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Organic Cultivation of *Triticum turgidum* subsp. *durum* Is Reflected in the Flour-Sourdough Fermentation-Bread Axis

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***Triticum turgidum* subsp. *durum* was grown according to four farming systems: conventional (C_{ONV}), organic with cow manure (O_{MAN}) or green manure (O_{LEG}), and without inputs (NO_{INPUT}). Some chemical and technological characteristics differed between C_{ONV} and organic flours. As shown by two-dimensional electrophoresis (2-DE) analysis, O_{MAN} and O_{LEG} flours showed the highest number of gliadins, and O_{MAN} flour also had the highest number of high-molecular-mass glutenins. Type I sourdoughs were prepared at the laboratory level through a back-slopping procedure, and the bacterial ecology during sourdough preparation was described by 16S rRNA gene pyrosequencing. Before fermentation, the dough made with C_{ONV} flour showed the highest bacterial diversity. Flours were variously contaminated by genera belonging to the *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. Mature sourdoughs were completely and stably dominated by lactic acid bacteria. The diversity of *Firmicutes* was the highest for mature sourdoughs made with organic and, especially, NO_{INPUT} flours. Beta diversity analysis based on the weighted UniFrac distance showed differences between doughs and sourdoughs. Those made with C_{ONV} flour were separated from the other with organic flours. Lactic acid bacterium microbiota structure was qualitatively confirmed through the culturing method. As shown by PCR-denaturing gradient gel electrophoresis (DGGE) analysis, yeasts belonging to the genera *Saccharomyces*, *Candida*, *Kazachstania*, and *Rhodotorula* occurred in all sourdoughs. Levels of bound phenolic acids and phytase and antioxidant activities differed depending on the farming system. Mature sourdoughs were used for bread making. Technological characteristics were superior in the breads made with organic sourdoughs. The farming system is another determinant affecting the sourdough microbiota. The organic cultivation of durum wheat was reflected along the flour-sourdough fermentation-bread axis.**

Organic farming is gaining broad recognition as a system that complies well with sustainability, an overarching principle that should drive agriculture now and in the coming year (1). Under organic farming practices, crops grow without using chemical pesticides, herbicides, and fertilizers and the cultivation relies mainly on crop rotation, compost, and green manure (e.g., N_2-fixing plants), organic fertilizers, and plant-based pesticides. Raw materials or foods, which are sold as organics in the European Union (EU), have to be produced strictly respecting European legislation (2). Based on these characteristics and constraints, consumers perceived organic foods as healthier and more eco-sustainable than conventional ones (3). In the period 2006 through 2010, certified organic production in Europe has increased by more than 40% (4). According to the Italian Institute for Food and Agricultural Market, organic farming in Italy contributes to over 25% of the organically cultivated products in Europe (5).

Cereals represent the most important organic food category, with 18% of all EU organic land and ca. 80% of the total organic arable crop area (4). Among cereals, organic and conventional durum wheat (*Triticum turgidum* subsp. *durum*) is cultivated on over 3.7 million ha in the EU, and more than one-half of this acreage lies in the Mediterranean area, wherein Italy and Spain are the first and second top producers (6). In Italy, ca. 67% of the production of durum wheat comes from the Southern regions, where it is mainly processed into pasta and bread (7). Within the EU, Italy is the most important producer of organic durum wheat, with 87,795 ha designated for its cultivation (8). The Sicily and Apulia regions supply more than 40% of the national production (9). Recently, indigenous and ancient varieties of durum wheat (e.g., cv. Senatore Cappelli) have been reevaluated because of their low input and suitability for organic farming (10). These characteristics guarantee superior nitrogen (N) extraction under low-N environments, good competitiveness regarding weed control (due to the high plant height), and high resistance to biotic and abiotic stresses (11). Overall, some of the main criticisms regarding organic crops are the price (organic durum wheat costs ca. 10% more than the conventional wheat) (5), low yield (the yield of organic durum wheat is ca. 21% lower than that of conventional wheat) (12), and lack of efficiency in terms of resource usage.

Although largely debated, the most recent literature (13, 14) has provided evidence that organic farming of wheat varieties has several positive effects on toxicological, nutritional, and technological properties of flours. Sourdough fermentation is the most natural and best-performing process to ensure the best sensory

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TABLE 1 Fertilization regimen, protection, and time of application used for conventional and organic farming of *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli

Farming system[a]	Fertilizer (amt, kg ha^{-1})	Time of application	Protection system (amt)	Time of application	Fertilization (kg ha^{-1})		
					N	P	K
C_{ONV}	Ammonium sulfate (190), single superphosphate (278), potassium sulfate (278)	November	Herbicide, iodosulfuron-methyl sodium (75 ml ha^{-1})	March	40	50	70
	Ammonium sulfate (190)	April	Fungicide, azoxystrobin + cyproconazole (0.8 liters ha^{-1})	May	40		
	Ammonium sulfate (190)	May			30		
O_{MAN}	Cow manure (6,056); soft ground rock phosphate-fosforite (62); crude potassium sulfate salt-kalisop (54)	November	Manual weeding	March	40	50	70
	N-based fertilizer (leather meal)-Dermazoto N11 (364)	April	Fungicide, sulfur (7 liters ha^{-1})	May	40		
	N-based fertilizer (leather meal)-Dermazoto N11 (272)	May			30		
O_{LEG}	Soft ground rock phosphate-phosphorite (185); crude potassium sulfate (233)	November	Manual weeding	March		50	70
	Faba bean (3,089 [dry matter])	April	Fungicide, sulfur (7 liters ha^{-1})	May	70[b]		
NO_{INPUT}	No application		No application				

[a] Treatments were set according to a completely randomized design with three field replicates. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs (control).
[b] Based on N budget, the rest of N was present in the soil in residual amounts from the previous year where faba bean was also shown (44).

and nutritional characteristics and fits perfectly into the processing chain that starts with the organic farming. None of the above studies has evaluated the effect of farming system on the structure of the wheat flour microbiota, which in turn may affect cereal sourdough fermentation and bread making. Sourdough fermentation processes depend on specific determinants, which have to be strictly controlled to obtain standardized and agreeably flavored products (15). Recently, it has been shown that the autochthonous microbiota of wheat flour, the chemical composition of the raw materials, and interactions between the microorganisms, together with fermentation parameters (e.g., temperature, inoculum size, and dough yield), determine the constitution and the stability of microbial consortia in sourdoughs (16, 17). In turn, the mature and stable sourdough microbiota, the chemical composition and technological aptitude of the flour, and the processing parameters reflect the desired characteristics in the baked products.

The aim of the present study was to characterize the microbiological, technological and chemical characteristics of durum wheat flour (*T. turgidum* subsp. *durum* cv. Senatore Cappelli) grown under organic (cow manure or green manure) and conventional farming conditions. A 16S rRNA gene-based high-throughput-sequencing approach targeting the 16S rRNA gene was used to describe the microbial ecology prior to and after sourdough preparation at the laboratory level. The technological characteristics of related sourdough breads were also determined.

MATERIALS AND METHODS

Description of site, plant cultivation, and experimental design. Durum wheat (*Triticum turgidum* subsp. *durum* cv. Senatore Cappelli) was grown in an experimental field belonging to the Mediterranean Agronomic Institute of Bari in Valenzano, which is located in the Apulia Region, southern Italy (41°03′N, 16°53′E, 72 m in altitude). The experimental field is a part of a medium-term field trial that started during the growing season from 2011 to 2012. Durum wheat was grown from November 2012 to July 2013. The soil site has a silty loam texture, the pH (1:2.5 soil to water) is 8.3, and the available P, exchangeable K, and organic matter are 11, 394, and 18 g kg^{-1}, respectively. Three treatments were set according to a completely randomized design, with three field replicates: one conventional system (C_{ONV}) and two organic systems with cow manure (O_{MAN}) and green manure (O_{LEG}). One field without inputs (NO_{INPUT}) was used as the control. Green manure was represented by faba bean (*Vicia faba*), which was sown at the rate of 10 seeds/m^2. The area of each plot was 16 m^2 (4 m by 4 m), separated by 1 m of buffer zone. The crop was sown at the rate of 350 seeds/m^2 (double rows at a row distance of 15 cm and with a distance of 30 cm between each double row and the next row). Farming systems differed mainly with respect to types of inputs, fertilizers (organic and inorganic), amendments, and plant protection products. The list and quantity of inputs, agricultural practices, and timing are reported in Table 1. The amounts of amendments and fertilizers used were based on nutritional requirements for durum wheat, plant density, and soil nutritional status. The conventional system was managed with the application of synthetic fertilizers, while herbicides and fungicides were applied when needed. The two organic systems (O_{MAN} and O_{LEG}) differed for types of practice used for fertility management in precropping and during the growing season. During the growing season, an additional fertilization was carried out on O_{MAN} by organic mineral fertilizers. Sulfur was used as a fungicide, and weeding was done manually. The inputs used for both organic systems were approved according to EU Regulations EC no. 834/2007 and 889/2008. No fertilizers or plant protection products were used for the control plot (NO_{INPUT}). The air temperature during the growing season was in the range 11 to 20°C, and the seasonal rainfall amounted to 600 mm. Wheat was grown under rain-fed conditions, which is typical for the Mediterranean area. Whole wheat grains from each treatment were put into separate bags and transferred to the milling plant.

Flour composition. Milling was carried out at the pilot plant of Tandoi S.p.A. (Corato, Bari, Italy). Cross-contaminations were avoided through a deep cleaning of the pilot plant after each flour milling with compressed air and sodium hypochlorite. Moisture, ash, protein, gluten index, and dry gluten were determined according to AACC International standard methods 44-15.02, 08-01.01, 46-12.01, 56-81.03, and 38-12.02 (18). Flour strength (*W*), tenacity (*P*), and extensibility (*L*) were determined through the Chopin Alveograph (MA82; Villeneuve-la-Garenne, France).

2-DE. Proteins were selectively extracted from the wheat flour, according to the method of Weiss et al. (19). Two-dimensional electropho-

resis (2-DE) was carried out with the immobiline-polyacrylamide system, as described by Di Cagno et al. (20). Aliquots of proteins (30 μg) were used for the electrophoretic run. Isoelectric focusing (IEF) was carried out on immobiline strips. A nonlinear pH gradient from 3.0 to 10.0 (immobilized-pH-gradient [IPG] strips; Amersham Pharmacia Biotech, Uppsala, Sweden) for the glutenin fraction and a linear pH gradient of 6 to 11 for the gliadin fraction were carried out using an IPG-phore at 20°C. The second dimension was carried out in a Laemmli system on 12% polyacrylamide gels (13 cm by 20 cm by 1.5 mm) at a constant current of 40 mA/gel and at 15°C for approximately 5 h, until the dye front reached the bottom of the gel. 2-DE protein standards (Bio-Rad Laboratories) were used for isoelectric point (pI) and molecular mass estimation. Gels were silver stained (20). Image analysis of the gels, acquired by a gel scanner (Amersham Pharmacia Biotech, Uppsala, Sweden), was carried out with the University of Texas Health Science Centre—San Antonio (UTHSCSA) ImageTool software (version 2.0; available from maxrad6.uthscsa.edu). Gray scale (0 to 255) images of the gels, at 600 dots per inch, were obtained and processed. The intensity of the spots was calculated as the black pixel area using a threshold method (21).

Dough and sourdough propagation. Dough was prepared and sourdough was propagated according to traditional protocols (22), without using starter cultures or baker's yeast. Dough preparation was as follows: durum wheat flours (187.5 g) and tap water (112.5 ml) were used to create 300 g of dough (where a dough yield of 160 = dough weight × 100/flour weight) with a continuous high-speed mixer (60 × *g*, dough mixing time of 5 min) (Chopin & Co., Boulogne, Seine, France). This preparation refers to the dough prior to fermentation and before becoming sourdough. The mixer was wiped with ethanol before being used it to mix doughs made from different flours. Daily, each sourdough was subjected to fermentation (propagation) at 25°C for 5 h. The only exception was the first fermentation, which lasted 8 h, according to traditional protocols (22). Between the daily fermentations, the sourdoughs were stored at 4°C for ca. 18 h as frequently used by bread manufacturers. Sourdough propagation was according to the back-slopping (refreshment) procedure, where the sourdough from the previous day was used, after homogenization, as the starter (20% [wt/vol] of the inoculum) to ferment a new mixture of flour (140.62 g) and tap water (84.38 ml), having a dough yield of 160. The propagation lasted 10 days, which was considered the time required to obtain a mature and stable sourdough, at least from a biochemical point of view (pH, total titratable acidity [TAA], organic acids, and soluble carbohydrates) (16, 23). The sourdoughs were cooled down to 4°C and analyzed within 2 h after collection. Preparation and propagation were carried out in triplicate.

Physicochemical, biochemical, and microbiological analyses. The pH values were determined online with a pHmeter (model 507; Crison, Milan, Italy). Total titratable acidity (TTA) was determined after homogenization of 10 g of dough with 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH needed to reach a pH value of 8.3.

The water-salt-soluble extract (WSE) of wheat flour was prepared according to Weiss et al. (19) and used to analyze organic acid, peptides, sugars, and free amino acids (FAA). Organic acids were determined by high-performance liquid chromatography (HPLC) using an ÄKTA Purifier system (GE Healthcare, Buckinghamshire, United Kingdom) (22). The fermentation quotient (FQ) was determined as the molar ratio between lactic and acetic acids. The peptide concentration was determined by the *o*-phthaldialdehyde (OPA) method (24). The concentrations of glucose, fructose, maltose, and sucrose were determined through HPLC analysis using an ÄKTA Purifier system (GE Healthcare) equipped with a Spherisorb column (Waters, Millford, MA) and a PerkinElmer 200a refractive index detector (25). Free amino acids were analyzed by a Biochrom 30 series amino acid analyzer (Biochrom Ltd., Cambridge Science Park, United Kingdom), as previously described by Nionelli et al. (25).

Ten grams of sample was homogenized with 90 ml of sterile peptone water solution (0.1% [wt/vol] peptone, 0.85% [wt/vol] NaCl). Lactic acid bacteria were counted at 30°C for 48 h, under anaerobiosis, using sourdough bacterium (SDB) agar medium supplemented with cycloheximide (0.1 g liter^{-1}). The number of yeast cells was estimated at 30°C for 48 h on Sabouraud dextrose agar (SDA) medium (Oxoid, Basingstoke, Hampshire, United Kingdom) supplemented with chloramphenicol (0.1 g liter^{-1}).

Total bacterial genomic DNA and RNA extraction. Ninety milliliters of potassium phosphate (50 mM [pH 7.0]) buffer was added to 10 g of sample and homogenized for 5 min, and DNA extraction was carried out as previously described (26). Total RNA was extracted using the RiboPure-Bacteria kit (Ambion RNA, Life Technologies Co., Carlsbad, CA), according to the manufacturer's instructions. The purified RNA (100 ng; final volume, 20 μl) was incubated at 42°C for 2 min in 2 μl of genomic DNA (gDNA) Wipeout buffer 7X (QuantiTect reverse transcription kit; Qiagen srl, Milan, Italy) and RNase-free water (final volume, 14 μl). The cDNA was obtained by the QuantiTect reverse transcription kit (Qiagen), according to the manufacturer's instructions. Three independent replicates of each sample were used for DNA and RNA extraction, cDNA synthesis, and amplicon generation.

PCR and DGGE analysis. Since the diversity of yeasts in mature sourdoughs was very low (26), only denaturing gradient gel electrophoresis (DGGE) analysis was carried out to describe the dynamics of yeast cells during sourdough preparation. DNA (40 ng) was amplified with primers NL1 and LS2, corresponding to the D1-D2 region of the 26S ribosomal DNA (rDNA) gene, to identify the yeast community. The PCR core program was carried out as described elsewhere (27). Amplicon separation by DGGE (Bio-Rad DCode system; Bio-Rad Laboratories, Milan, Italy) and band purification were performed as previously described (26). DNA sequencing reactions were carried out by MWG Biotech AG (Ebersberg, Germany). Sequences were compared using the GenBank database and the BLAST program (28).

Amplicon library preparation and pyrosequencing. cDNA was used to study bacterial diversity by pyrosequencing of the amplified region from V1 to V3 (amplicon size, 520 bp) (16). PCRs were carried out using cDNA as the template as previously described (16); amplicons obtained from the triplicate RNA extractions were pooled before further processing. PCR products were purified twice with an Agencourt AMPure kit (Beckman Coulter, Milan, Italy) and then quantified using the QuantiFluor system (Promega, Milan, Italy) prior to further processing. Amplicons were used for pyrosequencing on a GS Junior platform (454 Life Sciences, Roche Diagnostics, Milan, Italy) according to the manufacturer's instructions by using Titanium chemistry.

Bioinformatics. A preliminary filtering of the results was performed using the 454 amplicon signal processing, and then sequences were analyzed by using QIIME 1.5.0 software (29). After the split library script performed by QIIME, the reads were excluded from the analysis if they had an average quality score lower than 25, if they were shorter than 300 bp, and if there were ambiguous base calls. Sequences that passed the quality filter were denoised, and singletons were excluded. Operational taxonomic units (OTU) were defined by a 97% identity; the taxonomy assignment and alpha and beta diversity analyses were performed through QIIME as previously described (16). Weighted UniFrac distance matrices were used for principal coordinate analysis (PCoA) analysis. The OTU taxonomy table generated by QIIME was used to produce pseudo-heat maps in the R environment using the gplots package.

Isolation and genotypic identification of lactic acid bacteria. Ten grams of each dough and sourdough was suspended in 90 ml of sterile sodium chloride (0.9% [wt/vol]) solution and homogenized with a Classic blender (PBI International Milan, Italy) for 2 min at room temperature. At least 10 colonies of presumptive lactic acid bacteria, possibly with different morphologies, were randomly isolated from the SDB plates containing the two highest sample dilutions. Gram-positive, catalase-negative, nonmotile rod and coccus isolates were cultivated in the broth medium at 30°C for 24 h and restreaked into the same agar medium. All isolates considered for further analyses were able to acidify the culture medium. To identify presumptive lactic acid bacterial strains, two primer

TABLE 2 Chemical and technological characteristics of grain and flour from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions[a]

Farming system[b]	Yield (tonnes ha^{-1})	1,000-kernel wt (g)	Moisture (%)	Protein (% of dry matter)	Gluten (%)	Gluten index (%)	Ash content (% of dry matter)	Falling no. (s)	Flour strength (*W*)	Tenacity/ extensibility (*P/L*)
C_{ONV}	2.9 ± 0.1 A	58.9 ± 1.1 A	11.7 ± 0.1 A	17.8 ± 0.3 A	14.7 ± 0.2 A	90 ± 4.5 A	1.0 ± 0.1 A	557 ± 31 A	137 ± 17 A	0.49 ± 0.1 A
O_{MAN}	2.9 ± 0.2 A	58.9 ± 1.8 A	11.8 ± 0.2 A	17.7 ± 0.1 A	14.7 ± 0.1 A	70 ± 2.7 B	0.9 ± 0.1 A	570 ± 24 A	126 ± 14 B	0.48 ± 0.1 A
O_{LEG}	2.8 ± 0.3 A	59.5 ± 1.8 A	11.7 ± 0.2 A	16.8 ± 0.2 B	13.7 ± 0.1 B	60 ± 2.4 C	1.0 ± 0.2 A	511 ± 29 B	118 ± 11 C	0.51 ± 0.1 A
NO_{INPUT}	2.6 ± 0.2 B	58.6 ± 1.6 A	11.7 ± 0.1 A	16.6 ± 0.2 B	14.6 ± 0.3 A	60 ± 3.1 C	1.0 ± 0.2 A	513 ± 19 B	124 ± 19 B	0.51 ± 0.1 A

[a] Data are means from three independent experiments ± standard deviations ($n = 3$). Values in the same column followed by different letters differ significantly ($P < 0.05$).
[b] Treatments were set according to a completely randomized design with three field replicates. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs (control). Further details are included in Materials and Methods.

pairs (Invitrogen Life Technologies, Milan, Italy), LacbF/LacbR and LpCoF/LpCoR, were used for amplification of the 16S rDNA (30). Electrophoresis was carried out on agarose gel at 1.5% (wt/vol) (Gellyphor; EuroClone), and amplicons were purified with the GFXTM PCR DNA and gel band purification kit (GE Healthcare). Sequencing electropherogram data were processed with Geneious. rDNA sequence alignments were carried out using the multiple-sequence alignment method, and identification queries were fulfilled by a BLAST search (28) in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/).

Total phenols and antioxidant activity. The concentration of total phenols was determined as described by Slinkard and Singleton (31) and is expressed as the gallic acid equivalent. The free radical scavenging capacity was determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·), as reported by Yu et al. (32). The scavenging activity was expressed as follows: DPPH scavenging activity (%) = [(blank absorbance – sample absorbance)/blank absorbance] × 100. The absorbance value was compared with that of 75 ppm butylated hydroxytoluene (BHT) as the antioxidant reference.

Determination of free and bound phenolic acids. Free and bound phenolics were extracted and analyzed through HPLC analysis (25). The analyses of vanillic, syringic, *p*-coumaric, ferulic, and sinapic acids (Sigma-Aldrich, Steinheim, Germany) were carried out at UV wavelengths of 280, 310, and 320 nm. A scan mode ranging from 230 to 450 nm was used. Identification of each peak was confirmed by retention time and absorbance spectrum of each pure compound. By spiking the sample with a known amount of pure compound and performing the same extraction and analytical procedures, recoveries were determined.

Phytase activity. Phytase activity was determined on the WSE by monitoring the rate of hydrolysis of *p*-nitrophenyl phosphate (*p*-NPP) (Sigma, 104-0) as previously described by De Angelis et al. (33). The *p*-nitrophenol released was determined by measuring the absorbance at 405 nm. One unit of activity was defined as the amount of enzyme required to release 1 μmol/min of *p*-nitrophenol under the assay conditions.

Bread making. Breads were manufactured at the pilot plant of the Department of Soil, Plant and Food Science of the University of Bari (Italy). Sourdoughs were used at 20% (wt/wt). Baker's yeast was added to all the doughs (2% [wt/vol], corresponding to a final cell density of ca. 10^7 CFU g^{-1}). Doughs were mixed at 60 × *g* for 5 min with an IM 5-8 high-speed mixer (Mecnosud, Flumeri, Italy), and fermentation was at 30°C for 1.5 h. All breads were baked at 220°C for 30 min (Combo 3; Zucchelli, Verona, Italy). Fermentations were carried out in triplicate, and each bread was analyzed twice.

Instrumental texture profile analysis (TPA) was carried out with a TVT-300XP texture analyzer (TexVol Instruments, Viken, Sweden), equipped with a P-Cy25S cylinder probe. Texture Analyzer TVT-XP 3.8.0.5 software (TexVol Instruments) was used. The specific volume of breads was measured by the BVM-test system (TexVol Instruments). The hardness value (maximum peak force), the chromaticity coordinates of the bread crust, and the crumb characteristics of the breads evaluated after 24 h of storage were determined as previously described by Nionelli et al. (25).

Statistical analysis. Analyses were carried out on three independent replications of each sample. Each replication was analyzed twice. Data were subjected to one-way analysis of variance (ANOVA); pairwise comparison of treatment means was obtained by Tukey's procedure at $P < 0.05$, using the statistical software Statistica 8.0 (StatSoft, Inc., Tulsa, OK). Chemical and rheology properties of flours were analyzed through PCA using the software Statistica 8.0.

Nucleotide sequence accession number. The sequence data have been submitted to the Sequence Read Archive database of the National Center for Biotechnology Information under accession no. SRP044788.

RESULTS

Chemical and technological characteristics of grains and flours. The yield of durum wheat was in the range 2.6 to 2.9 tonnes ha^{-1}, the lowest ($P < 0.05$) being obviously for the NO_{INPUT} system (Table 2). No significant ($P > 0.05$) differences were found among the flours regarding the moisture, ash and *P/L* values. The total protein contents were the same ($P > 0.05$) for both the C_{ONV} and O_{MAN} flours, while the NO_{INPUT} and O_{LEG} flours showed the lowest ($P < 0.05$) concentration. The latter farming system also gave the lowest concentration of gluten. The gluten index was significantly higher ($P < 0.05$) for C_{ONV} (90% ± 4.5%), followed by O_{MAN} (70% ± 2.7%), and NO_{INPUT} and O_{LEG} (ca. 60%) flours. The values of "falling number" were in the range of 511 ± 29 to 570 ± 24 s, the lowest and the highest being for NO_{INPUT} and O_{LEG} and C_{ONV} and O_{MAN} flours, respectively. As expected, the rheology parameter *W* (flour strength) was the highest for C_{ONV} flour and slightly but significantly ($P < 0.05$) lower for the farming systems O_{MAN} and NO_{INPUT}.

2-DE analysis of the gliadin fraction showed significant ($P < 0.05$) variations of the total number of polypeptides. O_{MAN} and O_{LEG} flours had the highest spot numbers (76 and 74 spots, respectively) (Fig. 1A to D; see Table S1 in the supplemental material). The lowest number (61 spots) was found for NO_{INPUT} flour. Within gliadins, the S-rich subunits (α-, β-, and γ-gliadins), having molecular masses of 30 to 45 kDa (34), were the most abundant: 62 spots were detected for O_{MAN} flour, followed by 56 spots for O_{LEG}, 51 for C_{ONV}, and 48 for NO_{INPUT}. S-poor gliadin subunits (ω-gliadins), which are usually located in the range of molecular masses of 46 to 74 kDa (34), were present at low levels. Contrarily to what is usually observed in the lower part (30 kDa) of the electrophoresis gel, including copious numbers of polypeptides, all flours showed a few spots. According to previous studies (34), glutenins ranged from 40.0 to 112.5 kDa and from pI 3.6 to 9.8 (Fig. 1E to H; see Table S1). The total number of polypeptides

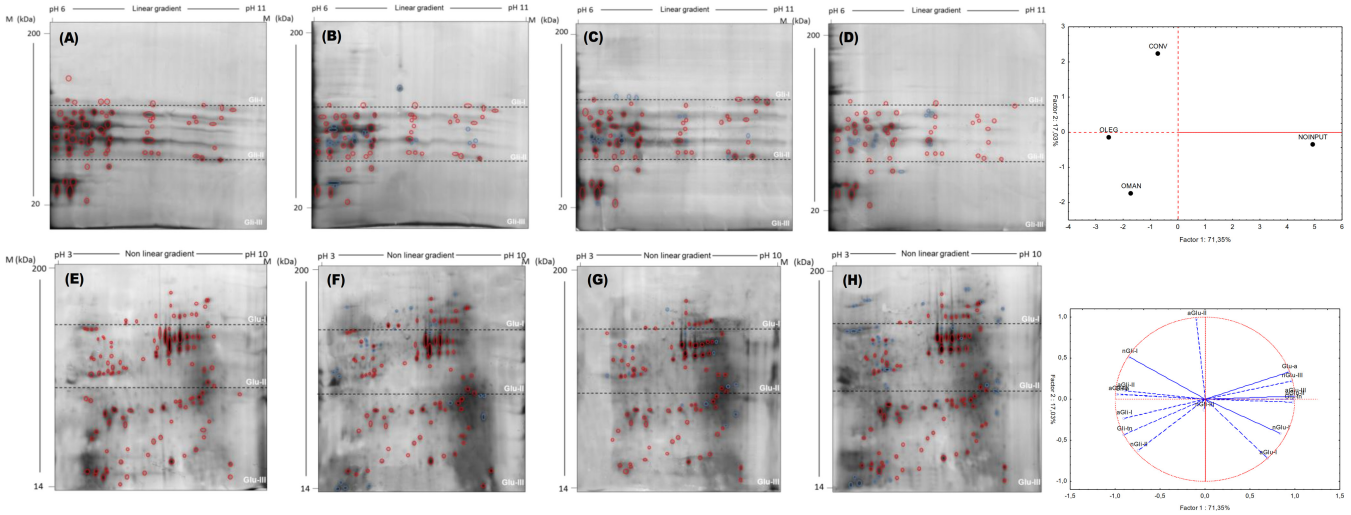

FIG 1 Two-dimensional gel electrophoresis (2-DE) analysis of gliadins (A to D) and glutenins (E to H) extracted from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions. C_{ONV}, conventional system (A and E); O_{MAN}, organic system with cow manure (B and F); O_{LEG}, organic system with green manure (C and G); NO_{INPUT}, without inputs (D and H). Further details are included in Materials and methods. The principal component biplot, based on the number (*n*) and the intensity of the polypeptides calculated as pixel area (*a*) by image analysis of the gels, is shown. The numbers and areas of the spots were calculated for three clusters of molecular masses, separated by dashed lines: gliadins, cluster I, >45 kDa (nGli-I and aGli-I, respectively), cluster II, 30 to 45 kDa (nGli-II and aGli-II, respectively), and cluster III, <30 kDa (nGli-III and aGli-III, respectively); glutenins, cluster I, 70 to 140 kDa (nGlu-I and aGlu-I, respectively), and cluster II, 30 to 70 kDa (nGlu-II and aGlu-II, respectively). Total intensities for gliadins and glutenins are represented by Gli-a and Glu-a, respectively.

resolved by 2-DE varied from 134 (NO_{INPUT}) to 94 (O_{LEG}). Spots were grouped into two clusters: 70 to 140 kDa, which corresponded to high-molecular-mass (HMM) glutenins, and 30 to 70 kDa, which referred to low-molecular-mass (LMM) glutenins (34). The highest number of HMM glutenins was found for NO_{INPUT} and O_{MAN} flours. NO_{INPUT} flour showed also the highest number of LMM glutenins. The number and intensity of gliadins and glutenins (pixel areas of the gel image detected by the threshold method) clustered based on molecular masses (gliadins, I, >45, II, 30 to 45, and III, <30 kDa; glutenins, I, 70 to 140, II, 30 to 70, and III, <30 kDa), which were subjected to PCA (biplot of Fig. 1). The first and second factors explained 88.38% of the total variance. Based on the farming system, flours were spread into the plane. O_{LEG} and O_{MAN} flours were grouped into the same area of the biplot and were clearly separated from C_{ONV} and, especially, NO_{INPUT} flours.

Sourdough fermentation. Before fermentation, the doughs had pH values that ranged from 6.03 to 6.10, corresponding to TTA of 1.3 to 1.8 ml of 0.1 M NaOH/10 g of dough (Table 3). The number of presumptive lactic acid bacteria was 2.5 to 2.9 log CFU/g. The number of yeast cells was 4.0 to 4.3 log CFU/g. After 10 refreshments, mature sourdoughs had low pH values (4.26 to 4.47). TTA ranged from 8.9 to 11.5 ml 0.1 M NaOH/10 g of dough. The concentrations of lactic and acetic acids were 104 to 139 mmol/kg and 10 to 16 mmol/kg, respectively. The fermentation quotient was 6.9 ± 0.3 for O_{MAN} to 11.6 ± 0.4 for O_{LEG}. At the end of the sourdough preparation, the cell densities of presumptive lactic acid bacteria were very similar and varied from 8.7 to 9.0 log

TABLE 3 Biochemical and microbiological characteristics of the doughs (after mixing and before fermentation) and mature sourdoughs (after 10 refreshments) from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions[a]

Dough and farming system[b]	Amt (log CFU g^{-1}) of:				Concn (mmol kg^{-1}) of:			Concn (mmol kg^{-1}) of:			
	Lactic acid bacteria	Yeast cells	pH	TTA (ml of 0.1 M NAOH)	Lactic acid	Acetic acid	FQ	Glucose	Fructose	Maltose	Sucrose
Doughs											
C_{ONV}	2.9 ± 0.2 A	4.3 ± 0.3 A	6.03 ± 0.1 B	1.3 ± 0.2 B	NF	NF		2.0 ± 0.1 A	2.1 ± 0.1 A	4.5 ± 0.2 B	4.6 ± 0.1 A
O_{MAN}	2.9 ± 0.2 A	4.1 ± 0.3 A	6.04 ± 0.1 B	1.4 ± 0.1 B	NF	NF		2.0 ± 0.1 A	2.1 ± 0.1 A	4.3 ± 0.2 B	4.7 ± 0.4 A
O_{LEG}	2.8 ± 0.3 A	4.0 ± 0.2 A	6.05 ± 0.1 B	1.7 ± 0.2 A	NF	NF		2.0 ± 0.1 A	2.1 ± 0.1 A	4.8 ± 0.3 A	4.7 ± 0.2 A
NO_{INPUT}	2.5 ± 0.3 A	4.2 ± 0.2 A	6.10 ± 0.1 A	1.8 ± 0.1 A	NF	NF		1.9 ± 0.1 A	2.0 ± 0.1 A	5.5 ± 0.2 A	4.7 ± 0.1 A
Sourdoughs											
C_{ONV}	8.7 ± 0.4 A	7.0 ± 0.3 A	4.47 ± 0.1 A	8.9 ± 0.4 C	106 ± 11 B	10 ± 0.3 C	10.6 ± 0.2 B	0.5 ± 0.1 A	0.5 ± 0.1 A	6.3 ± 0.2 B	2.3 ± 0.1 A
O_{MAN}	8.7 ± 0.2 A	6.9 ± 0.2 A	4.35 ± 0.1 B	10.1 ± 0.2 B	104 ± 12 B	15 ± 1.2 A	6.9 ± 0.3 D	0.5 ± 0.1 A	0.5 ± 0.1 A	5.9 ± 0.1 B	2.4 ± 0.1 A
O_{LEG}	8.9 ± 0.3 A	7.1 ± 0.3 A	4.26 ± 0.1 C	10.3 ± 0.3 B	139 ± 13 A	12 ± 0.7 B	11.6 ± 0.4 A	0.6 ± 0.1 A	0.4 ± 0.1 A	6.2 ± 0.1 B	2.5 ± 0.1 A
NO_{INPUT}	9.0 ± 0.5 A	7.3 ± 0.4 A	4.28 ± 0.1 C	11.5 ± 0.4 A	130 ± 11 A	16 ± 0.4 A	8.1 ± 0.2 C	0.6 ± 0.2 A	0.6 ± 0.1 A	7.5 ± 0.3 A	2.3 ± 0.1 A

[a] Data are means from three independent experiments ± standard deviations ($n = 3$). Values in the same column followed by different letters differ significantly ($P < 0.05$). NF, not found.

[b] Treatments were set according to a completely randomized design with three field replicates. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs (control). Further details are included in Materials and Methods.

CFU g^{-1}. Yeast cells were in the range 6.8 to 7.3 log CFU g^{-1}. The ratio between lactic acid bacteria and yeasts stabilized to ca. 100:1 for almost all of the sourdoughs. Compared to the doughs, the concentrations of glucose, fructose, and sucrose of all of the sourdoughs markedly ($P < 0.05$) decreased. The opposite was found for maltose, which increased, probably due to relevant flour enzyme activities. The concentrations of free amino acids (FAA) did not differ ($P > 0.05$) (536.6 ± 24 to 551.2 ± 25 mg kg^{-1}) among the doughs (see Table S2 in the supplemental material). All of the sourdoughs had concentrations of total FAA that were significantly ($P < 0.05$) higher. The increases varied from 50.5% for O_{MAN} to 55.7% for NO_{INPUT} (see Table S2). Almost all FAA increased, with the highest variations observed for Val, Met, Ile, Leu, Orn, and Lys.

PCR-DGGE analysis of the yeast community. DGGE profiles of amplicons from the D1-D2 region of 26S rRNA gene were similar for doughs and sourdoughs (see Table S3 in the supplemental material). Sequencing of the bands (lengths in the range of 180 to 210 bp; average of query coverage, 98%) revealed the common presence of *Saccharomyces cereviasiae*, *Candida humilis*/*Kazachstania barnettii*, *Saccharomyces bayanus*/*Kazachstania* sp., *Candida* sp., *Rhodotorula glacialis*, and *Alternaria* sp.

Pyrosequencing data analysis and alpha diversity. After 454 amplicon signal processing and QIIME filtering protocols, the number of 16S rRNA sequence reads obtained was 40,273, with an average of 5,034 reads/sample (a pool of the three replicates) and an average length of 486 bp, as calculated after primer removal. The number of OTU, the Chao1 and Shannon indices, and the Good's estimated sample coverage (ESC) are reported in Table S4 in the supplemental material. As expected, the highest diversity was found for the doughs. The dough made with C_{ONV} flour had the highest diversity indices, followed by the O_{LEG} dough. After 10 days of propagation, the microbial diversity indices decreased markedly and the highest values were found for the sourdoughs made with the O_{MAN} and, especially, NO_{INPUT} flours. As shown by the ESC value, satisfactory coverage was obtained for all of the samples.

Microbiota structure of doughs and sourdoughs. The bacterial sequences from 16S rRNA genes assigned to bacterial phyla and their relative abundance (%) varied slightly, depending on the farming system (data not shown). All of the doughs were dominated by *Proteobacteria* (68.4 to 96.6%), with very low abundances of *Actinobacteria* and other phyla. The dough made with C_{ONV} flour differed for the relatively high abundance of *Firmicutes* (29%), which were harbored at very low percentages in doughs made with O_{MAN} (1.23%), O_{LEG} (4.05%), and NO_{INPUT} (0.33%) flours. As expected, all mature sourdoughs were completely dominated by *Firmicutes*.

Most of the OTU, which were classified at the genus level and occurred at a relative abundance of >0.05%, are shown in Fig. 2. *Pantoea* (54.1%), *Leuconostoc* (19.3%), *Pediococcus* (3.8%), *Pseudomonas* (2.8%), *Lactobacillus* (2.4%), and *Enterobacteriaceae* family (1.4%) were the main genera found in the dough made with C_{ONV} flour (Fig. 2A). The profiles of the doughs made with O_{MAN} and O_{LEG} flours mainly differed in terms of the low abundance or absence of *Leuconostoc* and *Pediococcus* and the higher levels of *Enterobacteriaceae*, *Erwinia*, and *Pseudomonas*. The *Enterobacteriaceae* family was mainly present in the dough made with NO_{INPUT} flour. In the mature sourdoughs, lactic acid bacteria were almost the only detectable microbial group. The sourdough made with C_{ONV} flour only contained *Leuconostoc* (52.6%), *Lactococcus* (39.2%), and *Lactobacillus* (8.1%). The same genera were present in the sourdoughs made with O_{MAN} and O_{LEG} flours, but the proportions varied and other lactic acid bacteria were found. *Leuconostoc* (54.0%) also dominated the O_{MAN} sourdough, followed by *Lactobacillus* (37.8%), *Lactococcus* (7.4%), and *Pediococcus* (0.4%). The O_{LEG} sourdough mainly differed in the abundance of *Lactobacillus* (58.2%) and *Leuconostoc* (37.8%). The sourdough made with NO_{INPUT} flour harbored *Weissella* (1.9%) and the highest incidence of *Pediococcus* (13.7%).

Figure 2B displays the abundance of *Firmicutes* alone. Taxonomic details up to the species level were supplied where such assignment was possible. The *Lactobacillus plantarum* group was the only one commonly found in all the samples from doughs to mature sourdoughs. However, compared to the mature sourdough made with C_{ONV} flour (5%), the abundance of the *L. plantarum* group was markedly higher in the O_{MAN} and O_{LEG} sourdoughs (37.8 and 58.2%, respectively). *Leuconostoc citreum* was present in all the mature sourdoughs. Except for the sourdough made with O_{LEG} flour, *Leuc. citreum* was the species found at the highest abundance (52.6 to 61.8%). All the mature sourdoughs harbored *Lactococcus lactis*. It was the second most dominant species (39.1%) for sourdough made with C_{ONV} flour but was poorly abundant when organic and, especially, NO_{INPUT} flours were used. *Lactobacillus brevis* (3.1%) was only found in mature C_{ONV} sourdough. On the contrary, the mature sourdoughs made with organic and, especially, NO_{INPUT} flours harbored *Pediococcus* sp. The mature NO_{INPUT} sourdough showed the larger subpopulation diversity. *Lactobacillus* sp. (6.3%), *Weissella* sp. (1.4%), *Weissella cibaria* (0.5%), and *Lactobacillus sanfranciscensis* (0.3%) were identified. Contaminants such as *Bacillus* sp., *Exiguobacterium* sp., *Paenibacillus* sp., *Bacillus psychrodurans*, and *Staphylococcus succinus*, which were exclusively present in doughs made with organic or NO_{INPUT} flours, disappeared during refreshments. The principal coordinate analysis (PCoA), which was based on the weighted UniFrac distance matrix, clearly clustered the doughs and sourdoughs (see Fig. S1 in the supplemental material). Moreover, within each of the two clusters, the doughs or sourdoughs made with C_{ONV} flour were separated from those made with organic flours.

Identification of lactic acid bacteria. In order to qualitatively confirm the results of sequencing, Gram-positive, catalase-negative, nonmotile cocci and rods able to grow at 15°C and to acidify SDB broth were identified by partial sequencing of the 16S rRNA (Table 4). The doughs harbored the following species: *Lactobacillus plantarum* group (27 isolates), *Pediococcus pentosaceus* (7), *Lecuconostoc citreum* (4), and *Enterococcus lactis* (2). Dough made with C_{ONV} flour differed for the presence of *P. pentosaceus*, which was absent in the doughs made with O_{MAN} and NO_{INPUT} flours. The *L. plantarum* group was found in the doughs made with organic O_{MAN} and O_{LEG} (10 and 9 isolates) and NO_{INPUT} (6 isolates) flours, while two isolates were found in the dough made with C_{ONV} flour. *Leuc. citreum* was found both in the dough made with C_{ONV} flour (2 isolates) and in the one made with NO_{INPUT} flour (2 isolates). *E. lactis* was found only in the dough made with NO_{INPUT} flour. Almost all the species found in the doughs were also found in the sourdoughs. The *L. plantarum* group was the only one found in all samples, from doughs to mature sourdoughs, whereas *Leuc. citreum* was present in all of the mature sourdoughs. *Lactobacillus brevis* (1 isolate) and *Weissella cibaria* (1

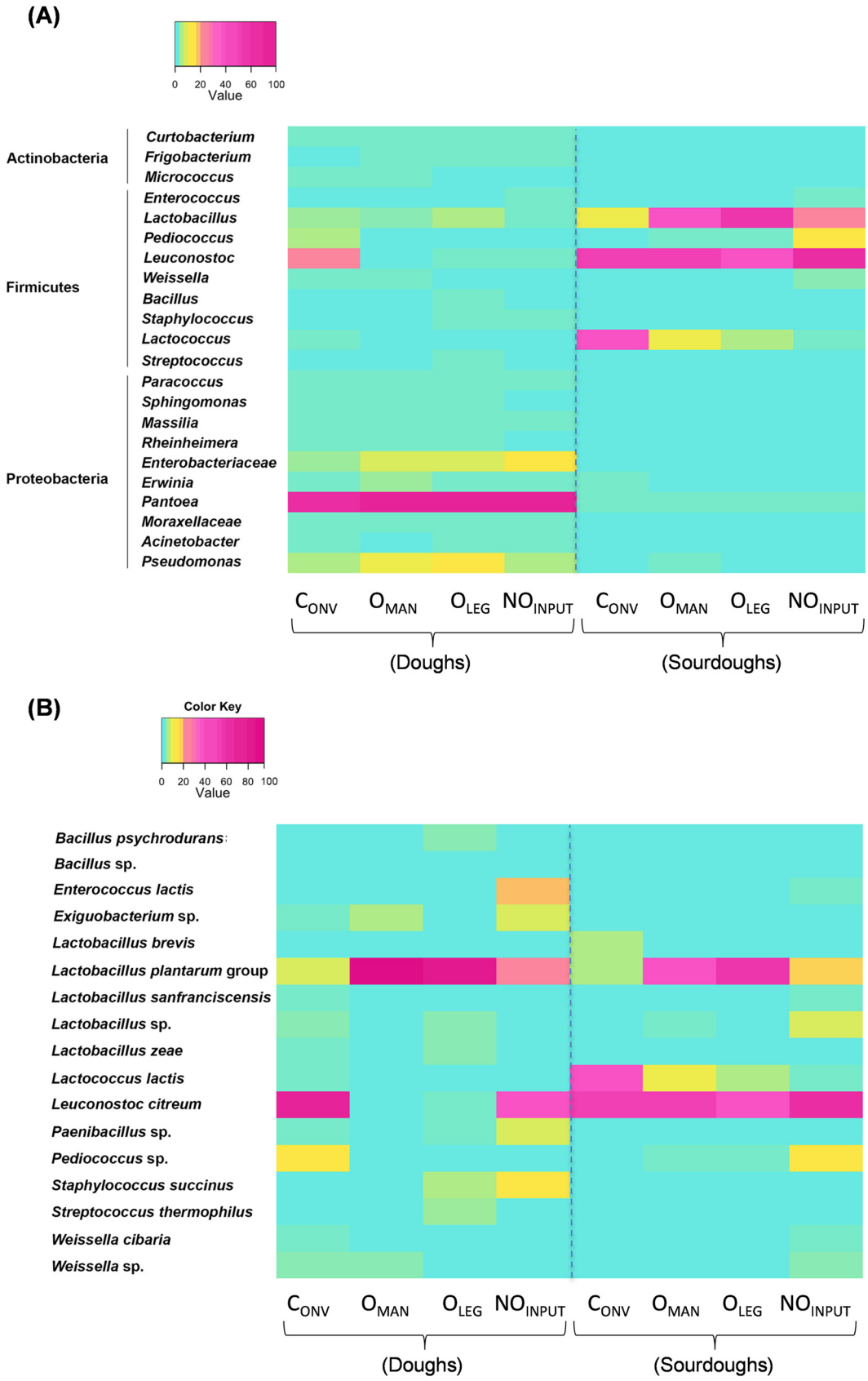

FIG 2 Pseudo-heat map depicting the distribution (%) of the bacterial genera (A) and species (B) of the doughs (after mixing and before fermentation) and mature sourdoughs (after 10 days of propagation) from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs. Further details are included in Materials and methods. Only OTU with an incidence above 0.05% in at least one sample are shown. The color key defines the percentage of OTU in the samples.

TABLE 4 Species of lactic acid bacteria identified from the doughs (after mixing and before fermentation) and mature sourdoughs (after 10 refreshments) from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions

Farming system[a]	Closest relative (% identity [no. of isolates])[b]	Closest GenBank accession no.
Doughs		
C_{ONV}	*Pediococcus pentosaceus* (100 [6])	JN851779.1, JN851781.1
	Lactobacillus plantarum (99–100 [2])	KJ187143.1
	Leuconostoc citreum (100 [2])	JN851747.1, JN851753
O_{MAN}	*L. plantarum* group (99–100 [10])	KJ187143.1, KM670024.1, HM101316.1, KJ187133.1, KJ802480.1
O_{LEG}	*P. pentosaceus* (100 [1])	KM005150.1
	L. plantarum (99–100 [9])	KJ187133.1, KM670024.1
NO_{INPUT}	*Enterococcus lactis* (100 [2])	DQ255948.1
	L. plantarum (99% [6])	KM670024.1, KJ187133.1
	Leuc. citreum (100 [2])	JN851753.1
Sourdoughs		
C_{ONV}	*P. pentosaceus* (99 [1])	JN851781.1
	L. plantarum group (99–100 [3])	EF439682.1, KJ187143.1, KJ802483.1
	Leuc. citreum (99–100 [6])	JN851747.1, JN851753.1
O_{MAN}	*P. pentosaceus* (100 [1])	KM005150.1
	L. plantarum (99–100 [4])	KM670024.1, EF439682.1
	Leuc. citreum (99–100 [5])	JN851753.1, JN851747.1
O_{LEG}	*L. plantarum* (99–100 [7])	HM101316.1, KM670024.1, KJ187143.1
	Leuc. citreum (99–100 [2])	JN851753.1
	Lactobacillus brevis (100 [1])	KJ187173.1
NO_{INPUT}	*P. pentosaceus* (99 [1])	KM005150.1
	L. plantarum (99–100 [2])	KM670024.1, AB819499.1
	Leuc. citreum (99–100 [6])	JN851753.1, JN851747.1
	Weissella cibaria (100 [1])	JN851745.1

[a] Treatments were set according to a completely randomized design with three field replicates. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs (control). Further details were included in Material and methods.
[b] Species showing the highest percentage of identity to the strain isolated from sourdough. The percentage of identity was that shown by performing multiple sequence alignments in BLAST.

isolate) were found only in mature sourdoughs made with O_{LEG} and NO_{INPUT} flours, respectively.

Phenolic compounds. Before fermentation, the highest concentrations of total phenols (46.6 ± 0.7, 47.6 ± 0.5, and 48.5 ± 0.5 mg liter^{-1}) were found in the doughs made with the O_{LEG} and O_{MAN} and NO_{INPUT} flours. The C_{ONV} dough contained total phenols at levels of 41.9 ± 0.4 mg liter^{-1}. Whatever the flour type, increases higher than 70% were found after sourdough preparation (data not shown). The concentrations of several individual phenolic compounds, in the free and bound forms, were determined (Table 5). The doughs made with organic flours contained the highest ($P < 0.05$) levels of bound phenolic compounds. The concentrations of total bound phenols increased markedly in the mature sourdoughs, and the highest levels were found with the O_{MAN}, O_{LEG}, and NO_{INPUT} flours. Whatever the flour type, the highest increases were found for bound sinapic acid. This phenolic acid, together with ferulic acid, was already the most abundant acid in the doughs. The concentrations of bound vanillic, syringic, and *p*-coumaric acids slightly decreased during sourdough preparation. The concentrations of phenolic compounds in the free form were markedly lower than those found in the bound status, and their variations were limited.

Antioxidant and phytase activities. The scavenging activity toward the DPPH radical was used to determine the antioxidant properties. During this assay, the colored stable DPPH radical is reduced to nonradical DPPH-H when in the presence of an antioxidant or a hydrogen donor. After 10 min of reaction, the scavenging activity of DPPH was 75.5% ± 0.3% for BHT. The antioxidant activities were found to be similar in the doughs made with C_{ONV}, O_{MAN}, and O_{LEG} flours (Fig. 3A). The statistically significant ($P < 0.05$) highest value was found for NO_{INPUT} dough. The antioxidant activity moderately increased in mature sourdoughs, while the greatest increase was obtained using O_{LEG} flour.

The phytase activity of the water-soluble extracts of the doughs made with organic and NO_{INPUT} flours was markedly ($P < 0.05$) higher than that found in C_{ONV} flour doughs (Fig. 3B). No significant ($P > 0.05$) increases in the phytase activity were found in the mature sourdoughs.

Correlations among microbiota and biochemical properties, phenolic compounds, and antioxidant activity. All the significant (false discovery rate [FDR] of <0.05) correlations between OTU at the species level and biochemical properties (pH, TTA, organic acids, FQ, carbohydrates, and total and individual FAA), total and individual bound phenolic acids, and

TABLE 5 Concentrations of total and individual free and bound phenolic acids of doughs (after mixing and before fermentation) and mature sourdoughs (after 10 refreshments) from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions[a]

Farming system[b]	Phenolic acid concn (μg g^{-1})											
	Vanillic acid		Syringic acid		*p*-Coumaric acid		Ferulic acid		Sinapic acid		Total	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Doughs												
C_{ONV}	19.0 ± 0.3 C	17.5 ± 0.3 B	5.5 ± 0.3 C	28.1 ± 0.8 B	ND	7.3 ± 0.5 B	1.2 ± 0.2 D	314.4 ± 11.6 C	ND	115.8 ± 7.3 G	25.7 BC	483.1 E
O_{MAN}	19.2 ± 0.4 C	8.1 ± 0.2 C	5.4 ± 0.3 C	33.5 ± 0.5 A	ND	8.7 ± 1.3 A	3.5 ± 0.1 B	329.6 ± 5.7 C	ND	146.8 ± 10.0 F	28.1 B	526.7 D
O_{LEG}	22.0 ± 0.4 B	ND	8.9 ± 0.2 A	34.3 ± 0.3 A	ND	8.9 ± 0.9 A	2.4 ± 0.2 C	339.6 ± 7.8 BC	ND	169.4 ± 11.2 E	33.9 A	552.2 C
NO_{INPUT}	27.7 ± 0.4 A	ND	ND	34.7 ± 0.8 B	ND	8.4 ± 1.3 C	5.1 ± 0.4 A	335.2 ± 15.6 D	ND	169.0 ± 5.5 G	32.8 A	547.6 F
Sourdoughs												
C_{ONV}	6.3 ± 0.1 F	3.4 ± 0.3 E	8.3 ± 0.3 A	26.8 ± 0.2 B	ND	7.5 ± 0.2 AB	1.5 ± 0.1 D	342.5 ± 4.6 BC	1.9 ± 0.1 B	227.8 ± 5.6 D	18.0 D	608.0 B
O_{MAN}	9.5 ± 0.2 D	6.4 ± 0.2 D	6.8 ± 0.2 B	21.9 ± 0.7 C	ND	6.1 ± 0.2 C	5.0 ± 0.3 A	354.9 ± 14.2 B	1.9 ± 0.1 B	321.9 ± 1.3 A	23.2 C	711.2 A
O_{LEG}	7.9 ± 0.2 E	2.0 ± 0.2 E	8.3 ± 0.1 A	27.8 ± 0.5 B	ND	7.8 ± 0.2 AB	2.2 ± 0.3 C	397.2 ± 5.4 A	9.4 ± 0.3 A	284.1 ± 12.3 C	27.8 B	718.9 A
NO_{INPUT}	7.8 ± 0.1 E	19.6 ± 0.3 A	7.3 ± 0.2 AB	22.8 ± 0.2 C	ND	7.6 ± 0.3 AB	2.1 ± 0.2 C	361.2 ± 11.2 B	ND	294.6 ± 10.4 B	17.2 D	705.4 A

[a] Data are means from three independent experiments ± standard deviations ($n = 3$). Values in the same column followed by different letters differ significantly ($P < 0.05$). ND, not detected.

[b] Treatments were set according to a completely randomized design with three field replicates. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic with green manure; NO_{INPUT}, without inputs (control). Further details are included in Materials and Methods.

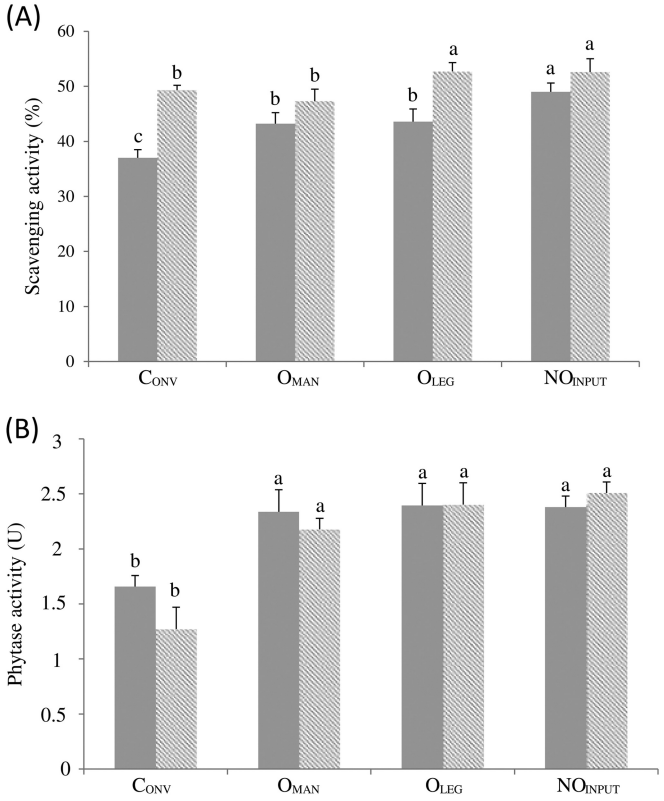

FIG 3 Antioxidant (A) and phytase (B) activities of the doughs (after mixing and before fermentation) (solid gray bars) and mature sourdoughs after 10 refreshments) (striped bars) from *Triticum turgidum* subsp. *durum* cv. Senatore Cappelli under conventional and organic farming conditions. C_{ONV}, conventional system; O_{MAN}, organic system with cow manure; O_{LEG}, organic system with green manure; NO_{INPUT}, without inputs. Further details are included in Materials and Methods. Antioxidant activity, calculated as scavenging activity on the DPPH radical, was expressed as follows: DPPH scavenging activity (%) = [(blank absorbance – sample absorbance)/blank absorbance] × 100. One unit of phytase activity was defined as the amount of enzyme required to liberate 1 μmol min^{-1} of *p*-nitrophenol under the assay conditions. Columns with different letters (a to c) differ significantly ($P < 0.05$).

antioxidant activity are shown in Fig. 4. The *L. plantarum* group, *Leuc. citreum*, and *Lc. lactis* were the only species that showed an abundant number of positive correlations with the above characteristics. In particular, the *L. plantarum* group was positively correlated with total FAA, several individual FAA (e.g., Val, Ile, Leu, Tyr, Phe, His), total bound phenols, and bound ferulic and sinapic acids. Several of the above correlations were also shown by *Lc. lactis* and *Leuc. citreum*. The latter species was also positively correlated with the antioxidant activity and almost all FAA. As expected, the above species showed positive and negative correlations, respectively, with the synthesis of lactic and acetic acids and the consumption of glucose, fructose, and sucrose. The abundance of the species belonging to the phylum *Proteobacteria* was mostly negatively correlated with all sought after dough and sourdough qualities.

Bread making. The use of sourdoughs influenced the pH values of the breads, which were in the range 4.9 ± 0.3 to 5.2 ± 0.2. Compared to bread made with C_{ONV} sourdough (2.78 ± 0.02 cm^3 g^{-1}), the specific volume of the breads made with organic (2.94 ± 0.03 to 2.98 ± 0.02 cm^3 g^{-1}) and NO_{INPUT} (3.22 ± 0.04 cm^3 g^{-1}) sourdoughs was significantly ($P < 0.05$) higher. The breads made with C_{ONV}, O_{MAN}, and O_{LEG} sourdoughs showed similar hardness values (4,256 ± 16 to 4,296 ± 25 g). The lowest hardness (4,050 ± 21 g) was found when NO_{INPUT} sourdough was used. Image analysis technology was used to assay the crumb structure. Digital images were preprocessed to estimate crumb cell-total area through a binary conversion. Compared to the C_{ONV} sourdough bread (48.22% ± 0.3%), the cell-total area (corresponding to the black pixel total area) of the O_{MAN} and O_{LEG} (53.05% ± 0.3% and 54.29% ± 0.3%) and, in particular, of the NO_{INPUT} (60.74% ± 0.3%) sourdough breads was higher. The highest crust lightness value (L) was found for the bread made with O_{MAN} (40.12 ± 0.1) and O_{LEG} (39.98 ± 0.2) sourdoughs. No significant ($P > 0.05$) differences were found between the C_{ONV} and NO_{INPUT} sourdough breads (38.79 ± 0.6 and 38.24 ± 0.4). No significant ($P > 0.05$) differences between the C_{ONV} and NO_{INPUT} sourdough breads were found for the parameter dE^*_{ab} (57.52 ± 0.3 and 57.88 ± 0.3), whereas the O_{MAN} and O_{LEG} sourdough breads showed lower values (56.29 ± 0.4 and 56.27 ± 0.4), respectively. The chromaticity coordinates of the bread crust (obtained with a

FIG 4 Spearman's rank correlation matrix of significant relationships between OTU (at the species level) and biochemical properties (pH, TTA, organic acids, carbohydrates, and total and individual FAA), total and individual bound phenolic acids, and antioxidant activity. The colors of the scale bar denote the nature of the correlation, with 1 indicating perfect positive correlation (dark green) and −1 indicating perfect negative correlation (dark red) between two microbial species and the above parameters. Only significant correlations (FDR of <0.05) are plotted.

Minolta CR-10 camera) were also reported in the form of a color difference, dE^*_{ab}, as follows:

$$dE^*_{ab} = \sqrt{(dL)^2 + (da)^2 + (db)^2}$$

where d*L*, d*a*, and d*b* are the differences between sample and reference for *L*, *a*, and *b* values (a white ceramic plate having an *L* value of 93.4, an *a* value of −0.39, and a *b* value of 3.99).

DISCUSSION

Previous studies have clearly demonstrated that sourdough positively affects the sensory, nutritional, and functional properties of baked goods (35, 36). It is also accepted that the microbial composition and performance of sourdough are influenced by a copious number of environmental and technological determinants (35, 37, 38). Among these determinants, the house microbiota contaminating the bakery environment seems to be one of the most important (35, 39). On the other hand, the most recent literature on the taxonomic structure and monitoring of the dominant population of lactic acid bacteria during sourdough propagation refers mainly to propagation at the laboratory level (16, 17, 23, 40–43). This is a prerequisite, especially when the goal of the study is to highlight the effect of one variable (e.g., temperature, dough yield, or flour type) (16, 42, 43) on sourdough microbiota. Spontaneous sourdough fermentations carried out at the laboratory level under semi-aseptic conditions indicate that the flour used for propagation is one of the possible sources of the microbiota (23, 35). Based on the above considerations, sourdough propagation under laboratory conditions was chosen to address the following questions. (i) Are the farming system and, in particular, the organic cultivation of durum wheat one of these determinants? (ii) Overall, may the organic cultivation of durum wheat reflect the flour-sourdough fermentation-bread axis?

The yield of the organic grain was similar to that of conventional grain. Plots for the cultivation of C_{ONV} and O_{MAN} grains received, at the same time, the same amount of nitrogen (N). The N supply to the O_{LEG} plot was ensured by the previous cultivation of faba beans (44). The ancient variety Senatore Cappelli (*T. turgidum* subsp. *durum*) was confirmed to be particularly suitable for organic farming (11). The conventional farming system favored the production of flour having the highest protein content, gluten index, and *W*. When green manure was used for organic farming,

the flour had a lower content of protein than that found under cow manure. The mineralization of the leguminous plant during growing season released N slowly, which could be responsible for this difference (45). All the flours had gluten index values of ≥60%, which were recommended for bread making (13), and "falling number" values representing low α-amylase activity and low sprout damage (13). The results of this study highlight certain aspects of sourdough production. First, the effect of the farming system on the gliadin and glutenin composition of *T. turgidum* subsp. *durum* was highlighted. Studies have shown that gliadins control dough viscosity, while glutenins are responsible for the elastic properties (25). The O_{MAN} and O_{LEG} flours showed the highest number of gliadins, especially the S-rich subunits. The O_{MAN} flour also had the highest number of HMM glutenins, which influence dough workability (46). LMM glutenins, which influence dough resistance and extensibility (34), were the most abundant for NO_{INPUT}, followed by the C_{ONV} and O_{MAN} flours. When the number and intensity of gliadins and glutenins were subjected to PCA (Fig. 1), the farming systems were clearly separated, meaning some differences in terms of the technological characteristics.

Sourdough is the ecosystem along the above-mentioned axis that has received most in-depth investigation. In this study, type I sourdoughs were prepared (back-slopped) under laboratory conditions, in accordance with traditional protocols for making breads (22, 26, 37). In agreement with the literature data (16, 47), the sourdoughs became mature after 10 days of propagation, and their biochemical characteristics (e.g., consumption of soluble carbohydrates and synthesis of organic acids) were found to be those of an evolved composition of the microbiota. As shown by plating, presumptive lactic acid bacteria had reached a stable number of ca. 9.0 log CFU g^{-1}, and the ratio between lactic acid bacteria and yeasts remained constant at ca. 100:1 (48). All the mature sourdoughs harbored species belonging to the genera *Saccharomyces*, *Candida*, *Kazachstania*, and *Rhodotorula*, as seen in the literature (16, 26, 35). However, the bacterial ecology was more complex and differences emerged among farming systems. As shown by PCoA (see Fig. S1 in the supplemental material), which was based on the weighted UniFrac distance matrix, the clustering of both the doughs and the mature sourdoughs showed a certain degree of differentiation between organic and conventional farming. Before sourdough preparation, the highest diversity was found for the C_{ONV} flour. This could be due to less competitive environmental conditions, which are also related to the use of fungicide compounds (49). Compared to the organic flours, the dough made with C_{ONV} flour showed greater abundance of *Firmicutes* and lower percentages of *Proteobacteria*. Usually, *Proteobacteria* are found in wastewater, forage feeding, and soils (16). The quantitative differences for the above phyla might be explained from an ecological point of view based on the concept of copiotrophic (*Firmicutes*) versus oligotrophic (*Proteobacteria*) bacteria (50). Genera belonging to *Proteobacteria* (e.g., *Pantoea* and *Pseudomonas*) of the family *Enterobacteriaceae* were mainly identified in the organic and NO_{INPUT} flours. During organic farming, *Pseudomonas* has the capacity to fix N_2, thus maintaining a suitable level of this element in the soil (51). *Firmicutes* were represented by *Lactobacillus*, *Pediococcus*, and *Leuconostoc* as the main genera. The two latter genera were only identified in the C_{ONV} flour. Such differences might be related to the variable capacity of metabolic adaptation and versatility. The *Lactobacillus plantarum* group was the only group found in all the flours. The ecological adaptability to different habitats and the metabolic versatility of this species are documented in the literature (52). As previously shown for conventional wheat, rye, and spelt flours (16, 23, 53, 54), the mature sourdoughs were almost completely and stably dominated by *Firmicutes*, through a transient phase evolution during refreshments. The diversity of *Firmicutes* was the highest for mature sourdoughs made with organic (green manure) and, especially, NO_{INPUT} flours. The sequencing (55, 56) approach was used to allow the identification of both dominant and subdominant microbial populations. The species identified in the mature sourdoughs corresponded to those usually found for conventional durum wheat sourdoughs (16, 22, 26). *Leuconostoc citreum* dominated mature sourdoughs made with the C_{ONV}, O_{MAN}, and NO_{INPUT} flours. The mature C_{ONV} sourdough also harbored *Lactococcus lactis* and the *L. plantarum* group and *Lactobacillus brevis* at low percentages. The *L. plantarum* group and *Pediococcus* sp. were mainly or only found in the mature organic and NO_{INPUT} sourdoughs. The robustness and good technological performance of *L. plantarum* have been previously shown (52). *Weissella* sp., *W. cibaria*, and *Lactobacillus sanfranciscensis* were identified only in the mature NO_{INPUT} sourdough. The culturing method qualitatively confirmed the dough and sourdough microbial composition obtained through pyrosequencing analysis. These differences in the compositions of the sourdough microbiota resulted from a combination of determinants, including environmental contaminations (e.g., depending on farming system), parameters for sourdough preparation (the same for all flours), and chemical composition of the flours (variable for some constituents). As previously observed (57, 58), the farming system affected the phytase activity of the flours and, especially, the content of phenolic acids. The farming system appears to have affected the phytase activity of the flours and, especially, their phenolic acid content. Relevant concentrations of bound ferulic and sinapic acids were present in the organic flours (57). The highest abundance of the *L. plantarum* group was found in the doughs and sourdoughs made with these flours. It has been previously shown (43) that the competiveness and the composition of lactic acid bacteria microbiota may be strongly affected by the phenolic compounds of the matrix. In particular, it was shown that *L. plantarum*, able to withstand the inhibitory activity of phenolic compounds (59), was able to degrade some food phenolic compounds, giving compounds that positively affect food aroma or increase the antioxidant activity (59). As previously shown (59, 60), *L. plantarum* is well able to withstand the inhibitory activity of phenolic compounds. In this study, independently of flour type, sourdough fermentation increased the level of total bound phenolic acids. Lactic acidification improves the extraction of total phenols (25), and feruloyl-esterase and β-glucosidase activities have recently been described for lactic acid bacteria (25). In summary, it can be hypothesized that the above activities caused the hydrolysis of phenolic compounds and their glycosylated forms into corresponding phenolic acids as previously reported (59). The increased solubilization of phenolics might have influenced the increased level of the antioxidant activity, which was found in the mature sourdoughs. At the end of sourdough propagation, only the *Firmicutes* genera and species were positively correlated with various sought after qualities characterizing the sourdough fermentation.

To complete the flour-sourdough fermentation axis, all the

sourdoughs used for making breads were mature. Characteristics such as specific volume, crumb structure, and crust color were found to be superior in the breads made with the organic sourdoughs and flours. These effects could be attributed to the physicochemical, technological, and microbiological characteristics of the organic flours and sourdoughs. Surprisingly, the bread made with NO_{INPUT} sourdough and flour showed optimal characteristics. The yield of durum wheat using this farming system was the markedly the lowest.

Besides the acknowledged role of the introduction of flour into the bakery environment to build up the house microbiota (39), the farming system is undoubtedly another determinant able to affect the flour microbiota and, consequently, the dynamics of a sourdough microbial community.

The organic cultivation of durum wheat reflects along the flour-sourdough fermentation-bread axis and could be considered a suitable alternative for making sourdough breads with specific characteristics.

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