	192.0	0.064	255.9	0.089	314.5
	15-30	0.059	261.9	0.059	194.9	0.058	248.3	0.083	298.8
Manure	0-15	0.064	211.0[a]	0.064	194.9	0.057	249.6	0.092	274.7 [a]
	15-30	0.060	259.5	0.060	182.2	0.055	241.4	0.067	290.1
Sludge	0-15	0.063	260.6	0.063	187.8	0.081[a]	246.5	0.075	262.2 [a]
	15-30	0.063	220.1 [a]	0.063	185.2	0.063	252.4	0.088	251.6 [a]

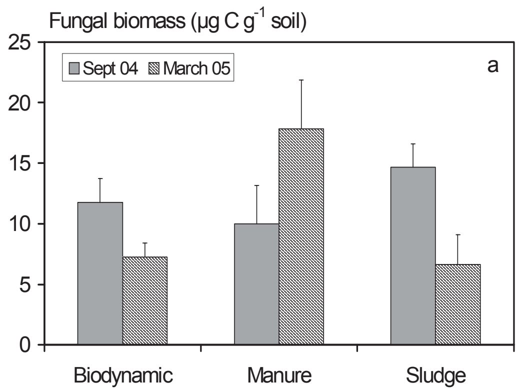
About community level physiological profile analysis, Biodynamic management presents high potential microbial activity in all sampling, Ig values in *Table 4*, while Manure and Sludge managements soils show always a lower potential microbial activity. In the same time it's possible to observe less potential activity in January 2005 for all sampling and managements. This evidence can be observed by equivalent values of Ig and kCLPP to indicate a similar behaviour of microbial communities in all managements during cold months.
These results fit very well with organic matter mineralization curves.
We conclude that functional diversity of microbial communities in the investigated soils is not affected by the different management practices. Probably, more information about genetic composition of microbial communities could reveal changing as reported in literature for heavy metals or human activity impact (Ovreas and Torsvik, 1998; Schloter et al., 2005).

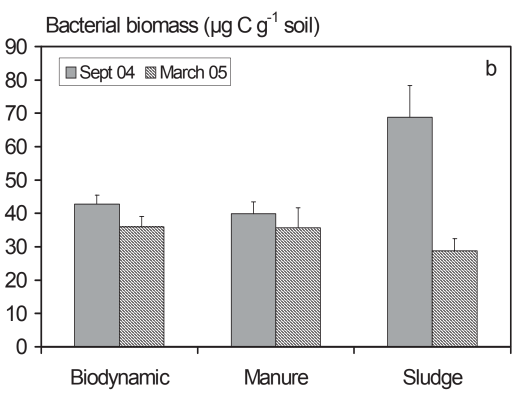
Although fungal biomass was significantly higher in the Manure treatment in March (*Figure 4a*), and bacterial biomass was significantly higher in the Sludge treatment in September (*Figure 4b*), there was not a consistent difference in fungal and bacterial biomass between the three sites. However, for a firm conclusion more than two sampling dates are needed.
In our experience nitrogen mineralization and hot water extractable carbon are less variable than amounts of bacteria and fungi. Both, potentially mineralizable N (labile N) and hot water extractable carbon (labile C) showed a consistent pattern with significantly lower values in the Sludge treatment (one way analysis of variance, $p < 0.05$). This was found on both dates with both parameters which are related to the availability of easily decomposable organic matter. Higher values are supposed to be more "sustainable" (Gani et al., 2003). A higher amount of nitrogen available for mineralization by soil microbes (mineralizable N) indicates higher biological soil fertility because nitrogen is usually the main limiting factor for crop production. A higher amount of hot water extractable carbon indicates a higher availability of food for micro organisms. Carbon is usually the growth limiting factor for soil micro organisms. More intensive land-use involving soil tillage, fertilization and grazing, stimulates microbial decomposition and tends to result in a net decrease in the labile carbon pool and ultimately in a decrease in total soil organic matter, aggregate stability and biodiversity.

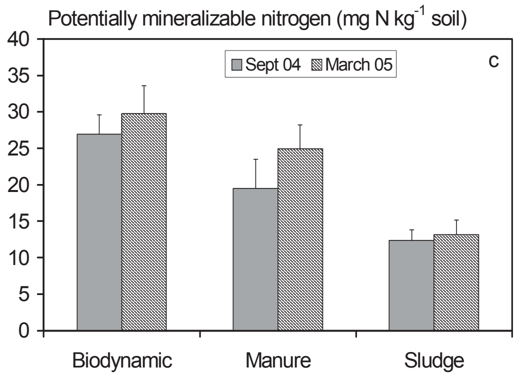
The levels of fungal and bacterial biomass of 10 and 40 µg C/g soil, respectively, are relatively low and characteristic for regularly ploughed arable fields. Less tilled grassland soils usually contain at least two-fold higher levels around 20 µg fungal and 100 µg bacterial C per gram soil (Bloem et al., 2006). Also the levels of mineralizable N (10-30 mg N/kg soil) and hot water extractable carbon (200-700 µg C/ g soil) are relatively low. Considerably higher levels of 100-200 mg N/kg and 1000-3000 µg C/g are characteristic for grassland soils (Gani et al., 2003; Sparling et al., 2003; unpublished results of soil quality monitoring in the Netherlands)..

The significantly lower levels of mineralizable nitrogen and hot water extractable carbon in the Sludge treatment compared to the Biodynamic and Manure treatments are in agreement with significantly lower amount of total extractable carbon and the lower humic and fulvic acid fraction of organic carbon (*Table 1*).

The differences between the Biodynamic site and the Manure site are less consistent. Potentially mineralizable nitrogen tends to be higher in Biodynamic, but this is statistically not significant *(Figure 4c*). However, the potentially mineralizable carbon (C_0, *Table 2*) was significantly higher in the Biodynamic treatment.







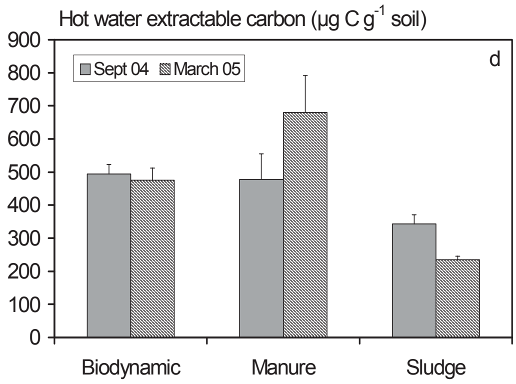


Figure N. 4. Biomass of fungi (a) and bacteria (b), potentially mineralizable nitrogen (c) and hot water extractable carbon (d) in soils with different management, sampled in September 2004 and March 2005

4. Conclusions

Microbial and biochemical indicators ware measured in samples of three agricultural fields on farms with different management:

- grassland under biodynamic management (Biodynamic)
- grassland with application of farm yard manure and mineral fertilizer (Manure)
- maize cultivation with sewage sludge amendment (Sludge)

There were no consistent differences in a range of microbial indicators between the three sites. Also the total soil organic carbon content did not show significant differences. However, potentially mineralizable N (labile N) and hot water extractable carbon (labile C) showed a consistent pattern with significantly lower values in the Sludge treatment. Soils with higher values are supposed to be more "sustainable". A higher amount of nitrogen available for mineralization by soil microbes (mineralizable N) indicates higher biological soil fertility. A higher amount of hot water extractable carbon indicates a higher availability of food for micro organisms. More intensive land-use involving soil tillage, fertilization and grazing, stimulates microbial decomposition and tends to result in a net decrease in the labile carbon pool and ultimately in a decrease in total soil organic matter, aggregate stability and biodiversity.

The lower levels of mineralizable nitrogen and hot water extractable carbon are in agreement with the significantly lower amount of total extractable carbon and the lower humic and fulvic acid fraction of organic carbon found in the Sludge treatment compared to the Biodynamic and Manure treatments. The differences between the Biodynamic site and the Manure site are less consistent. Potentially mineralizable nitrogen tends to be higher in Biodynamic, but this is statistically not significant. However, the potentially mineralizable carbon (C_0) was significantly higher in the Biodynamic treatment.

REFERENCES

Alianiello F and Benedetti A: Effects of agricultural practices on mineralization kinetics of organic nitrogen. In: J.J. Neeteson and J. Hassink (eds.) *Nitrogen mineralization in agricultural soils*. Proceedeing Symposium in Haren, NL, 19-20 April 1993, 1994.

Anderson TH and Domsch KH: Application of ecophysiological quotients (qCO2 and qD) on microbial biomass from soils of difference cropping histories. "*Soil Biology and Biochemistry"*, 1990, n. 10, p. 251-255.

Anderson T-H: Microbial eco-physiological indicators to asses soil quality. "*Agricolture Ecosystem and Environment"*, 2003, n. 98, p. 285-293.

Benedetti A: La sostanza organica e l'azoto dei fanghi di depurazione delle acque. In: Figliolia A. e Benedetti A. (eds.) *I fanghi di depurazione delle acque*. Vol. 4. Progetto Editoriale PANDA Ministero delle Politiche Agricole e Forestali, 2004.

Benedetti A and Sebastiani G: Determination of potentially mineralizable nitrogen in agricultural soil. "*Biology and Fertility of Soils"*, 1996, n. 21, p. 114-120.

Bloem J, Veninga M, Shepherd J: Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. *"Applied and Environmental Microbiology"*, 1995, n. 61, p. 926-936.

Bloem J, De Ruiter PC, Bouwman LA: Food webs and nutrient cycling in agro-ecosystems. In: Van Elsas JD, Trevors JT, Wellington E, eds, Marcel Dekker Inc. New York, *Modern Soil Microbiology*, 1997, p. 245-278.

Bloem J, Vos A: Fluorescent staining of microbes for total direct counts. In: Kowalchuk GA, De Bruijn FJ, Head IM, Akkermans ADL and Van Elsas JD, eds, Kluwer Academic Publishers, Dordrecht, 2nd edition. *Molecular Microbial Ecology Manual,* 2004, p. 861-874.

Bloem J, Schouten AJ, Sørensen SJ, Rutgers M, Van der Werf A, Breure AM: Monitoring and evaluating soil quality. In: Bloem J, Benedetti A, Hopkins DW eds), CABI, Wallingford, UK. *Microbiological Methods for Assessing Soil Quality,* 2006 p. 23-49.

Canali S, Benedetti A: Soil nitrogen mineralization. In: Bloem J, Benedetti A, Hopkins DW eds), CABI, Wallingford, UK. *Microbiological Methods for Assessing Soil Quality,* 2006, p. 127-135.

Ciavatta C, Govi M, Vittori Antisari L, Sequi P: Characterization of humified compounds by extraction and fractionation on soild polyvynilpyrrolidone. "*Journal. of Chromatography*, 1990, n. 509, p. 141-146.

Dommergues Y: La notion de coefficent de mineralisation du carbone dans les sols. *L'agronomie Tropicale*, 1960, n. 15, p. 54-60.

Doran JW, Parkin TB: Defining and Assessing Soil Quality, Soil Science Society of America, 677 Sogoe Rd., Madison WI 53711, USA. In: Defining Soil Quality for a Sustainable Environment. ISSS Special Publication n.35, 1994.

Gallardo JF, Martin V, Santa Regina I: Dynamics of leaf decomposition in forest ecosystem of the Sierra de Gata (Province of Salamanca, Spain), In: Proceedings of the 6th IHSS Congress, 1993 p.335 – 343.

Ghani A, Dexter M, Perrott KW: Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. "*Soil Biology and Biochemistry"*, 2003, n. 35, p. 1231–1243.

Garland JL, Mills AL: Classification and Characterization of heterotrophic microbial communities on the basis of patterns of Community-Level Sole-Carbon-Source Utilization. "*Applied and Environmental Microbiology"*, 1991, n. 57, p. 2351-2359.

Guckert JB, Carr GJ, Johnson TD, Hamm BG, Davidson DH, Kumagai Y: Community analysis by Biolog: curve integratin for statistical analysis of activated sludge microbial habitats. *"Journal of Microbiological Methods"*, 1996, n. 27, p. 183-197.

Hargreaves PR, Brookes PC, Ross GJS Poulton PR: Evaluating soil microbial carbon as indicator of long-term environmental change. *"Soil Biology and Biochemistry"*, 2003, n. 35, p. 401-407.

Holloway JD, Stork NE: The dimension of biodiversity: the use of invertebrates as indicators of human impact. In: Hawksworth D.L. (Ed.) *The biodiversity of microorganisms and invertebrates: Its role in sustainable agriculture*. WEFSA 1, Vol. 4, CAB International, London, 1991, p. 37-62.

Isermeyer H: Eine einfache Methode sur Bestimmung der Bodenatmung und der Karbonate im Boden. *Z. Pflanzanernah Bodenkunden*, 1952, n. 56, p. 26-38.

Keeney DR, Nelson DW: Nitrogen - Inorganic forms. In: Black CA, Evans DD, White JL, Ensminger LE, Clark FE (Eds.) *Methods of soil Analysis*, Part 2Madison WI: Am Soc Agron, 1982, p.682-687.

Lindstrom JE, Barry RP, Braddock JF: Microbial Community Analysis: a kinetic approach to constructing potential C source utilization patterns "*Soil Biology and Biochemistry"*, 1998, n. 30, p. 231-239.

Nannipieri P, Grego S, Ceccanti B: Ecological significance of the biological activity in soil. In: Bollag J-M, Stotzky G (Eds), *Soil Biochemistry*, Marcel Dekker, New York, 6, 1990, p. 293-355.

Ovreas L and Torsvik VV Microbial diversity and community structure in two different agricultural soil communities *Microbial Ecology,* 1998, n. 36, p. 303-315.

Riffaldi R, Saviozzi A, Levi-Minzi R: Carbon mineralization kinetics as influenced by soil properties. *"Biology and Fertility of Soil"*, 1996, n. 22, p. 293-298.

Riffaldi R, Saviozzi A, Levi-Minzi R, Cardelli R: Conventional crop management effects on soil organic matter characteristics. Agronomie 2003, n. 23, p. 45-50.

Schloter M, Dilly O, Munch JC: Indicators for evaluating soil quality. *"Agriculture Ecosystem and Environment"*, 2003, n. 98, p. 255-262.

Schloter M, Bergmuller C, Friedel J, Hartmann A and Munch JC: Effect of different farming practice on the microbial community structure *Applied and Environmental Microbiology (submitted).*

Sequi P: *Chimica del Suolo*, Patron Ed. Bologna, 1989, 615 pp.

Sparling G P, Lilburne L, Vojvodic-Vukovic M: *Provisional targets for soil quality indicators in New Zealand*. Landcare Research New Zealand, Palmerston North, N.Z., 2003.

Springer U, Klee J: Prüfung der Leistungsfähigkeit von einigen wichtigeren Verfahren zur Bestimmung des Kohlemstoffs mittels Chromschwefelsäure sowie Vorschlag einer neuen Schnellmethode. *Z. Pflanzenernähr. Dang. Bodenkundem*, 1954, n. 64, p. 1.

Stotzky G: Soil as an environment for microbial life. In: Van Elsas JD, Trevors JT, Wellington MH (Eds), *Modern soil microbiology*, Marcel Dekker, New York, 1997, p. 1-20.

Vance ED, Brookes PC, Jenkinson DS: An extraction method for measuring microbial biomass C. *"Soil Biology and Biochemistry"* 1987, n. 19, p. 703-707.

Yakovchenko V, Sikora LJ, Kaufman DD A biologically based indicator of soil quality. *"Biology and Fertility of Soils"*, 1996, n. 21, p. 245-251.