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A metagenomic-based, cross-seasonal picture of fungal consortia associated with Italian soils subjected to different agricultural managements

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ABSTRACT

This work pictures the biodiversity of fungal consortia inhabiting real agroecosystems, sampled in one production farm in two seasons (spring, autumn), coinciding with climate gradients and key moments of the agricultural cycle. Soil was sampled from three plots differently managed in terms of fertilization, pesticide and tillage application: conventional, organic, no-tillage. Metagenomic analyses on ITS1 amplicons depicted the highest indexes of richness for organic. No-tillage resulted in inhabitation by the most divergent communities, with their own composition, prevalence and seasonal trends. Ascomycota always predominated, with the exception of conventional, that had high abundance of a single basidiomycete species. Our results showed evidence that agricultural soils under organic and no-tillage systems harbour distinct mycobiota, even in neighbouring fields. From our results, fungal consortia altered even in the first year after the management change.

1. Introduction

Fungal communities (mycobiota) fulfil major ecological functions in many environments, among which are soils. Here, they seem to dominate the microbial biomass (Joergensen and Wichern, 2008) and participate in nutrient cycling, including natural carbon, nitrogen and phosphorus cycles (Christensen, 2013). Fungi are also involved in a range of other processes, integral for the ecological functioning of all soils: decomposition of organic matter, soil stabilization, plant productivity and protection against pathogens, and composition of the plant community (van der Heijden et al., 2008). Clearly they contribute to soil fertility and quality, and with resident bacterial communities, are considered crucial bioindicators (Schloter et al., 2003; Sharma et al., 2010).

The impact of soil bacteria and fungi is so deep that the ambitious Earth Microbiome Project has been launched, aiming to analyse 200,000 soil samples and construct a Gene Atlas of uncultured microbial diversity for all biomes on Earth (Gilbert et al., 2014). Also, the International Decade of Soils was launched in 2015 by the International Union of Soils Sciences, for addressing the complex relations between the exacerbated exploitation of soils and many compelling health, environmental and social issues (iuss.org).

These concepts apply *a fortiori* to agroecosystems. A solid understanding of microbiota and mycobiota dynamics and diversity is of pivotal importance in agroecosystems, together with a clear elucidation of its responses to natural fluctuations and

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management decisions. Management considerations include the diverging possibilities offered by conventional fertilization and pesticide application vs. low-input organic farming, or conventional tillage vs. low-disturbance no-tillage practices. The large increase in agricultural productivity has led to ecosystem and soil degradation, accumulation of pesticides, diminished availability and quality of water (Alvarez et al., 1995; Tilman et al., 2002; Foley et al., 2005), and is perceived as a main threat for global biodiversity (Convention on Biological Diversity, 2010). Another important point is that a deep understanding of soil bacterial and fungal communities constitutes the premise for managing them in terms of presence of beneficial species and absence of detrimental ones (Chaparro et al., 2012), and for understanding processes that affect fertility (Carbonetto et al., 2014).

The effects of farming management on soil mycobiota and microbiota are complex and appear variable (Bunemann et al., 2006; Carbonetto et al., 2014). It is usually reported that organic farming favours an higher abundance and diversity of macrobiota, but data on microbial communities, especially on fungi, are less complete (Postma-Blaauw et al., 2010) and often do not refer to 'real' fields under production. The adoption of limited tillage systems, less disturbing for soils, is known to augment soil organic matter, water content, and crop yields (Alvarez and Steinback, 2009), but again effects on mycobiota are still scarcely understood.

This work aims at picturing a broad spectrum of the biodiversity of fungal communities inhabiting different agroecosystems located in the same production farm. To our knowledge, this has been done only a few times so far, in completely different agricultural realities and with other experimental approaches (see for example Moeskops et al., 2010). The farm sampled in our study produces crops and is located in the Padan Plain area (Pavia province, Lombardy, Italy), one of the main European agricultural sites. The sampled plots are subjected to differential management practices that are applied in parallel in an effort to find which one better combines productivity, environmental sustainability and the addition of minor amounts of additives. These management protocols differ for the use of fertilization, pesticides and tillage. A second important objective of this work is to describe the influence of seasonality (e.g., climate fluctuations, but also seasonal agricultural treatments in key moments of the production cycle) on the structure of the mycobiota. These goals will be reached by high throughput deep sequencing using an Illumina MiSeq-based amplicon sequencing of the ribosomal internal transcribed spacer-1 (ITS1) region.

2. Methods

2.1. Management protocols applied to the plots: fertilization, pesticide application and tillage

Samples were collected from three fields belonging to the production farm 'La Calvenzana' (Rivanazzano Terme, Pavia, Italy). The first one, 'Pomocotogno', spans 5 hectares (ha) and will be hereafter referred to as 'conv' because it receives fertilizer and plant protection schemes based on conventional, high-throughput systems. Nitrogen fertilizer (urea, 58 kg ha^{-1}) is applied once a year (normally around mid-April) and plant protection is achieved using herbicides (3 treatments per year) employing the recommendations and thresholds of the EU Regulation (EC) No 1107/2009. Conventional tillage is applied. During recent years, this field has had a rotation of annual crops, with chickpea (harvested in 2015) and grain sorghum (harvested in 2016) most recently.

The 'Vallone 2' plot, spanning 4 ha, has been managed since 2010 following the EU Council Regulation No 834/2007 on organic agriculture. After the prescribed 5-year cultivation of alfalfa to enrich soil especially for nitrogen, this field (hereafter 'org') yielded its first certified organic production (barley, variety 'bio arda') in 2016. Plant protection is achieved here by using mechanical strategies. Conventional tillage is applied and no additional fertilization is given.

The 'Valloncino 3' plot (3 ha) has been subjected to conservational no-tillage (or sod-seeding) practices i.e., minimum soil disturbance combined with rotations, since 2015. It will be referred to as 'sod'. This plot receives two herbicide treatments per year and nitrogen fertilizer (58 kg ha^{-1}) as specified above for conv. The last crop rotation before sampling was sorghum-barley (Manara variety).

2.2. Sample collection

Soils were sampled during autumn (A, end of November 2015) and spring (S, end of April 2016). Three soil samples were collected from each plot to trace a triangle, with vertices placed as far apart as possible, but at least 10 m away from field edges. Soil samples (500 g each) were aseptically taken at 3 cm depth with a sterile spoon, after removal of vegetation cover, stones and other debris, and put in sterile polyethylene bags. Samples were returned to the laboratory in coolers and were kept at −20 °C (for the metagenomic analyses) or +4 °C (for the other analyses) for 24–48 h before processing. For metagenomics, samples referring to each plot were pooled before freezing.

2.3. Soil chemical analyses

Chemical properties of soils were determined by Minoprio Analisi e Certificazioni, Como, Italy, according to the Italian standard protocols (DM 13/09/99). The following parameters were evaluated: pH, organic matter, total nitrogen (N_{TOT}), organic carbon (C_{ORG}), C/N ratio, plant-available phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K), soil composition in sand, silt, clay and soil texture.

2.4. DNA extraction, ITS1 amplification and Illumina sequencing

Total DNA was extracted from 350 mg of 'A' (autumn) and 'S' (spring) samplings of conv, org and sod plots, using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's specifications. The extraction buffer SL1 was used, supplemented with 70 μl of SX enhancer. DNA was then quantified on a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA). For amplicon production, the ribosomal ITS1 region was targeted, by using primers BITS and B58S3 (Bokulich and Mills, 2013) linked to Illumina adapters. PCR was performed in a 50 μl volume containing 5–10 ng template DNA, 1x HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA), 0.5 μM of each primer. The cycling program, performed on a MJ Mini thermal cycler (Promega corp., Madison, WI), included an initial denaturation (95 °C for 3 min), followed by 25 cycles at 94 °C for 30 s, 55 or 60 °C for 30s, 72 °C for 30 s, and final extension (72 °C for 5 min). Amplicons obtained using the two annealing temperatures were pooled as suggested by Schmidt et al. (2013). Clean-up of amplicons was performed using Agencourt AMPure XP SPRI magnetic beads (ThermoFisher Scientific). Illumina sequencing libraries were finally constructed through the link of indexes (Nextera XT Index Kit, Illumina, San Diego, CA), quantified using a Qubit 2.0 Fluorometer (ThermoFisher Scientific), normalized and pooled. Libraries were subjected to paired-end sequencing (2 × 250 bp, nano format) on an Illumina MiSeq sequencer at BMR Genomics (Padova, Italy).