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Soil microbial dynamics in organic (biodynamic) and integrated apple orchards

B. P. Bougnom · B. Greber · I. H. Franke-Whittle · C. Casera · H. Insam

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Abstract Biodynamic (organic) and integrated apple orchards can differ in their soil chemical and microbiological properties which may have implications for disease control and long-term soil fertility. Soil samples were collected from three different apple orchards (each with biodynamic and integrated plots) during three different seasons. Physical and chemical characteristics of the soils under the two management systems did not show clear differences. Basal respiration and microbial biomass were higher in organic farms, while the metabolic quotient in organic farms was lower (or equal in one case) to that of integrated farms. There were also seasonal variations. Principal component analysis of the DGGE banding patterns of dominant fungal communities revealed no influence of the farming practices. Analysis of the bacterial communities with the COMPOCHIP microarray showed that certain probes targeting bacteria known to be degraders of biocides were predominantly found in integrated farming plots, while no difference between the two management practices concerning the presence of plant growth promoting rhizobacteria was evident. In conclusion, despite the reduced use of chemicals in integrated farming (compared to conventional), soil microbial properties (CO_2 production and biomass) were different to those found in biodynamic farming, while the microbial spectrum did not significantly change.

Keywords Biodynamic farming, organic farming, apple orchards · Microbial biomass · Microbial community · Microarray

B. P. Bougnom · B. Greber · I. H. Franke-Whittle · H. Insam (✉)
Institute of Microbiology, University of Innsbruck,
Technikerstraße 25 d,
6020 Innsbruck, Austria
e-mail: heribert.insam@uibk.ac.at

B. P. Bougnom
Laboratory of Microbiology, Department of Biochemistry,
University of Yaounde 1,
P.O. Box 812, Yaounde, Cameroon

C. Casera
Research Centre Laimburg,
Vadena,
39040 Posta Ora, Bolzano, Italy

Introduction

Soil microbes, the living part of soil organic matter, are involved in many biochemical processes, including the release of nutrients from organic matter, making the nutrients available to plants (e.g., N, P, and S) as well as the degradation of toxic materials (Dalal 1998; Friedel et al. 2001; Buckley and Schmidt 2003). This, in turn, likely promotes the soil fauna (e.g., earthworms, ants, or mites; Albiach et al. 2001). The build-up of a large and active soil microbial biomass is therefore critical for a qualified food web and ultimately for a more sustainable agriculture (Fließbach and Mäder 1997; Reganold et al. 2001; Oehl et al. 2004; Birkhofer et al. 2008).

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Organic agriculture is defined as a farm production system that sustains the productivity of soils and health of plants and relies upon a natural balance to conserve resources. It further aims at building fair producer–consumer relationships that account for social and environmental costs (IFOAM 2006). The use of mineral fertilizers as well as many pesticides and herbicides is not allowed, or most strongly restricted. Biodynamic agriculture moreover uses special fermented herbal preparations and mineral mixes as compost additives and plant treatments (Carpenter-Boggs et al. 2000) and targets at balancing the interrelationship of soil, plants, and animals and their holistic development.

Integrated farming can be described as integrating natural regulation processes with farming activities to replace off-farm inputs as much as possible. It aims at sustaining quality production with minimal external inputs and environmental hazards (Wijnands 1997). The use of mineral fertilizers and chemical crop protection is allowed but reduced to a minimum based on detailed soil and climate analyses as well as pest monitoring.

Since sustainability issues are taken more and more into consideration in agriculture, integrated and organic farming practices have been adopted for the management of apple orchards by many farmers. However, in South Tyrol, one of the major European apple-growing regions, it has been observed that apple replant disease (ARD) is found in integrated farms but to a much lesser extent in organic farms (Josef Dalla Via, personal communication). The causes of ARD are not known, but it has been hypothesized that it is connected to the occurrence of specific antagonistic microorganisms (Mazzola 1998). Understanding how soil microorganisms respond to different farming practices may add to our understanding of the integral consequences of organic versus integrated farming systems in affecting the soil ecosystem functioning. This study aims at investigating seasonal dynamics of soil microbial communities in south Tyrolean apple orchards and compares organic (more specifically, biodynamic) and integrated farming practices.

Materials and methods

Experimental design

The experiment was conducted at three established Golden Delicious apple farms in South Tyrol, Italy (46°38′ N, 11°29′ E). Each farm had a biodynamic (BIO; Demeter certification) and an integrated field (INT; according to CODEX), thus providing pairwise sites. Each field was further subdivided into five plots.

The trees in the biodynamic fields are about 30 years old. Two of the farms had been managed biodynamically since 1981, the third one since 1990. The trees in the integrated fields 1, 2, and 3 are about 20, 25, and 5 years old, respectively. Fields 1 and 2 have been managed in an integrated way for 20 years, while field 3 has been managed using an integrated approach for 5 years (since planting). In the biodynamic fields, cow manure was applied topically each year at a rate of 7 $t ha^{-1}$ in the fall. The integrated fields received soil-analysis-based mineral fertilizer at a rate of 60 kg N, 100 kg K, and 25 kg P_2O and the necessary chemical treatments mainly for control of fungal pathogens. Both biodynamic and integrated fields received an extensive treatment of 200 kg lime sulfur ha^{-1} as a fungicide using a motorized sprayer. Overhead irrigation was standard at all sites.

Soil sampling

Five composite soil samples were collected from each plot (experimental unit) using a 2.5-cm-diameter corer (0–20 cm depth). From each plot, five subsamples (2 cm diameter soil cores) were randomly taken from the tree rows and five subsamples from the drive lane. These ten subsamples were combined and constituted a composite sample. That was done five times, and these five composite samples were combined, constituting one observation.

The sampling dates were spring 2007 (May 25), prior to the onset of the main growth period, summer 2007 (July 18) during the main growth period, and autumn 2007 (October 17), prior to leaf senescence and prior to manure application. The soil samples were sieved (2 mm sieve) to remove larger roots, stones, and animals. One part of the samples was air-dried for physical and chemical analysis, another part was frozen at −20°C, and the remainder was kept at 4°C for biological analysis.

Physical and chemical analysis

Soil physical and chemical analyses were made using the official *Verband der Deutschen Landwirtschaftlichen Untersuchungs- und Forschungsanstalten* method (Hoffmann 1997). The different physical and chemical parameters measured are given in Table 1.

Table 1 Physical and chemical characteristics of the soils collected from three farms and their BIO and INT management plots

	Farm I		Farm II		Farm III	
	BIO I	INT I	BIO II	INT II	BIO III	INT III
Sand (%)	30	35	59	36	16	15
Silt (%)	63	60	37	54	76	76
Clay (%)	7	5	4	10	8	9
pH in $CaCl_2$	7.02	7.11	7.24	7.05	7.11	7.15
P_2O_5 (mg g^{-1})	0.25	0.41	0.14	0.23	0.46.	0.39
K_2O (mg g^{1})	0.20	0.15	0.17	0.17	0.24.	0.22
B (mg g^{-1})	0.65	0.77	0.50	0.73	0.94	1.05
Humic acids (C, mg kg^{-1})	8.5	4.3	7.9	11.4	5.3	3.4
Fulvic acids (C, mg kg^{-1})	3.6	2.8	8	6	5.1	3
Total organic carbon (C, mg g^{-1})	42.1	27	37.4	56.6	39.5	36.7
Humification rate (%)[a]	28.7	26.3	42.5	30.7	26.3	17.4
N (mg kg^{-1})	18	19	23	20	27	23
C/N	10.1	10.4	10.9	10.2	10	9.20
Ca (mmolc kg^{-1})	167	209	209	180	224	192
Mg (mmolc kg^{-1})	23.2	24	32.7	20.3	35.8	32.5
K (mmolc kg^{-1})	4.74	4.13	4.15	4.11	5.85	5.69
Na (mmolc kg^{-1})	0.64	0.66	1.35	0.47	0.69	0.75
CEC (cmol kg^{-1} dm)	19.5	23.8	24.7	20.5	26.7	23.2

BIO biodynamic, *INT* integrated

[a] Humification rate: HR (%)=(HA+FA)/TOC×100

Microbiological analysis

Basal respiration and substrate induced respiration

Basal respiration was measured as CO_2 evolution from moist soil samples (60% water holding capacity) at 22°C using a continuous flow infrared system (Heinemeyer et al. 1989). Readings were taken after 14 h incubation. Microbial biomass carbon was determined by substrate-induced respiration (SIR), as described by Sparling (1995) after the addition of 1% glucose (dry matter basis) to the samples and measuring CO_2 evolution for 6 h. The metabolic quotient qCO_2 (respiration-to-biomass ratio) was calculated (Anderson and Domsch 1993).

DNA extraction and polymerase chain reaction amplification

Soil DNA extraction was carried out using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentration was determined by fluorescence using a PicoGreen® dsDNA quantitation kit (Molecular Probes Inc., Eugene, OR, USA) and a fmax Fluorescence Microplate Reader (Molecular Devices, Sunnyvale, CA, USA), as described by the manufacturer. Polymerase chain reaction (PCR) targeting the 18S rRNA gene was conducted using the fungal primers described by Vainio and Hantula (2000), FF390 (5′CGATAACGAACGAGACCT3′) and FR1GC (5′CGATAACGAACGAGACCT 3′). A GC clamp was added to the 5′-end of the reverse primer to stabilize the melting behavior of the PCR product (Muyzer et al. 1993). The PCR mixture was prepared in 25 µl volumes with 0.5 ng of extracted DNA, 1× reaction buffer (16 mM$(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 1.5 mM $MgCl_2$, 0.01% Tween 20) (GeneCraft, Münster, Germany), 1 mM $MgCl_2$ (50 mM), 0.2 µM of each primer, 0.2 mM dNTPs, 2% DMSO, 0.5 µg BSA, 1.25 U Taq polymerase, and purified water. PCR amplifications were performed in

a ThermoHybaid PCR Express thermocycler. The amplification program was as follows: 5 min denaturation at 94°C, then 35 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. Thermal cycling was completed with an extension step at 72°C for 10 min. PCR products were checked by running 2 μl amounts of PCR products on 1.5% agarose gels and staining with ethidium bromide.

PCR product quantification and DGGE analysis

DNA concentration of PCR products was determined by fluorescence using the PicoGreen® dsDNA quantitation kit (Molecular Probes Inc., Eugene, OR, USA) and a fmax Fluorescence Microplate Reader (Molecular Devices, Sunnyvale, CA, USA), as described by the manufacturer. DGGE was conducted according to Duineveld et al. (2001), using 7.5% acrylamide gels with a denaturing gradient of 30% to 60%. PCR products (60 ng) of different replicates were loaded on the gel. DGGE was performed in 1× Tris–acetate–EDTA buffer at 60°C using an INGENYphorU electrophoresis tank (Ingeny International, Goes, the Netherlands) at a constant voltage of 100 V for 16 h. Gels were silver-stained with a Hoefer Automated Gel Stainer (Pharmacia Biotech, Germany), dried at room temperature, and scanned (Epson, Klosterneuburg, Austria).

Microarray analysis

The COMPOCHIP microarray, spotted with 401 probes targeting bacteria that have been previously reported in composting processes, as well as plant, animal, and human pathogens and plant disease suppressive bacteria, was used to analyze the soil samples. The sampling was carried out previously explained. Fluorescence labeling of target DNA, hybridization on aldehyde coated epoxy microscope slides, scanning, and image analysis was conducted as described by Franke-Whittle et al. (2005, 2009).

Statistics

For physical and chemical data, a principal component analysis (PCA) was conducted with the different observations made from the different plots as previously explained. The principal components were subjected to a one-way ANOVA, with farms as blocks, using Genstat 11.0 (VSN International Ltd., England). For basal respiration and SIR, the data obtained were subjected to a two-way analysis of variance (ANOVA), with treatment (BIO and INT), farms (Blocks), and time (autumn, spring, and summer) as factors.

From DGGE, composite datasets, based on the number of fungal bands in the different samples, were generated using the Gel-Compar II software package, version 4.0 (Applied Maths, Ghent, Belgium). A correlation matrix between the different treatments was analyzed by PCA, and PCA scores were analyzed by one-way ANOVA, with farms as blocks, using Genstat 11.0 (VSN International Ltd., England). Based on the total signal-to-noise ratio (SNR) of the microarray data, a PCA was conducted, a biplot was generated, and PCA scores were analyzed by one-way ANOVA, with farms as blocks, using CANOCO for windows 4.5 (Ter Braak and Smilauer 2002).

Results

Physical and chemical characteristics

Physical and chemical characteristics of the soils collected from the farms are given in Table 1. PCA (Fig. 1) showed components 1 and 2 to explain 69.5% of the total variance. For farms I and II, differences between integrated and organic farming were evident, and this was not the case for farm III. ANOVA of the two principal components (PCs) showed no significant differences between the two farming systems. Positive interactive effects of treatment and farm site were found along axis 1.

Microbial activity and biomass

CO_2 production, microbial biomass, and metabolic quotient from soil samples collected from the three farms in spring, summer, and autumn are summarized in Table 2. According to ANOVA, significant differences were found among the two farming types for CO_2 production ($\alpha \leq 0.05$) and microbial biomass ($\alpha \leq 0.001$), but not for the metabolic quotient. The differences were significant among the different times of sampling for CO_2 production ($\alpha \leq 0.01$), microbial biomass ($\alpha \leq 0.01$), and metabolic quotient ($\alpha \leq 0.050$). Basal respiration was higher in BIO plots; it was higher in summer than in spring and autumn. The

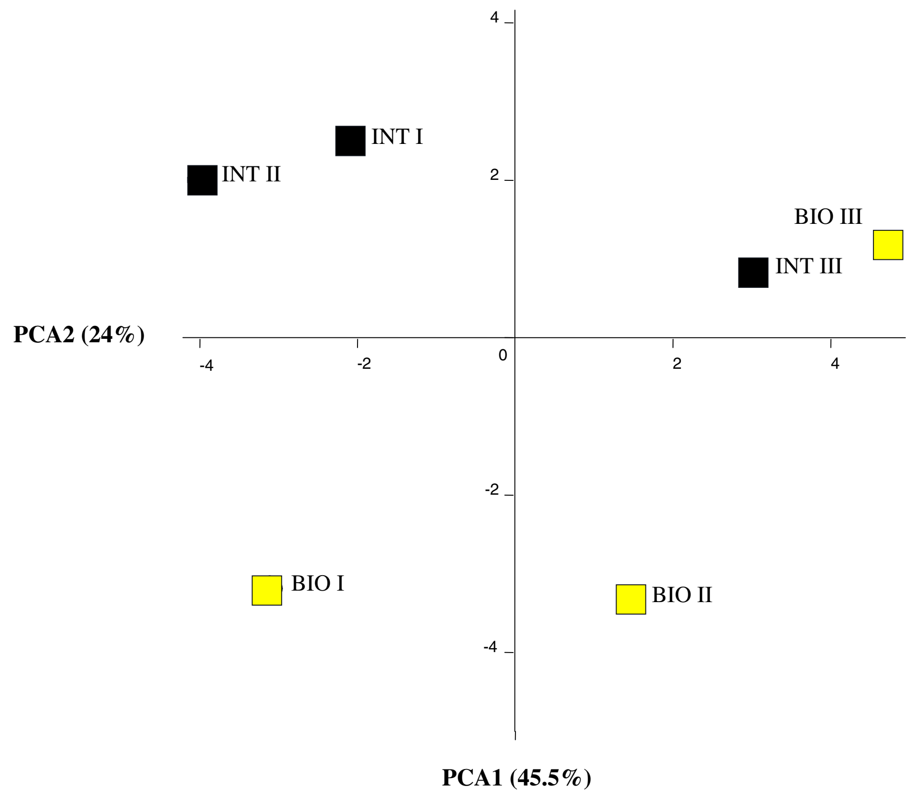

Fig. 1 PCA plots showing differences in the physical and chemical characteristics of the organic and integrated plots of the different farms. *Similar symbols* indicate the different replicates (n=3)

microbial biomass was greater in BIO plots; summer and spring samples exhibited a higher microbial biomass than the autumn samples. The metabolic quotient was significantly lower in spring than at the two

Table 2 Basal respiration, microbial biomass, and metabolic quotient in BIO and INT apple orchards samples at three different seasons

Time	Treatment	Basal respiration ($\mu g\ CO_2 g^{-1}\ h^{-1}$)	Microbial biomass ($\mu g\ g^{-1}$ soil)	Metabolic quotient ($mg\ CO_2–C\ g_{mic}{}^{-1} h^{-1}$)
Spring	BIO	7.23	2,464	2.9
	INT	4.40	1,487	3.1
Summer	BIO	9.01	2,491	3.7
	INT	8.40	1,730	4.9
Autumn	BIO	6.46	1,530	4.1
	INT	4.90	1,165	4.2
Mean variance, %		11.9	9.7	10.5
Treatment ($F_{1,18}$)		0.03[a]	<0.001[c]	0.24 NS
Time ($F_{2,18}$)		0.006[b]	0.003[b]	0.02[a]
Time×Treatment ($F_{2,18}$)		0.41 NS	0.26 NS	0.26 NS

Mean variance and significance levels of treatment, time, and interactive effects of management and sampling time are shown at the bottom of the table. Each value represents a mean of three replicate fields

BIO biodynamic, *INT* integrated, *NS* nonsignificant

[a] Significant differences at $\alpha \leq 0.05$

[b] Significant differences at $\alpha \leq 0.01$

[c] Significant differences at $\alpha \leq 0.001$

other sampling dates. In no case were interactive effects of management and sampling time found.

PCR-DGGE for fungi

DGGE profiles indicated a phylogenetically diverse spectrum of soil fungi. DGGE patterns showed numerous bands (20 to 28 per sample), with about three to four dominant bands common to all samples. Many bands appeared exclusively in the organic or integrated plots and could be identified as key organisms present in these plots. The PCA plots of the dominant fungal populations during the sampling time are shown in Fig. 2. In spring, summer, and autumn, the fungal community did not show clear differences with regard to the management practices. The organically managed fields were not found to separate from their integrated managed counterpart. The PCs clearly separated the different samples into three different groups corresponding to their time of sampling. ANOVA of the PCs revealed no significant differences among the different treatments along the two axes; the differences were significant among the different time of sampling along the axis 1 ($F_{(2,18)}$=5.94, $\alpha \leq 0.05$) and 2 ($F_{(2,18)}$=

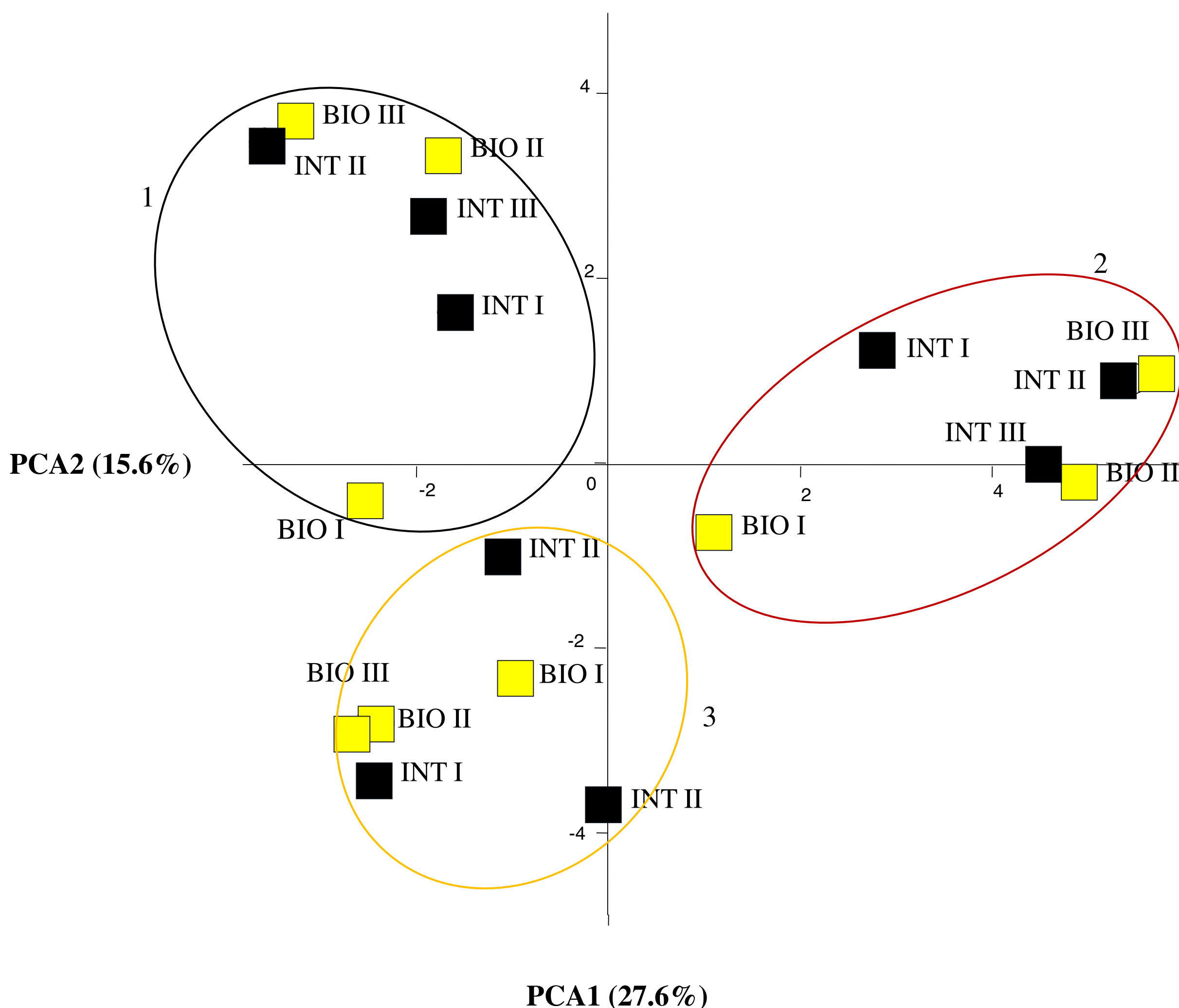

Fig. 2 Ordination plot of the first two component variates of fungal DGGE fingerprinting patterns after principal component analysis (PCA) based on DNA extracted from the different farms in spring (*1*), summer (*2*), and autumn (*3*). Replicates (*n*=3) are indicated by the *identical symbols*

21.72, $\alpha \leq 0.01$). No interactive effects of management and sampling time were found along either axis.

Microarray

Considerable variation in hybridization signals from sample replicates was found upon hybridization of PCR products with the COMPOCHIP microarray. PCA of the SNR values from hybridized PCR products from all farms in spring is shown in Fig. 3. The first two axes explain 86% of the total variance. One-way ANOVA of PCA scores showed no significant difference between the ORG and INT plots. However, it was apparent that for two of the integrated fields, stronger signals for a variety of bacteria were found than for all other fields. Probes with higher signals in the samples from the integrated farms included probes targeting *Xylella*/*Xanthomonas*/*Stenotrophomonas*, *Promicromonospora*, *Arthrobacter*, *Microbacterium*, *Flavobacterium*/*Flexibacter*, *Clostridium bifermentans*, *Clostridium butyricum*, *Azospirillum lipoferum*, α-Proteobacteria, and *Nitrosospira*/*Nitrosovibrio*/*Nitrosomonas*.

Discussion

Individual soil properties may fail to give an appropriate estimation of soil quality since a large number of physical, chemical, and biochemical properties that determine soil processes and their spatial and temporal variability contribute to soil quality sensu (Doran and Parkin 1994). Soil characteristics are influenced by the use of different management practices like ploughing depth, crop, or cover crop type and the management history of the soil (van Diepeningen et al. 2006). Organic farming has been reported to change the soil characteristics by increasing its organic carbon content, size, and stability of aggregates; water retention and infiltration; and decreasing bulk density, dispersion ratio, and soil strength (Singh et al. 2007; Herencia et al. 2008). Integrated farming aims at sustaining quality

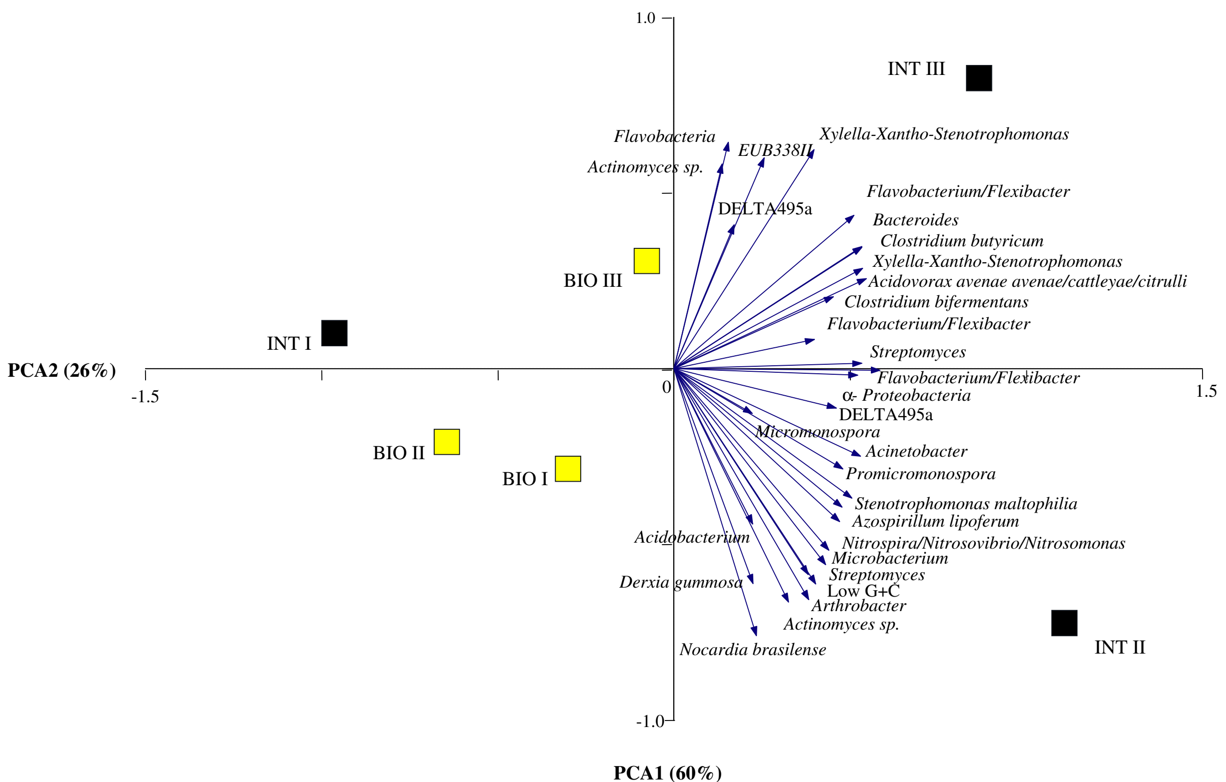

Fig. 3 PCA of the first two component variates of microarray data soil samples representing bacterial composition from the different farms in spring. Replicates (n=3) are indicated by the *identical symbols*

production with minimum external inputs and environmental hazards (Wijnands 1997), and the system is characterized by a reduced use of pesticides. This could explain why its impacts on soil physical and chemical characteristics were not statistically different from those of organic farming, as revealed in this study. The fact that BIO III and INT III showed more affinity could be related to the management history of the soil. This farm had the shortest history of integrated and organic farming. Many studies have reported a stimulation of microbial activity and biomass in organic farming management systems (Fließbach and Mäder 1997; Garcia-Gil et al. 2000), and our results are in accordance with these observations. The increase of microbial activity and biomass can be attributed to the incorporation of easily degradable materials, entering the soil from the different organic wastes which stimulate the indigenous microbial activity of the soil as well as to the incorporation of exogenous microorganisms therein (Perucci 1992; Fuchs 2009). Lower microbial biomass in agroecosystems is often due to reduced organic C in the soil (Wardle 1992). Since organic farming is characterized by big inputs of organic materials when compared to integrated farming, the higher microbial biomass generally observed in organic plots was not thus a surprise. Microbial biomass is generally considered an indicator of soil fertility; therefore, the values observed in organically managed apple orchard soils could be interpreted as an indication of improved soil quality (Franca et al. 2007). The fact that microbial biomass was higher in spring and summer samples can be explained by the fact that during this period of growth, more root derived C is usually available. Basal respiration represents the soil microbial energy requirement at a steady-state condition; higher values indicate an increase of microbial community energy needs (Canali et al. 2009). In contrast, in their 7-year side-by-side comparison of conventional, integrated, and organic apple cultivation, Reganold et al. (2001) did not find a difference between integrated and organic practice concerning soil quality. However, in terms of environmental and economic sustainability, they ranked organic management first, followed by integrated and conventional management. The metabolic quotient tended to be lower in organic plots, which indicates a more efficient C turnover, confirming the findings of others (Canali et al. 2009; Fließbach and Mäder 1997). Generally, the qCO_2 is found to be the highest when ecosystem stress level is high. The qCO_2 of agricultural soils usually ranges from 0.5 to 3.0 mg CO_2–C $g^{-1} C_{mic} h^{-1}$ (Böhme et al. 2005). The elevated metabolic quotient (>4) in integrated plots may thus express some sort of stress (Ros et al. 2006), potentially a reaction to biocide or fertilizer application.

DGGE involves the use of PCR to amplify DNA which is then subjected to electrophoresis. This method is thus not exempt from the biases which arise from the PCR amplification of mixed microbial communities. The selective and potentially biased nature of PCR amplification which can result in a disproportionate representation of certain sequences in a clone library has been well reported (Suzuki and Giovannoni 1996; Chandler et al. 1997). Nonetheless, it remains a very useful tool that used to detect changes in microbial community profiles in environmental samples (Muyzer et al. 1993; Felske and Akkermans 1998). The differences observed between the different farming practices were not statistically significant. This could be explained by the fact that integrated farming is characterized by a reduced use of pesticides and herbicides compared to conventional farming. For this reason, any difference to organic farming is difficult to verify. Another factor that should be considered is the use of chemicals; both farming systems received extensive application of lime sulfur, a fungicide. The different seasons may have had different impacts on the amount and/or chemical composition of the rhizodeposits and the nutrient availability, which could explain why the seasons differently affected the fungal community composition (Marschner et al. 2001). Environmental factors, such as moisture content, are known to cause microbial shifts (Dunfield and Germida 2003). These results are a confirmation of those of Shishido et al. (2008) and Smit et al. (2001), who showed that seasonal factors affected soil microbial community composition in apple orchards.

Microarray analysis using the COMPOCHIP array was conducted in order to investigate the presence of pathogens and plant growth promoting rhizobacteria (PGPR) in the soil samples. Bacterial probes targeting *Azospirillum*, *C. butyricum*, *Nitrosospira*/*Nitrosovibrio*/*Nitrosomonas*, and the group α-proteobacteria hybridized well with soil DNA samples from integrated plots and to a lesser extent with organic plots. Bacteria from these genera/groups (ammonium oxidizing bacteria) indicate the availability of reduced

nitrogenous compounds, be they organic or inorganic like ammonium from the mineral fertilizer (Watson et al. 1989). The different integrated farms were found to have higher levels of *Arthrobacter*, *C. bifermentans*, *Microbacterium*, and *Flavobacterium,* genera and species known to include pesticide degrading strains (Hammill and Crawford 1996; Arrault et al. 2002; Bieszkiewicz et al. 2002) which might indicate some recent history of pesticide use on the integrated farming sites. Probes targeting the genera *Xylella*, *Xanthomonas*, and *Stenotrophomonas* also hybridized well with DNA from soils of integrated plots; these bacterial genera include species that are also known as plant and human pathogens. Thus, although some species of these genera are also known to be common soil inhabitants, their presence could be of concern in these farms. Since we did not conduct any pathogenicity tests, we do not know if these bacteria could be pathogenic to apple trees. The COMPOCHIP microarray included probes targeting known PGPR such as *Bacillus subtilis*, *Bacillus pumilus*, *Burkholderia cepacia*, *Paenibacillus*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Stenotrophomonas maltophilia*, and *Streptomyces.* PGPR are known for their antagonism toward phytopathogenic microorganisms (Scher and Baker 1982; Shanahan et al. 1992). Probes targeting the PGPR were not found to hybridize with DNA from any soil sample, indicating the absence of such microorganisms in the farms, or the presence of these organisms at levels below the detection limit. This would indicate a very low natural protection of these soils against potential pathogens. The difference in the probe signals indicates that the communities of BIO I, BIO II, BIO III, and INT I were quite similar, but different to INT I and INT II. The differences in bacterial community between the two farming systems, although not significant, could be due to the reasons previously explained. The increase of microbial biomass and activity in the organic farming plots supports the results of Manici et al. (2003), who found increased fungal abundance in organically farmed orchards.

Conclusions

The results obtained in this study indicated that farms under organic and integrated management did not show significant differences in their physical and chemical characteristics. Organic farming stimulated microbial activity and biomass compared to integrated farming, but changes in the fungal and bacterial community composition were not evident. Seasonal variation had a clear impact on all these parameters; farm sites influenced the soil composition and the bacterial diversity. Bacteria known to be potential pathogens and degraders of biocides were predominantly found in integrated farming plots, while no difference between the two management practices concerning the presence of PGPR was evident. In which way, these data could be associated to sustainable management of apple orchards remains to be elucidated.

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